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Date: **Meenakshi Sharma**

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LIST OF ABBREVIATIONS

ATP	Adenosine triphospahte	**AA**	Arachidonic acid
AD	Alzheimer's disease	**ANOVA**	Analysis of variance
aprv.	Approved	**BP**	Blood Pressure
brs	Broad singlet	**C**	Carbon
CAM	Cell adhesion molecules	**C 5a/3a**	Complement components
$CDCl_3$	Deuterated chloroform	**COX**	Cyclooxygenase
Cl	Clinical trials	**CV**	Cardiovascular
CRH	Corticotrophin releasing hormone	**Calc.logP**	Calculated logarithm of partition coefficient
d	Doublet	**dc**	Discontinued
DMARDs	Disease modifying anti- rheumatic drugs	**DMF**	Dimethyl formamide
dd	Doublet of doublets	**ED_{30}**	Dose effective for 30% inhibiton
ER	Endoplasmic reticulum	**FDA**	Food and drug administration
GI	Gastrointestinal	**GluC**	Glucuronide conjugation
HOMO	Highest occupied molecular orbital	**HPA**	Hypothalamus pituitary adrenal axis
HETEs	Hydroxyeicosatetraenioc acids	**ip**	Intraperitoneal
IAEC	Institutional animal ethics committee	**IUPAC**	International union of pure and applied chemistry
IL	Interleukins	**IC_{50}**	Concentration for 50% inhibition
IgG	Immunoglobin G	**IR**	Infrared
LT	Leukotrienes	**LOX**	Lipooxygenase
LPS	Lipopolysaccharide	**LD_{50}**	Dose lethal for 50% animals
LUMO	Lowest unoccupied molecular orbital	**NSAIDs**	Nonsteroidal anti-inflammatory drugs
mp	Melting point	**NMR**	Nuclear magnetic resonance
N	Nitrogen	**ns**	Nonsignificant
OA	Osteoarthritis	**ORD**	Optical rotatory dispersion
PAF	Platelet activating factor	**PAR**	Protease activated receptors
PG	Prostaglandins	**PT**	Pharmaceutical tool
POAH	Preoptic area of anterior hypothalamus	**PM**	Parameterized method
PL	Phospholipase	**PPA**	Polyphosphoric acid
p.o.	peroral	**qr**	Quartet
ppm	Parts per million	**RA**	Rheumatoid arthritis
qn	Quintet	**Tx**	Thromboxane
SEM	Standard errors of mean	**TPA**	Tetradecanoyl phorbol acetate
TNF	Tumor necrosis factor	**TI**	Temperature index
TLC	Thin layer chromatography	**TMS**	Tetramethyl silane
ud	Underdevelopment	**wd**	Withdrawn

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ABSTRACT

Based on established facts variously substituted indan-1-alkanoic acids and their amide derivatives were synthesized as better and safer NSAIDs. The idea behind derivatization was to lower the side effects such as gastric irritation and ulceration, which are associated with free carboxyl group. Neutralization of the carboxyl group by amidation was done with the view that these would not only enhance absorption by increasing lipophilicity but would also impart COX-2 selectivity. Structures of the synthesized compounds were confirmed by their spectral and elemental analysis.

Synthesized compounds were tested for their anti-inflammatory and analgesic activities. Few selected compounds were also screened for their antipyretic, anti-arthritic and ulcerogenecity profiles. Anti-inflammatory, analgesic, antipyretic and anti-arthritic activities were determined using carrageenan-induced rat paw edema model, acetic acid-induced writhing model, lipopolysaccharide-induced pyresis model and adjuvant-induced arthritis model (prophylactic) respectively. These compounds were found to be long acting and their residual anti-inflammatory activity at 24^{th} hour exceeded that of Indomethacin. These compounds showed negligible gastrointestinal ulcerogenecity as compared to the standard drug indomethacin. It was found that increase in side chain length enhances the activity profile (acetic acid derivatives were more active than the carboxylic acid derivatives). Though some of the synthesized compounds showed good analgesic and antipyretic activity profile, however, these compounds were found to be less effective in the arthritis model. One of the test compounds showed a very interesting toxicity profile which if properly exploited may lead to a candidate drug or a pharmacological tool. The LPS induced pyresis model for antipyretic screening also showed that the inhibition of TNF-α is not involved in the mechanism of action of these compounds. Studies with the enzyme inhibitors revealed that probably the test compounds *per se* are the active species. The molecular orbital studies were also done in order to establish the relationship between HOMO and LUMO surfaces and their energies with the biological activity of these compounds.

CHAPTER 1

INTRODUCTION

Musculoskeletal disorders are among the most prevalent conditions worldwide accounting for a high proportion of those with disability in the work force as well as in the elderly. Musculoskeletal conditions cause more functional limitations in the adult population in most welfare states than any other group of disorders. The economic and social burden of these diseases is great and their impact on both individuals and society results from a decreased quality of life, lost productivity and increased costs of health care. The classification of these rheumatic conditions is hampered by the absence of firm etiological evidence for most of the diseases and heterogeneity in their clinical presentations. The term rheumatic diseases include rheumatoid arthritis (RA), osteoarthritis (OA), systemic lupus erythematosus and scleroderma, ankylosing spondylitis, osteoporosis, back pain, gout, fibromyalgia and tendonitis[1, 2](Table 1.1). Rheumatoid

arthritis is a chronic, generally progressive autoimmune disease that causes functional disability, significant pain and joint destruction, and leads to premature mortality. It is estimated to affect between 0.5 and 1.0% of the adult population worldwide and 0.75% of Indian population and increases in prevalence with age and affects more women than men[3, 4] . Available statistical data reveals that about 19 % of the rural Indian population suffers from musculoskeletal disorders[5].

Table 1.1. Epidemiology of major rheumatic diseases [1, 6]

Disease	Point Prevalence/1000	Incidence/1000	Age Ratio [65:25] yr	Gender Ratio [Female:Male]
Rheumatoid arthritis	8.0 **5.1**	0.5	6:5	2.5:1
Juvenile chronic arthritis	0.7	0.1	N/A	2:1 – 7:1
Osteoarthritis (knee)	100 **58**	N/A	0	2:1
Ankylosing spondylitis	2.0	0.07	0	1:3
Systemic lupus erythematosus	0.4	0.05	1.5:1	3:1 - 9:1
Systemic sclerosis	0.1	0.01	3:1	4:1
Gout	N/A **1.0**	1.0	2:1	1:6
Back pain	**131**			
Knee pain	**127**			
Shoulder pain	**74**			

Bold figures correspond to Indian data

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used to relieve pain and symptoms of acute and chronic musculoskeletal disorders. In addition to their antiinflammatory

and analgesic effects, NSAIDs also have unwanted side effects on the upper gastrointestinal tract. According to Food and Drug Administration (FDA) of United States, the prevalence of serious events (symptomatic ulcers, bleeding, perforations) is in 1-2% patients treated with NSAIDs for up to three months, while for those patients treated for a year, it can be seen in 2-5% of patients[7]. Hence development of better and safer NSAIDs is always desirable.

1.1. Inflammatory Process: Inflammation is a normal, protective, complex, homeostatic process. When tissue injury is caused by a single, nonlethal, finite event such as mechanical trauma, a thermal or chemical burn, or a single exposure to a nonreplicating antigen, the inflammatory and reparative process progresses smoothly from injury to healing. In contrast, persistent inflammation, such as seen in RA, may be harmful with destruction of cartilage and supporting joint structures as well as through systemic reactions[8].

The hallmarks of a localized acute inflammatory response are swelling (tumor), redness (rubor), heat (calor), pain (dolor), and loss of function (functio laesa). Inflammation in the early phase serves to destroy, inactivate, confine or neutralize the initiating irritant or etiologic agent while in the latter stages helps to clean the debris so that healing can occur. The initiation of the acute inflammatory process is dependent on the release of vasoactive and chemotactic agents from the injured cells and tissues. The vasoactive mediators cause the relaxation of vascular smooth muscle cells and an increase in vascular diameter (vasodilation). This results in an increase in the volume of blood in the area and a reduction in blood flow. The increased blood volume causes the tissue to redden and also heats the tissue. Inflammation also results in the release of mediators (as shown in Fig. 1.1) that cause endothelial cell contraction leading to increased vascular permeability in which fluid between the endothelial cells that form the blood vessels leaks into the underlying tissue, particularly at the postcapillary venules. This results in accumulation of fluid (edema) in the tissue and enables extravasation of leukocytes into the tissue that further contribute to the swelling and redness of the area[9].

Serotonin or histamine (effects last 12 h) and kinins (effects last 6 h) are released in the early phase of inflammation. The late phase mediators include eicosanoids (prostanoids and leukotrienes that steadily increase up to 24th h), lysosomal and fibrinolytic enzymes, free oxygen radicals, platelet activating factor (PAF), cytokines, e.g., tumor necrosis factor (TNF) and interleukins (IL-1β, IL-6), chemokines (IL-8), immunoglobulin, complement and other blood proteins. These are responsible for pain, for promoting cellular infiltration into the inflamed site, and for suppuration, malaise and fever. Local inflammation provides early immune protection by enabling white blood cells and plasma components to access the infected area. This effectively restricts tissue damage to the site of infection and enables the repair process to begin. Inflammation is initiated with the activation of the innate immune response which provides immediate protection for the host[10, 11]. The recent detection of protease-activated receptors (PARs) on peripheral and central neurons suggests that neuronal PARs might be involved not only in neurogenic inflammation and neurodegenerative processes, but also in nociception[12].

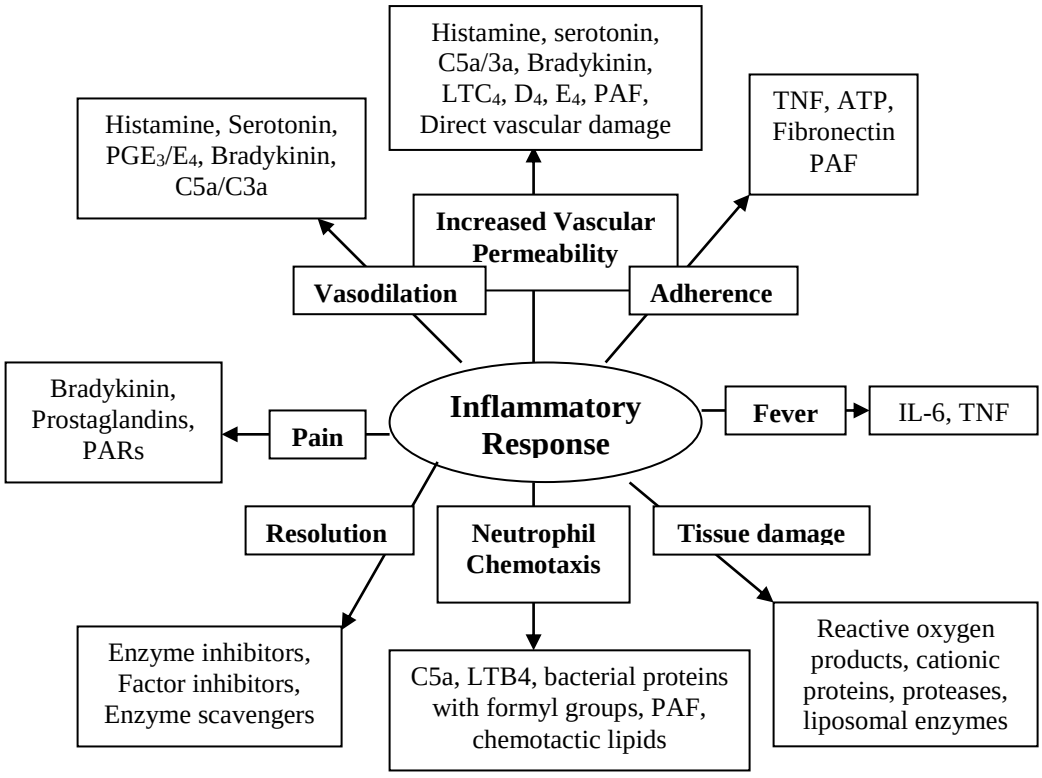


Figure 1.1. Various actions and the corresponding mediators of inflammatory response[11]

1.2. Proinflammatory Cytokines: All proinflammatory cytokines are pleiotropic in nature; that is, they can mediate different responses on different cell types. Proinflammatory

cytokines, such as TNF-α, IL-1β and IL-6 are endogenous pyogens, each of which acts on the hypothalamus to induce fever that inhibits the growth of a number of pathogens. In addition, all three cytokines independently induce the production of acute phase proteins in the liver that are subsequently released into the circulation. These acute phase proteins include C-reactive protein (a2 marker for sepsis), serum amyloid A, fibrinogen, mannose-binding protein and various complement components. These molecules are the raw materials needed for coagulation, phagocytosis and direct killing of the bacteria. Both TNF-α and IL-6 act on vascular endothelial cells and macrophages to induce secretion of colony stimulating factors (CSFs) that act within the bone marrow to induce hematopoiesis, resulting in increased numbers of white blood cells needed to fight the infection. Both TNF-α and IL-1β act on vascular endothelial cells to cause both increased vascular permeability and increased expression of cell adhesion molecules (CAMs) such as intercellular CAM (ICAM-1)**,** vascular CAM (VCAM-1), as well as endothelial CAM (ECAM or E-selectin)[13]. The various actions of these cytokines have been shown in Figure 1.2.

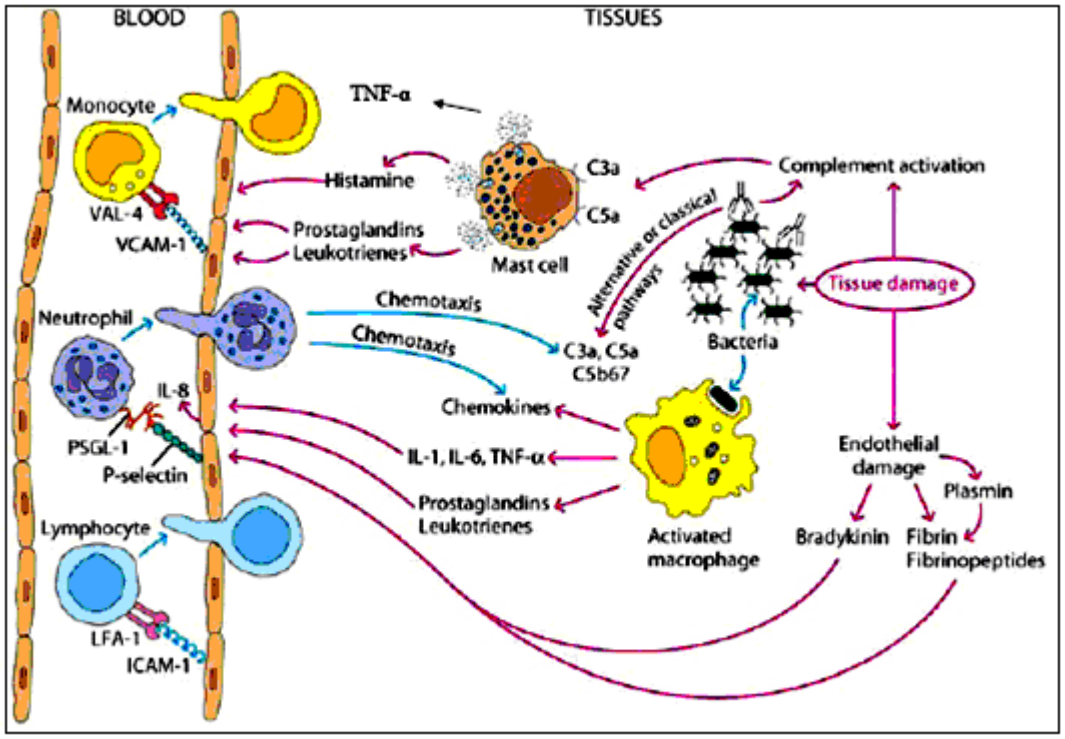
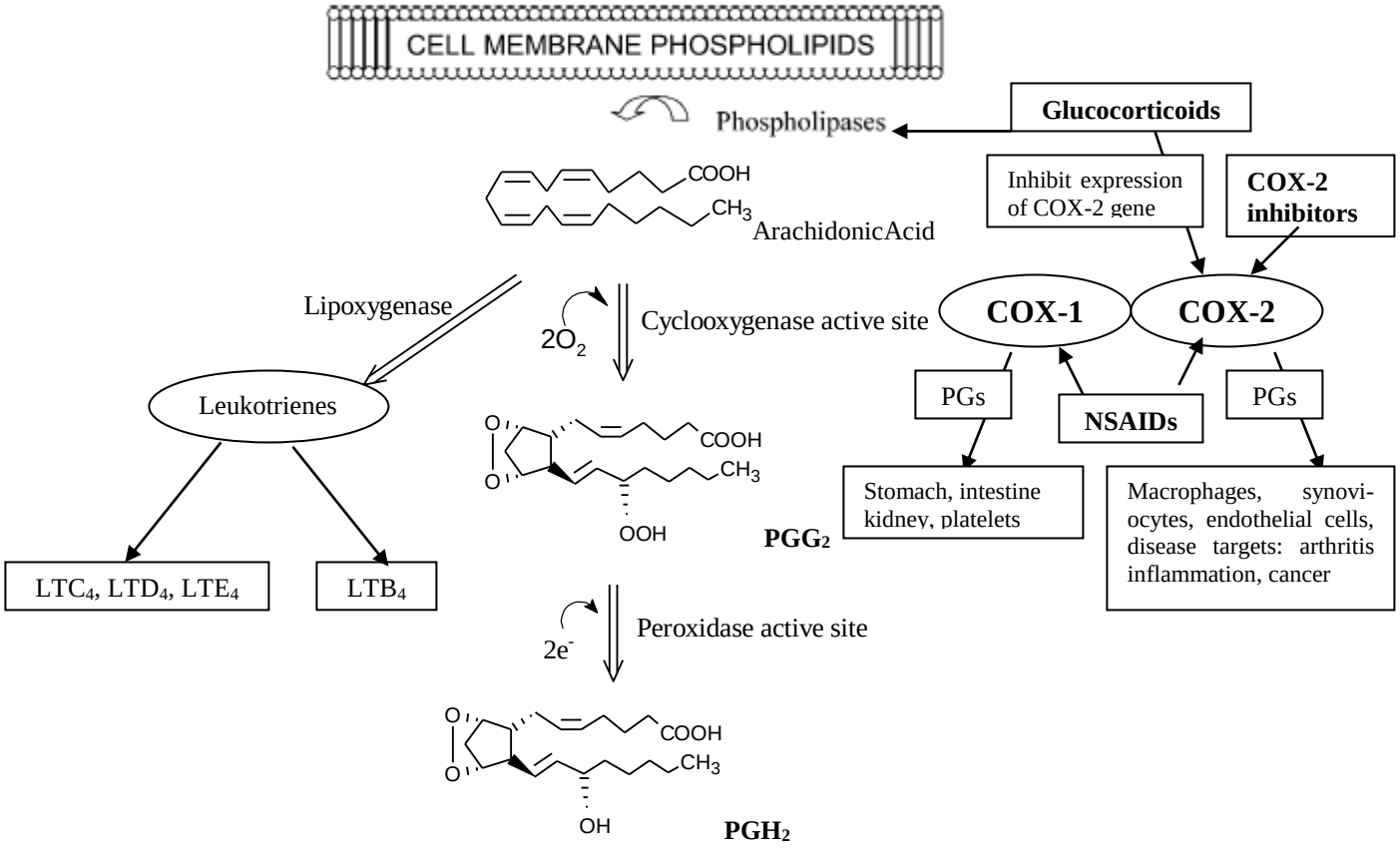


Figure 1.2. The inflammatory process[14]

1.3. Arachidonic Acid Cascade: Eicosanoids are biologically active metabolites derived from arachidonic acid (AA). Following its release from membrane phospholipids in response to a variety of nonspecific activation stimuli (physical, chemical, hormonal, cytokines, etc.),

AA is mobilized from the membrane phospholipid bilayer through the action of phospholipases (PL) and converted to prostaglandin (PG)H_2 by PGH-synthase-1 or -2, also referred to as cyclooxygenases (COX)-1 and -2. Cyclooxygenase isozymes catalyze a two-step reaction, first cyclizing AA to form PGG_2, and then reducing the 15-hydroperoxy group to form PGH_2. Cell-specific PG synthases catalyze the conversion of PGH_2 to biologically active end-products including PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 and thromboxane (Tx)A_2, known collectively as prostanoids (Figure 1. 3). In addition, AA can be metabolized by different lipoxygenases (LOX) and converted to hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LT). Eicosanoids modulate or, in some instances, mediate the action of a variety of biological responses. They are produced ‘‘on demand’’, rather than released from stores in the cell of origin, act primarily as autacoids on the parent and/or neighboring cells and have very short biological half-lives[15]. Once generated, prostaglandins exert their actions on other cells through various G-protein coupled receptors. The functions of various eicosanoids have been described in Figure 1.4.



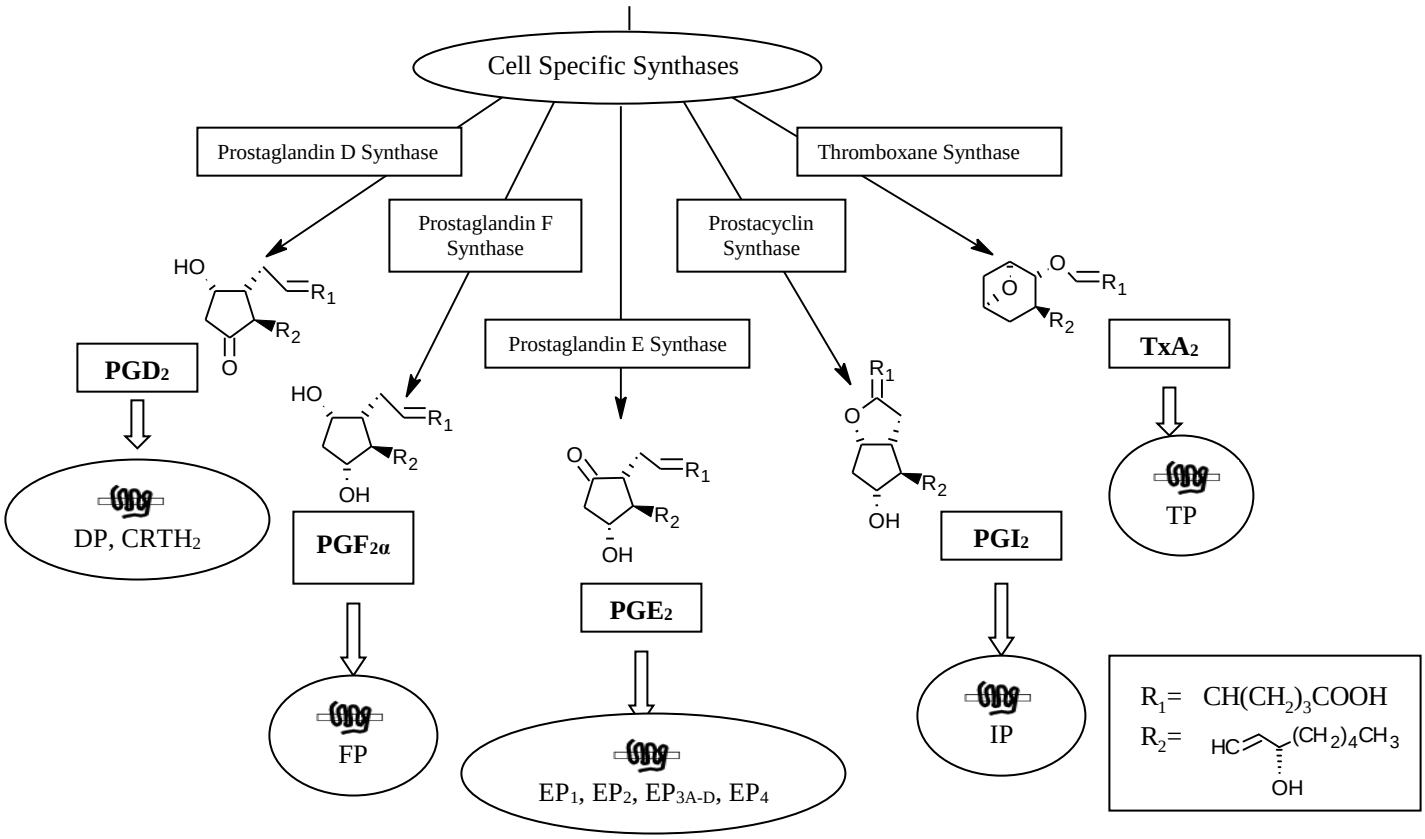


Figure 1. 3. Arachidonic acid cascade and site of action of anti-inflammatory drugs[16 - 20]

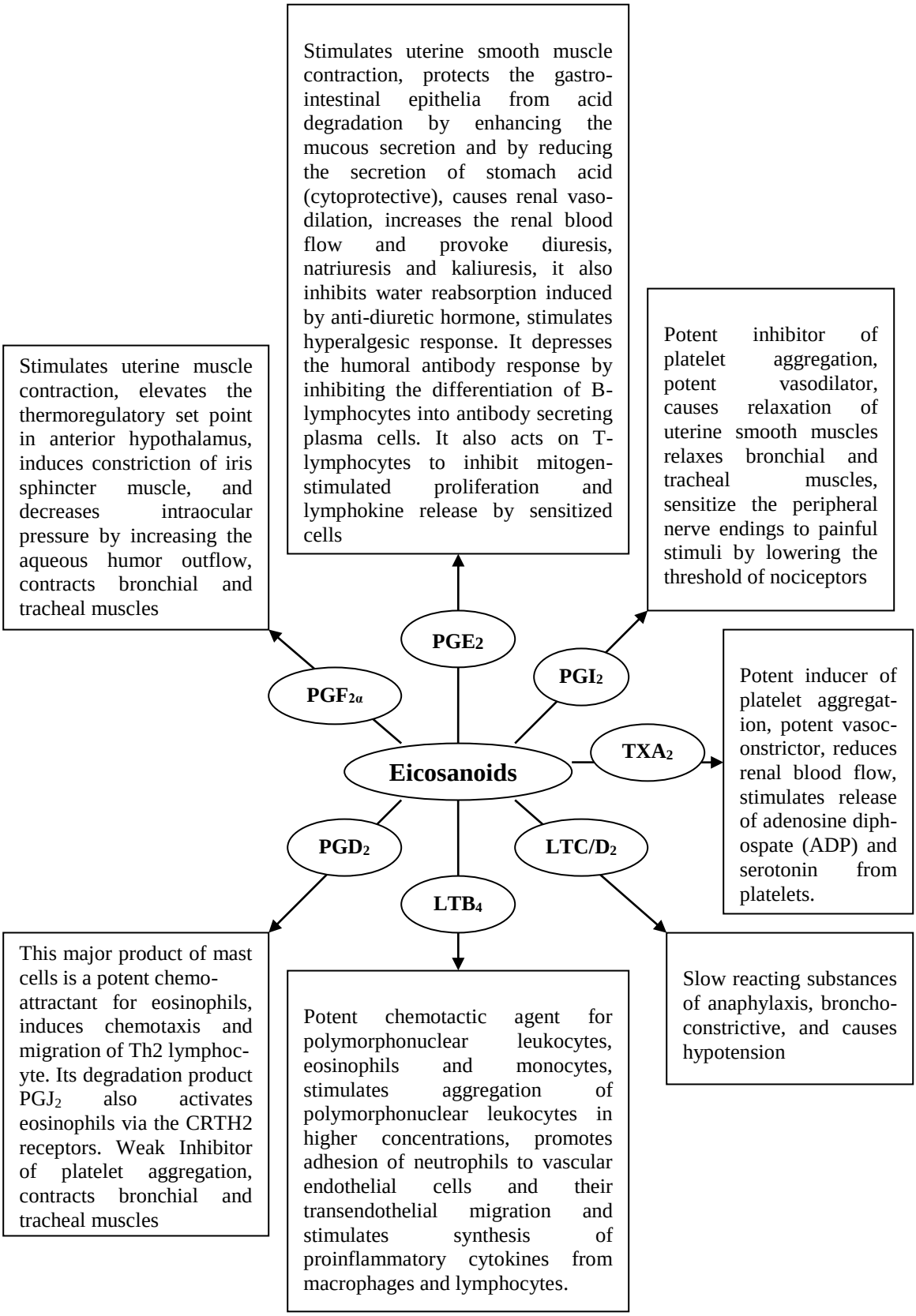


Figure 1.4. Pharmacological actions of various eicosanoids [20 - 22]

1.4. Cyclooxygenases: In relatively recent years, two different isoforms of cyclooxygenase (COX) have been identified. Cyclooxygenase-1 (COX-1) can be detected in most tissues and is typically expressed at constant levels throughout the cell cycle. Cyclooxygenase-2 (COX-2) is undetectable in most mammalian tissues, but its expression can be induced rapidly in cells involved in inflammation like fibroblasts, monocytes, and vascular endothelium in response to growth factors, tumor promoters, hormones, bacterial endotoxin and cytokines. Therefore COX-1 has become known as the constitutive isoform and COX-2 as the inducible one. Thus COX-1 generates 'good' prostaglandins for physiological 'housekeeping functions', including platelet dependent homeostasis, gastric mucosal integrity, and regulation of renal blood flow, while the COX-2 forms the 'bad' prostaglandins involved in inflammatory reactions and is responsible for inflammatory signs like fever, pain, capillary edema and vasodilatation. As a direct consequence, the specific inhibition of COX-2 is expressed to cause significant anti-inflammatory relief without interfering with prostaglandin-mediated physiological processes, especially gastrointestinal and renal functions. On these foundations pharmaceutical industry made efforts to develop and market highly selective COX-2 inhibitors with little or no COX-1 inhibitory activity. However, more recent evidence has shown that both COX-1 and COX-2 are involved in physiological as well as pathological processes[23].

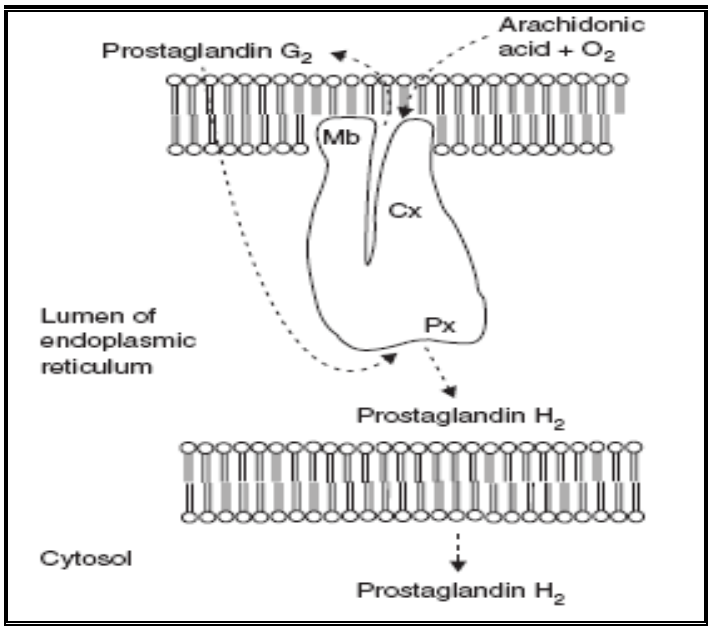


Figure 1.5. Cross-section of cyclooxygenase monomer in the lumen of endoplasmic reticulum, showing two distinct catalytic sites. Cx, cyclooxygenase catalytic site; Mb, membrane binding domain; Px, peroxidase catalytic site.

Structurally, the COX enzyme is composed of three independent folding units: an epidermal growth factor-like domain, a membrane-binding motif and an enzymatic domain that contains both the COX and peroxidase active site as shown in Figure 1.5. The active site of the COX enzyme consists of a long hydrophobic channel with the amino acids tyrosine 385 and serine 530 at its apex. This channel extends from the centre of the catalytic domain to the outer surface of membrane binding site. Although the genes for COX-1 and COX-2 are different, the proteins share approximately 60% homology at the amino acid level. Since the amino acid sequences of both enzymes are closely related the structures are very similar. Cyclooxygenase-1 and -2 enzymes are homodimers. Both COX-1 and COX-2 are membrane bound proteins that reside, after synthesis and transport, primarily in the endoplasmic reticulum (ER). The lumen of endoplasmic reticulum is important for both the structure and function of COXs; its oxidative potential allows the formation of the disulfide bonds of the enzymes, and N-linked glycosylation which occurs in the ER appears to be necessary for proper protein folding. Moreover, the final product of COXs, PGH_2, is sufficiently nonpolar to diffuse through the membrane of the ER to isomerases located on the cytosolic surfaces of the ER or in the cytosol [24, 25].

Most NSAIDs compete with arachidonic acid for binding at the COX active site. Eight amino acid residues play an important role for the substrate and the inhibitor binding in the COX channel (Figure 1.6). The amino acid residues surrounding the active site had been described as inner first shell residues that are in direct contact with the inhibitors or to the second shell residues which are not in direct contact with the inhibitors. The amino acid numbering refers to the COX-1 and COX-2 enzyme coding (for the residues in COX-2 one has to subtract 14 to reach the homologous amino acid residue in COX-1)[25]. The amino acid residues and their corresponding interactions have been described in Table 1.2.

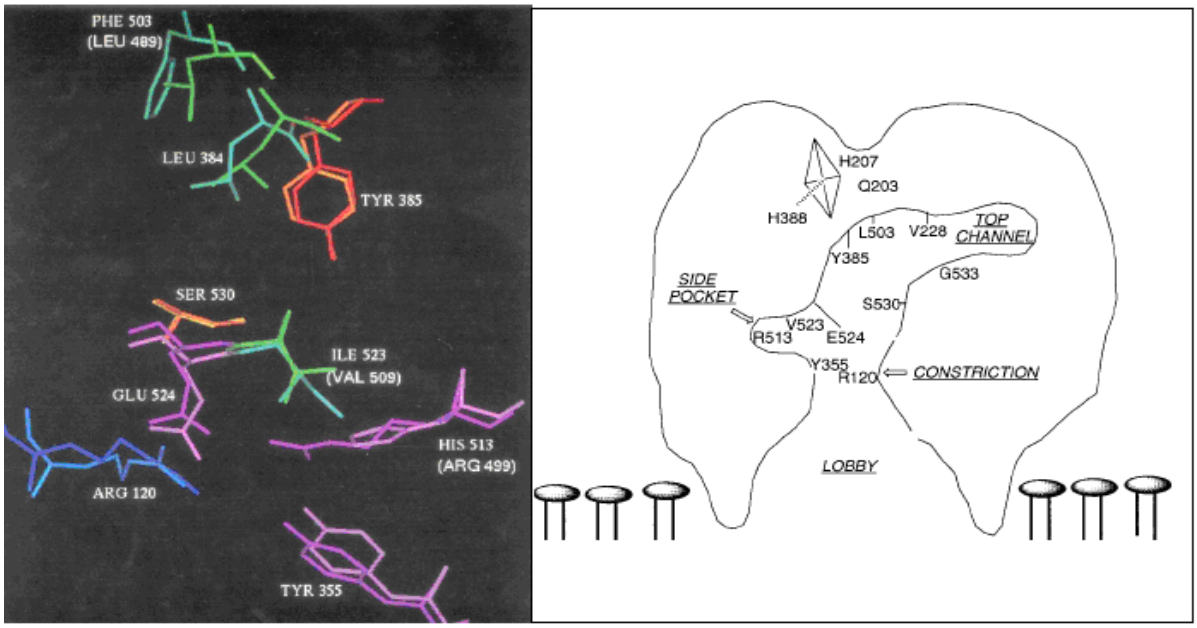


Figure 1.6. COX subunit with key structural elements and important amino acid residues[25, 26]

Table 1.2. Various sites of COX and possible interations[25, 27 - 29]

VARIOUS SITES OF COX	RESPONSIBLE AMINO ACID	INTERACTIONS
Catalytic Centre (apex of channel)	Tyr385 (Tyr371in COX-2)	13-pro *S* hydrogen of arachidonic acid interacts with phenolic oxygen of Tyr 385 and is at a distance of 2.5 A from the latter
Acylation Centre	Hydroxyl group of serine 530 (516 in COX-2)	Acylation and irreversible inhibition of COX-1 by aspirin; binding of benzoyl group of indomethacin, aniline NH of diclofenac and fenamates
Side Pocket (adjacent to central COX-channel)	Substitution of Ile523 in COX-1 by the smaller Val in COX-2 (Val 509)	Sulfone part of COX-2 inhibitors binds to this pocket giving COX-2 selectivity
Extra Space (Top of the channel)	Phe503 in COX-1 is replaced by Leucine in COX-2 (Leu 489) The smaller residue in COX-2 allows the first shell amino acid Leu384 to create an extra space	This extra space allows to accommodate and bind to the larger structures of the COX-2 inhibitors
Ionic Binding Site	Arg 120 (Arg 106 in COX-2) is one of the very few charged amino acids within the active site	AA as well as acidic COX-inhibitors bind via their carboxylate anion to the guanidinium cation of Arg 120. Does not influence binding of nonacidic inhibitors
H-Bonding Dynamics	Arg 120 and His 513 in COX-1 (Arg 106 and Arg 499 in COX-2) are involved together with Tyr 355 and Glu 524 (Tyr 341 and Glu510 in COX-2) in a hydrogen bonding network.	Plays a dominant role for the irreversible substrate binding status, responsible for the allosteric activation of the COX enzyme and for the time dependency of COX-2 inhibition

1.5. Non-steroidal anti-inflammatory drugs (NSAIDs): As outlined above, COX-2 appears to be the target for the anti-inflammatory effects of NSAIDs and COX-1 for their side effects. Many studies since the early 1990's have shown that the broad range of classical NSAIDs inhibit both COX-1 and COX-2 although with a general tendency towards COX-1 selectivity.

This appears to be associated with gastrointestinal toxicity; the more COX-1 selective drugs appear to have a tendency to cause more gastrointestinal damage. This has provided the rationale for the development of selective inhibitors of COX-2(Figure 1.7). The major mechanism by which NSAIDs elicit their therapeutic effects is the inhibition of prostaglandin (PG) synthesis. Specifically NSAIDs competitively inhibit COX, the enzyme that catalyses the synthesis of cyclic endoperoxides from arachidonic acid (AA) to form prostaglandins[30, 31].

NSAIDs display an unusually high degree of structural diversity. Various classes of NSAIDs (Figure 1.8 and Tables 1.3, 1.5 and 1.6) have been identified with compounds from different classes bearing little or no resemblance in their binding to the active sites of COX-1 and COX-2. Only slight chemical modifications to a single compound can drastically change its COX-binding characteristics. Most of the NSAIDs in clinical use belong to the aryl-acetic and -propionic acid class[25]. The roles of COX-1, COX-2 and the NSAIDs are depicted in Figure 1.9.

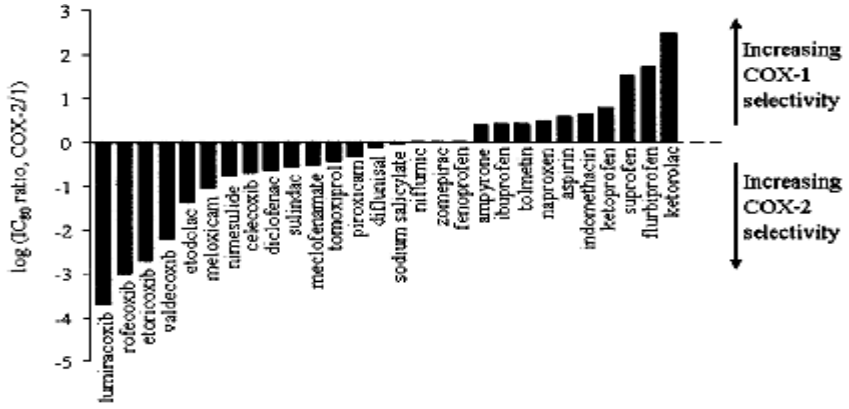


Figure1.7. Relative selectivity of agents as inhibitors of COX-1 and COX-2[30]

Table 1.3. Classification of NSAIDs according to their interaction with the enzyme protein[16]

Type	Drugs
Irreversible inhibitors of COX-1 or COX-2	Aspirin or *o*-(acetoxyphenyl)hept-2-inyl-sulfide (APHS) acetylates the amino acid serine so that endogenic AA is prevented from reaching the catalytic center.
Reversible, competitive inhibitors of COX-1 and COX-2	Inhibitors such as ibuprofen, piroxicam or mefenamic acid compete with AA to bind at the catalytic center
Slow time-dependent, reversible inhibitors of COX-1 and COX-2	Indomethacin and flurbiprofen seem to act by ionic interactions between their carboxylic function and the arginine residue of the enzyme. This effect seems to influence the helix D of the protein followed by a remarkable loss of flexibility of the enzyme protein.
Slow, time-dependent irreversible inhibitors of COX-2	Representatives of this group are selective COX-2 inhibitors such as celecoxib, rofecoxib and others. They are weak competitive inhibitors of COX-1, but inhibit COX-2 in a slow time-dependent process.

1.6. Management of NSAIDs related gastrointestinal toxicity [32, 33]**:** Because of the prevalence and severity of NSAID-related gastrointestinal complications, recent efforts have been directed at the prevention of mucosal injury induced by NSAIDs. The best way to prevent mucosal injury is to avoid the use of NSAIDs or substitute an agent which is less toxic such as acetaminophen, salsalate, or magnesium salicylate. In patients with inflammatory arthritides disease modifying anti-rheumatic drugs should be the first line of treatment. Nevertheless a potent NSAID is commonly preferred; in such cases administration of concomitant medication to protect the gastric mucosa from injury should be undertaken (Table 1.4). This concomitant therapy includes sucralfate, H_2-receptor antagonists, proton pump inhibitors, prostaglandins and COX-2 inhibitors

Table 1.4. Choice of long-term use of NSAIDs in rheumatoid arthritis and osteoarthritis patients [34]

Risk factors	Without CV risk	With CV risk
No GI risk	NSAID	NSAID with caution
Intermediate GI risk	Coxibs or NSAIDs together with either PPI or misoprostol	NSAIDs together with either PPI or misoprostol
High GI risk (previous ulcer complications)	No coxib and no NSAIDs	No coxib and no NSAIDs

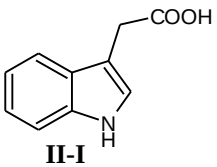
PPI: proton pump inhibitor

CHAPTER 2

LITERATURE SURVEY

2.1. Indan derivatives as non-steroidal anti-inflammatory agents

Of all the chemical classes that have been examined in relatively recent years as potential non-steroidal anti-inflammatory agents, none has received wider attention than the aryl- and heteroarylalkanoic acids. Features of a typical molecule, which are important for anti-inflammatory activity, include a carboxyl group separated by one or more carbon atoms from a flat aromatic nucleus that is further substituted by a lipophilic group. The lead compound for aryl acetic acids is ibufenac from which ibuprofen and (S)-(+)-2-(3-chloro-4-cyclohexylphenyl) propionic acid have come up[79]. Ring chain modification of the aryl propionic acid has led to the development of indan moiety. Indan ring system has been found to act as an inert carrier, which serves to hold biologically active functional moieties in a stereospecific manner[80]. This congener of indene is isosteric and bioisosteric with indolidine and indole respectively that includes important classes of anti-inflammatory compounds. Thimann[81] while working on auxin indole-3-acetic acid (**II-I**) observed that 3-indenylacetic acid (**II-II**) also acted as a mild plant hormone. Indan-1-carboxylic acid (**II-III**) inhibited root growth of wheat seedlings.



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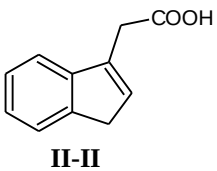

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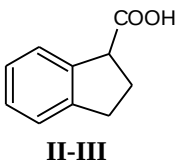
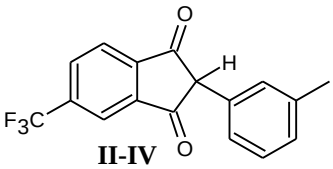


II-III

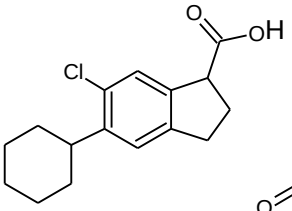


II-IV

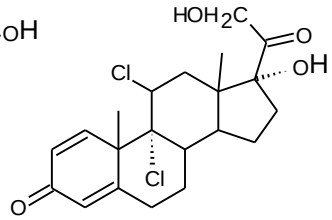
Lombardino *et al.*[82] prepared appropriately substituted 2-aryl-1,3-indandiones without anticoagulant activity but retaining the desired anti-inflammatory activity. They found that fairly large meta substituents on 2-aryl function diminished anticoagulant activity. Certain substituents in the indan ring successfully removed anticoagulant activity while retaining anti-

inflammatory activity in many. 5-triflouromethyl-2-(3'-methyl)phenyl-1,3-indandione (**II-IV**) was the most active member of such a series.

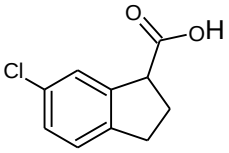
Noguchi *et al*.[83] described the design and synthesis of 6-chloro-5-cyclohexylindan-1-carboxylic acid (clidanac) (TAI-284) (**II-V**). The structure of this compound was compared with the anti-inflammatory corticosteroids. A compound having chlorine at 11β in the steroid nucleus, 9α, 11β-dichloro-17α,21-dihydropregna-1,4-diene-3,20-dione (**II-VI**) showed good separation of anti-inflammatory from glucocorticoid activity. Thus variously substituted 6-chloroindan-1-carboxylic acids (**II-VII**) were prepared and their biological activity was evaluated.



II-V

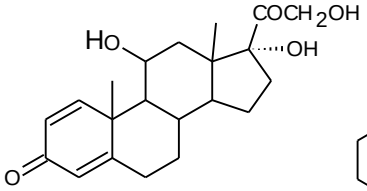


II-VI

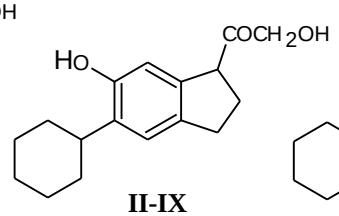


II-VII

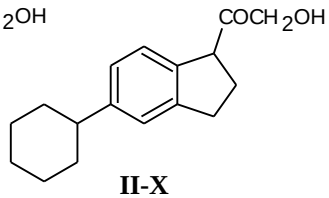
Juby *et al***.**[84] prepared two racemic 1-hydroxy acetylindans (**II-IX**, **II-X**) with the view that these bear even closer resemblance to the steroid nucleus. Earlier Noguchi *et al*[83] suggested that structural analogy between clidanac and anti-inflammatory corticosteroids, e.g., **II-VIII** is responsible for anti-inflammatory activity of clidanac. These hydroxy acetyl compounds were considerably less active than the corresponding carboxy compounds. Results did not support the view that the anti-inflammatory activity of the indan-1-carboxylic acids is due to steroid like structure.



II-VIII

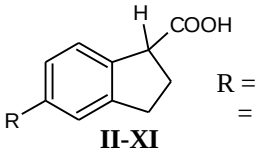


II-IX



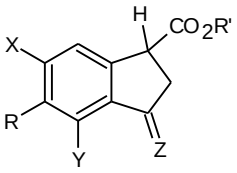
II-X

Allen *et al*.[85] prepared a series of 5-substituted-1-indancarboxylic acids as analogs of active phenylacetic acids in which the carboxylic group is conformationally fixed. 5-isopropyl- and 5-cyclohexyl-1-indancarboxylic acid (**II-XI**) were found to be most active in suppressing carrageenan-induced paw edema in rats and U.V-induced erythema in the guinea pig. These compounds were less potent than indomethacin and did not suppress adjuvant-induced arthritis.



R = isopropyl
= cyclohexyl

II-XI

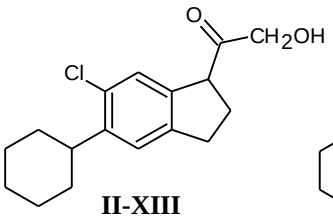


II-XII

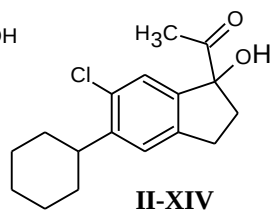
R= H, alkyl, cycloalkyl
Z= O, H_2, OH
X= H, Cl, NO_2,NH_2, AcNH, F, OH, MeO, AcO, cyclohexyl
Y= H, NH_2, Cl, OH
R'=H, alkyl

Juby *et al*.[79] also synthesized a series of indan-1-carboxylic acids and their derivatives (**II-XII**) as potential anti-inflammatory agents. Substitution at position 5 with cycloalkyl groups resulted in marked improvement with optimum activity being shown by the 5-cyclohexyl derivative. Incorporation of a variety of both electron withdrawing and releasing groups at position 6 gave products with improved or comparable activity. 3-keto and 3-hydroxy analogs and the indene analogs showed only weak activity. Corresponding alcohol and methyl ester derivatives showed comparable activity. Most of the activity resided in the isomer with S configuration at position 1. The most active compound in the series was (1*S*)-(+)-6-chloro-5-cyclohexylindan-1-carboxylic acid which had an ED_{30} of 0.85mg/kg p.o. and LD_{50} of 35mg/kg p.o. in rats.

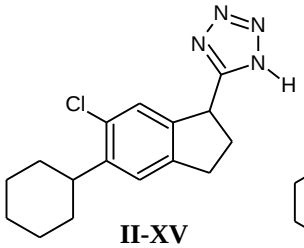
Noguchi *et al*.[86] prepared various indan compounds to develop the structure activity relationships of 6-chloro-5-cylcohexylindan-1-carboxylic acid (TAI-284). However, TAI-284 was found to be the most active (its potency was comparable to that of indomethacin) compound amongst these. The replacement of cyclohexyl moiety at C-5 by other alkyl groups such as isobutyl or isopropyl, or the removal of the chlorine at C-6 resulted in considerable reduction of the activities. It was found that anti-inflammatory activity resided in the dextro isomer, which was assigned S configuration by optical rotatory dispersion (ORD) spectrum. Noguchi[87] also studied some 1-substituted 6-chloro-5-cyclohexyl indans and prepared 6-chloro-5-cyclohexyl-1-hydroxymethyl carbonylindan (**II-XIII**), 1-acetyl-6-chloro-5-cyclohexyl-1-indanol (**II-XIV**), 5-[(6-chloro-5-cyclo hexylindan)-1yl]tetrazole (**II-XV**) and 6-chloro-5-cyclohexyl-1-methyl indan-1-carboxylic acid (**II-XVI**). These compounds were found to be less active than TAI-284.



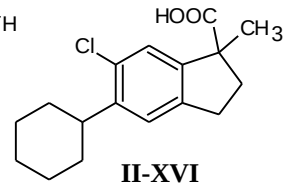
II-XIII



II-XIV



II-XV



II-XVI

Kishimoto *et al*.[88] carried out the synthetic study of metabolites of TAI-284. 5-(*cis* and *trans*-3'-hydroxycyclohexyl)indan-1-carboxylic acids were stereoselectively prepared by sodium borohydride reduction and catalytic hydrogenation of corresponding 3'-oxo and 4'-oxo compounds, respectively. Chlorination of these compounds with molecular chlorine in acetonitrile gave their 6-chloro derivatives (**II-XVII** – **II-XX**), which were correlated with metabolites of TAI-284. The metabolite **II-XIX** was almost equivalent to TAI-284 regarding anti-inflammatory activity as estimated by the carrageenan edema test, and was more active than TAI-284 in the analgesic activity estimated by the phenylquinone writhing test and determination of the ulcerogenic activity. The metabolite **II-XVIII** showed half the anti-inflammatory activity and one third to one quarter the analgesic activity of TAI 284; however the degree of ulcerogenicity was considerably lower than that of TAI 284. Compound **II-XVII** was less active, and compound **II-XX** showed the weakest activity and was comparable to that of phenylbutazone[89].

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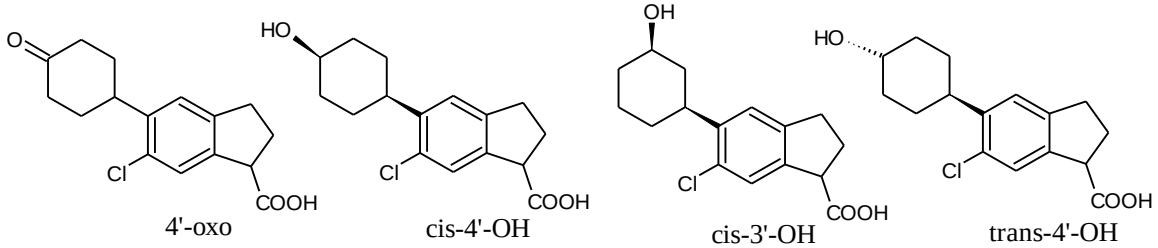
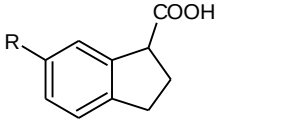
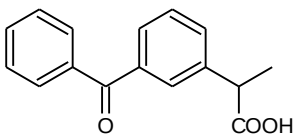


4'-oxo **II-XVII** | cis-4'-OH **II-XVIII** | cis-3'-OH **II-XIX** | trans-4'-OH **II-XX**

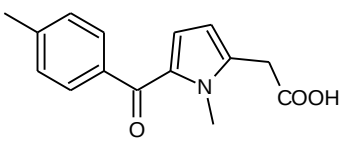
Aono *et al*.[90] studied the relative reactivity in each position of 1-indancarboxylic acid towards the electrophilic substitution reactions such as chlorination, bromination, acylation, sulfonation and nitration and it was found that 6-position was the most reactive (**II-XXI**). By these reactions, pharmacologically interesting meta-substituted phenyl acetic acid derivatives were readily obtained. Meta-substituted arylacetic acids, e.g. ketoprofen (**II-XXII**) and tolmetin (**II-XXIII**) supported the fact that new anti-inflammatory compounds could be obtained by appropriate substituents at either 4 or 6 positions of 1-indancarboxylic acid, which corresponds to the meta position in phenylacetic acid.



R= -Cl, -Br, -acyl, $-SO_2Cl$,$-NO_2$ **II-XXI**

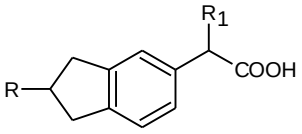


II-XXII

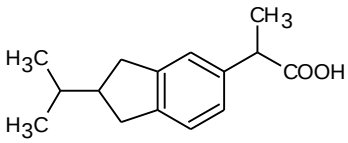


II-XXIII

Teulon *et al*.[91] prepared various 2-alkyl-α-methyl and 2-alkylindan-5-acetic acids (**II-XXIV**). Propionic acids were found to be more active than corresponding acetic acids. 2-isopropyl and 2-ethyl derivatives are better than 2-methyl but not significantly different from each other. The most active compound found was 2-isopropyl-α-methylindan-5-acetic acid (**II-XXV**). Also it was found that stereochemistry has no influence and the enantiomers have the same activity.

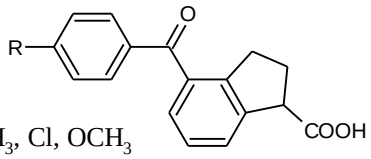


II-XXIV

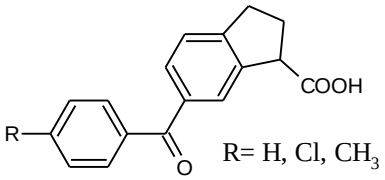


II-XXV

Aono *et al*.[92] synthesized 4-aroyl-1-indancarboxylic acids (**II-XXVI**) and isomeric 6-aroyl-1-indancarboxylic acids (**II-XXVII**) in an attempt to obtain information on the receptor sites of anti-inflammatory aroyl-acetic acids. 4-aroyl compounds showed potent anti-inflammatory activity, the activity of 6-aroyl compounds were significantly weaker. The results suggest that locations and conformations of the functional groups in **II-XXVI** are considerably closer to actual conformation of an aroyl acetic acid required for exerting anti-inflammatory activity



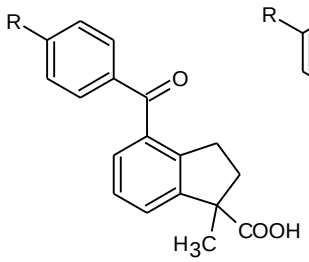
II-XXVI



II-XXVII

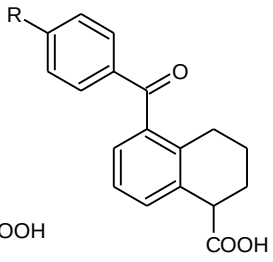
They synthesized 4-aroyl-1-indancarboxylic acids from the corresponding 4-aroyl-1-indanones[93] by the one carbon homologation reaction using p-toluenesulfonylmethyl isocyanate via 4-aroyl-1-indancarbonitriles. Some of the compounds were resolved into their enantiomers and it was found that the anti-inflammatory activity virtually resided in the levo isomers, which was assigned S configuration the by ORD.

Aono *et al*.[94] also made some modifications on 4-aroyl-1-indancarboxylic acids (**II-XXVI**) for elucidating the receptor site of anti-inflammatory aryl acetic acids. Modifications were introduced on the characteristic part of **II-XXVI** and 1-methylated derivatives (**II-XXVIII**), tetralin analogs (**II-XXIX**), benzyl analogs of **II-XXVI** (**II-XXX**) were also prepared. However these compounds showed weaker activities than the parent compound **II-XXVI**. The results suggested that 1-indancarboxylic acid is the pharmacologically effective derivative of aryl acetic acid.



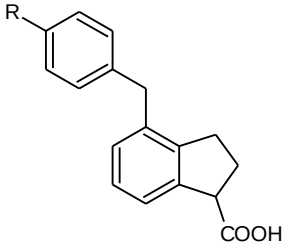
R= H, CH_3, Cl

II-XXVIII



R= H, CH_3, Cl

II-XXIX

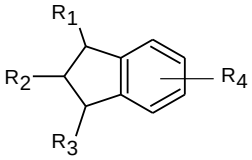


R= H, CH_3, Cl, OCH_3

II-XXX

Smith *et al*.[95] synthesized some compounds (**II-XXXI**) with indan as parent nuclei for anti-inflammatory testing. Compound **II-XXXII** showed 52% reduction in swelling of the non-injected foot in comparison to control compound phenylbutazone which showed 42% reduction at the dose level of 100 mg/kg and 33 mg/kg for 16 days respectively. The reference drug indomethacin showed 57% reduction at the dose level of 1mg/kg for 16 days. 2-(4-chlorobenzylidene)-3-oxo-6-indanacetic acid (**II-XXXIII**) showed 43% reduction and its methyl derivative showed 53% at 100 mg/kg for 16 days in an anti-arthritic assay.

Roy *et al*.[96, 97] synthesized a series of indan-1-acids (**II-XXXIV**) and screened for anti-inflammatory activity. Varying degree of anti-inflammatory activity was found in carrageenan-induced paw edema test. These compounds also exhibited appreciable antipyretic and analgesic activity. Among these compounds 6-methoxyindan-1-acetic acid and 5,6-dimethoxyindan-1-



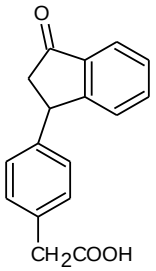
R_1= O; H_2

R_2= H_2; PhCH; 4-ClC_6H_4CH

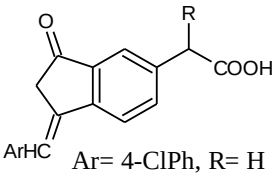
R_3= H_2; H, 4-$C_6H_4CH_2COOH$;
H, Me; H, c-C_6H_{11}

R_4= 6-CH_2COOH; 6-Ph; 5-Ph;
6-CH_2COONa; 6-CH(Me)COOH

II-XXXI



II-XXXII

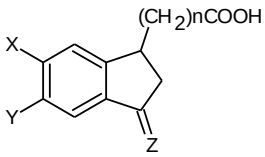


Ar= 4-ClPh, R= H

Ar= 4-ClPh, R=Me

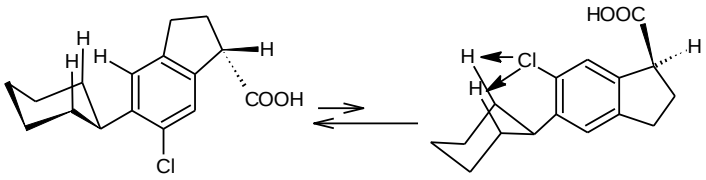
II-XXXIII

acetic acid showed activity profile close to that of phenylbutazone having a prolonged action and lower toxicity than the latter. The acute oral LD_{50} was found to be greater than 1000mg/ kg and minimum ulcerogenic dose was 300mg/kg.



X= H, OCH_3
Y= H, OCH_3
Z= O, H_2
n= 0, 1

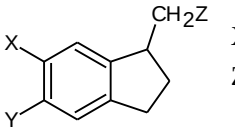
II-XXXIV



***anti*-form** ⇌ ***syn*-form**

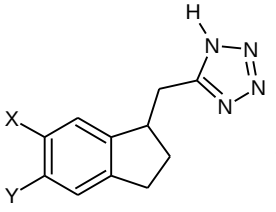
Tamura *et al.*[98] studied inhibition of prostaglandin biosynthesis by clidanac, its metabolites and some analogs. The (+) isomer was found to 1000 times more active than (-) isomer in inhibiting PG synthetase activity. Metabolites of clidanac, 6-chloro-5-(cis 4'-hydroxycyclohexyl)-1-indan carboxylic acid, 6-chloro-5-(cis 3'-hydroxycyclohexyl)-1-indan carboxylic acid and 6-chloro-5-(trans 4'-hydroxycyclohexyl)-1-indan carboxylic acid were significantly less potent than the parent compound in inhibiting the PGE_2 synthesis. Structure activity studies with clidanac analogs showed that the compounds with halogen at C-6 and bulky groups such as cyclohexyl or cyclopentyl at C-5 was of considerable significance for the conformational requirement for binding to the enzyme. Earlier Kamiya *et al.*[99] had demonstrated that the cyclohexyl ring of clidanac was deviated away from the chlorine at C-6 and out of plane with indan ring, and its spatial features were common to that of indomethacin. Based on this it was also shown that the *anti*-form in which the conformation of the functional group (cyclohexyl ring) and carboxyl group are directed to the opposite sides is more favorable in binding to the enzyme than the *syn*-form.

Roy *et al.*[100] synthesized esters, amides and nitrile derivatives of 6-methoxyindan-1-acetic acid and 5,6-dimethoxyindan-1-acetic acid (**II-XXXV**) in an attempt to minimize gastric ulcerogenecity. It was found that the anti-inflammatory activity of ethyl esters were almost equal to those of parent acids and phenylbutazone while other derivatives were less potent. The ethyl ester derivatives were found to be less ulcerogenic than phenylbutazone.



X=Y= H; OCH_3
Z= $COOCH_3$, $CONH_2$, CN
$CONHCH_3$, $CONHC_2H_5$

II-XXXV



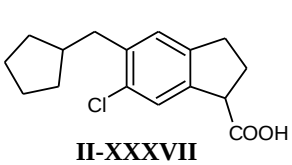
1. X= Y= H
2. X= OCH_3, Y= H
3. X= Y= OCH_3

II-XXXVI

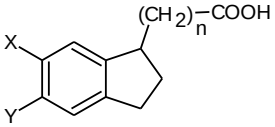
They also attempted to develop relatively less toxic and more potent anti-inflammatory agents by synthesizing few tetrazole derivatives[101] of the type **II-XXXVI** including 5-(indan-1'-yl)methyl tetrazoles (**II-XXXVI-1**), 5-(6'-methoxy-indan-1'-yl)methyltetrazoles (**II-XXXVI-2**) and 5-(5',6'-dimethoxy-indan-1'-yl)methyltetrazoles (**II-XXXVI-3**). The idea was that

being bioisosteric with the carboxyl group these tetrazole derivatives would also have significant activity. These compounds showed anti-inflammatory activity close to that of phenylbutazone in both acute (carrageenan-induced edema) and chronic (adjuvant-induced arthritis) animal test models. Oral LD_{50} in mice was found to be greater than 1000mg/kg for compounds **II-XXXVI-2** and **II-XXXVI-3**.

Boettcher *et al.*[102] prepared an isomer of clidanac, 6-chloro-5-(cyclopentylmethyl)indan-1-carboxylic acid (**II-XXXVII**). This acid showed good anti-inflammatory and analgesic activities without producing irritation in the gastrointestinal tract up to the highest tested dose of 400mg/kg in rats under both acute and chronic conditions.

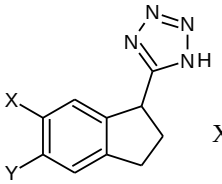


II-XXXVII



n= 0, 1, 2, 3
X= H, OCH_3
Y= H, OCH_3

II-XXXVIII



X=Y= H, OCH_3

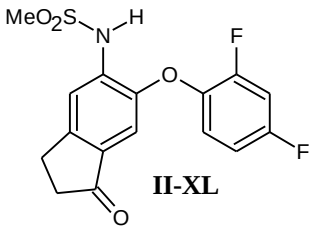
II-XXXIX

Mukhopadhyay *et al.*[103, 104] synthesized some indan-1-acids analogs with longer aliphatic chain structure (**II-XXXVIII**) to impart greater lipophilicity and better transport facility through biomembrane. It was found that indiscriminate chain lengthening is not beneficial for biological activity, as the activity seems to reside in a small structural framework. Indan-1 acetic acid was found to show most prominent anti-inflammatory activity and methoxyl substitutions at 5 and 6 position potentiates the biological activity. 3-propionic acid and 2-propionic acid derivatives were also prepared and were found to have longer duration of action and lower ulcerogenic liability.

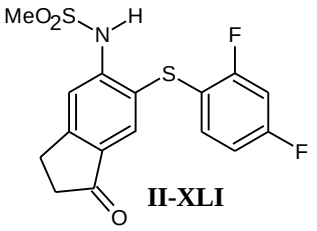
Ray *et al.*[105, 106] synthesized few 5-(indan-1'-yl)tetrazoles (**II-XXXIX**) and screened for anti-inflammatory and related biological properties. These compounds were found to be superior to phenylbutazone in established adjuvant-induced arthritis and yeast-induced pyrexia biomodels in rats. The results indicated 5-(indan-1'-yl)tetrazole as the most promising compound in chronic anti-inflammatory and antipyretic tests. The anti-inflammatory activities of corresponding carboxamides have also been reported by them.

Klein *et al.*[107] reported on a novel selective inhibitor of COX-2, CGP-28238 (6-(2,4-difluorophenoxy)-5-methyl-sulfonylamino-1-indanone) (**II-XL**). With an IC_{50} value of 15 nM, **II-XL** blocked COX-2 activity in a similar concentration range to that of other potent NSAID such as indomethacin and diclofenac (IC_{50} = 1.17-8.9 nM). However, in contrast to

these reference NSAIDs, **II-XL** was at least 1000-fold less potent in inhibiting COX-1. This was a lead compound for a new generation of potent anti-inflammatory drugs with an improved side-effect profile.



II-XL

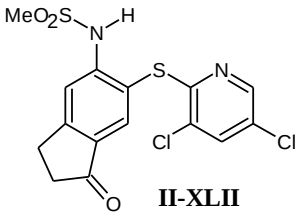


II-XLI

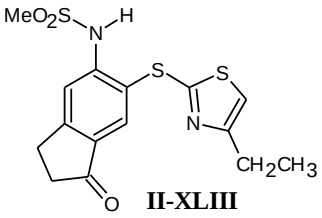
Chun-Sing Li *et al.*[108] identified the thioether analog of flosulide, L-745,337, 6-[(2, 4-diflourophenyl)thio]-5-methanesulfonamido-1-indanone (**II-XLI**) as a potent, selective and orally active COX-2 inhibitor. Ortho and para substituted 6-phenyl substituents were found to be optimal for *in vitro* potency. Replacement of this phenyl group by a variety of heterocycles (2-pyridyl and 2-thiazolyl) analogs gave compounds that were less active. The methanesulfonamido group seemed to be optimal at the 5-position of the indanone system. This compound showed efficacy profile superior or comparable to that of nonselective indomethacin and was non-uclerogenic within the dosage ranges required for functional efficacy. This compound was equipotent to flosulide in the *in vitro* assays but more potent in the rat paw edema assay and less ulcerogenic than the latter in rats.

Quimet *et al.*[109] have shown that appropriately substituted 2-mercaptopyridine and 2-mercaptothiazole were suitable replacements of the thiophenol moiety of L-745,337 in the flosulide class of COX-2 inhibitors. For the indanone analogs, the result of *in vitro* activity suggest that small alkyl substituents at the 4-position on the 2-mercapto thiazole ring improved COX-2 potency and substitutions at the 5-position decreased it. A potent member of this series, the 3,5-dichloropyridyl derivative (**II-XLII**), showed ED_{30} of 0.7 mg/kg in the rat paw edema assay. Halogen substitution at the 3/5 positions on the pyridine ring led to improved *in vitro* COX-2 inhibitor potency compared to the unsubstituted analog or the methyl analog. In this 2-mercaptothiazole series, the 4-ethylthiazolyl compound, (**II-XLIII**), had the best *in vitro* overall profile. The *in vivo* efficacy study showed an ED_{30} of 0.3 mg/kg in rat paw edema assay. Few benzofuranone analogs were also prepared and in this case, the structure activity relationship was focused toward the size and the orientation of the 4-substituents on thiazole ring. Many large 4-alkyl substituents like propyl, isopropyl, tert-butyl, or phenyl were inactive against COX-2. Superior inhibitor activity and selectivity was observed with substituents having comparable size to the ethyl substituent. The 4-

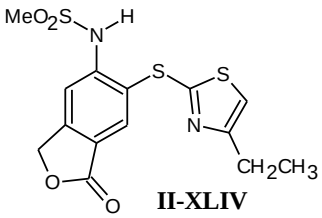
ethylthiazole compound (**II-XLIV**) was the best analog prepared in the benzofuranone series. This compound showed superior *in vitro* potency and selectivity than L-745,337 (**II-XLI**). In the rat paw edema assay, an ED_{30} of 0.16 mg/kg was observed. It also exhibited a high *in vivo* efficacy in biomodels of pain and fever and no significant GI toxicity in rats.



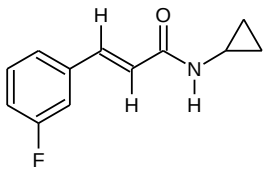
II-XLII



II-XLIII

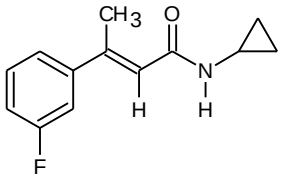


II-XLIV

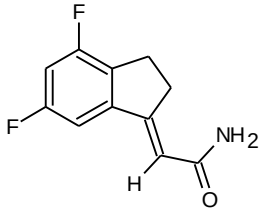


II-XLV

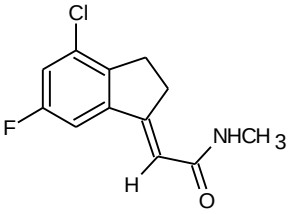
Musso *et al.*[110, 111] designed rigid cyclic analogs of cinnamides, (E)-N-cyclopropyl-3-(3-fluorophenyl)prop-2-enamide (**II-XLV**), (E)-N-cyclopropyl-3-(3-fluorophenyl)but-2-enamide (**II-XLVI**), which led to the discovery of the potent, centrally acting muscle relaxant (E)-2-(4,6-difluoro-1-indanylidene)acetamide (**II-XLVII**). This compound also possesses potent anti-inflammatory and analgesic activity. Various analogs of this compound were prepared and structure activity relationships were obtained. The compound **II-XLVII** has been taken into phase I clinical trials and did not produce sedation at doses up to 250mg/ kg orally.



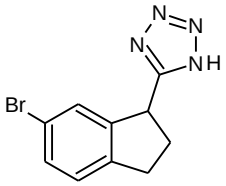
II-XLVI



II-XLVII



II-XLVIII



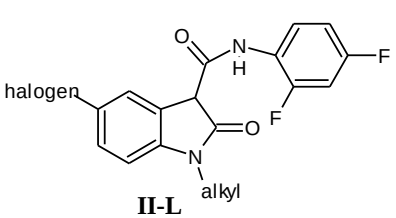
II-XLIX

In further extension of structure activity relationship studies, (E)-2-(4-chloro-6-flouro-1-indanylidene)-N-methylacetamide (**II-XLVIII**) was discovered as a potent anti-inflammatory and analgesic agent without centrally acting muscle relaxant activity.

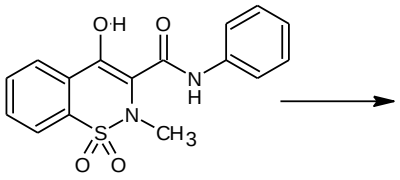
Bachar *et al.*[112] synthesized chloro and bromo substituted indanyltetrazoles and indanylmethyltetrazoles from the respective acids through amide and nitrile route. These compounds were tested for their analgesic activity against acetic acid induced writhing assay. 5-(6'-bromoindan-1'-yl)tetrazole (**II-XLIX**) exhibited significant analgesic activity at 50mg/kg when administered orally and was comparable to that of phenylbutazone and Indomethacin.

2.2. Amide derivatives as anti-inflammatory agents

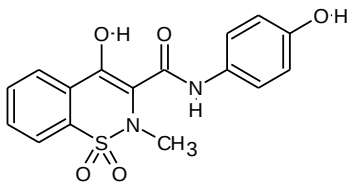
Wiseman *et al.*[113] prepared some carboxanilides of the oxindole ring system and described their anti-inflammatory activities and plasma half lives. N-alkylation and 5-halogenation in a series of 2',4'-difluorooxindole-3-carboxanilides (**II-L**) showed enhanced anti-inflammatory activity. Earlier Beckett and Dorman[114] had reported that N-alkyl oxindoles are metabolized both by N-dealkylation and by hydroxylation at the 5 position of the oxindole ring system. Therefore, the importance of N-alkylation and 5-halogenation in a series of 2',4'-difluorooxindole-3-carboxanilides was examined with respect to anti-inflammatory potency and plasma half life.



II-L



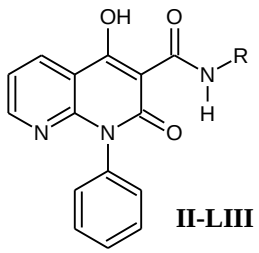
II-LI



II-LII

Ainie *et al.*[115] studied the metabolism of the nonsteroidal anti-inflammatory agent, 4-hydroxy-2-methyl-2*H*-l, 2-benzothiazine-3-carboxanilide-1,l-dioxide (**II-LI**). It was found to have a plasma half-life of 6 hr in the rat, 30 hr in the dog, 4.5 hr in the monkey, and 21 hr in man. The principal metabolite in man, monkey, and rat, formed by hydroxylation of the carboxanilide moiety (**II-LII**), is excreted in the urine as an acid-labile conjugate. The dog eliminates the drug in the urine mainly as a water-soluble conjugate of the parent drug.

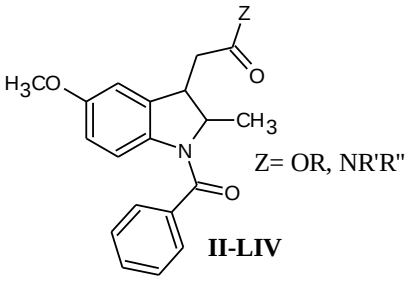
Kuroda *et al.*[116] designed and synthesized 4-hydroxy-2(1*H*)-oxo-l-phenyl-l, 8-naphthyridine-3-carboxamides (**II-LIII**) as new anti-inflammatory agents. The nature of substituents on the amide nitrogen showed a pronounced effect on anti-inflammatory activity. Studies of structure-activity relationships led to compounds bearing a pyridine ring on the amide nitrogen. 3-pyridyl and 4-pyridyl derivatives were active against carrageenan -, zymosan-, and arachidonic acid-induced rat paw edemas and also potently inhibited the reverse passive Arthus reaction in rats. Thus, these compounds were endowed with a broader spectrum of anti-inflammatory activity than the classical nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and piroxicam.



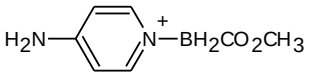
II-LIII

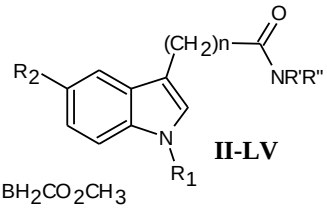
R=3-pyridyl
R=4-pyridyl

Kalgutkar *et al.*[26] studied the structure activity relationship involved in transformation of the indomethacin into a COX-2 inhibitor. Many of the structurally diverse indomethacin esters and amides (**II-LIV**) inhibited purified human COX-2 with IC_{50} values in low nanomolar range but did not inhibit ovine COX-1 activity at concentrations as high as 66μM. Inhibition kinetics revealed that indomethacin amides behave as slow, tight binding inhibitors of COX-2 and that selectivity is a function of the time dependent step. Conversion of indomethacin into esters and amides derivatives provides a facile strategy for generating highly selective COX-2 inhibitors and eliminating the gastrointestinal side effects of the parent compound. Moreover, the amide derivatives were found to be stable in human plasma and human liver microsomes and were not found to undergo hydrolytic cleavage to form the parent acid.



II-LIV

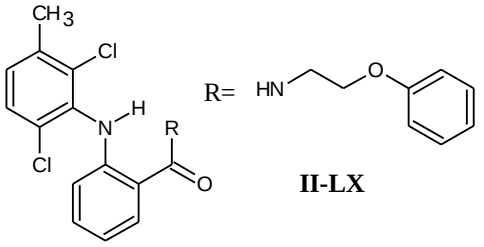
	R_1	R_2	n	NR'R"
II-LVI	H	H	1	4 -aminopyridyl
II-LVII	H	H	2	4 -aminopyridyl
II-LVIII	H	F	2	4 -aminopyridyl
II-LIX	H	H	2	



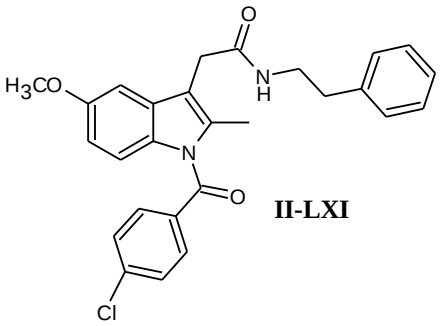
II-LV

Duflos *et al.*[117] prepared *N*-substituted-(indol-3-yl)carboxamides and alkanamides of the type **II-LV** and screened for evaluation of their anti-inflammatory activity. None of the considered carboxamides exhibited significant inhibitory effect in the carrageenan-induced rat paw edema after oral administration of 0.1 mM kg^{-1}; however, introduction of an alkyl chain lead to the formation of alkanamides which showed moderate to high activity with 46–95% inhibition. The efficacy of these compounds in the inhibition of topical inflammation was confirmed by measuring reduction of ear thickness in the acute tetradecanoyl phorbol acetate (TPA)-induced mouse ear swelling assay. The introduction of a methylene and especially an ethylene chain at C-3, leading to acetamide (**II-LVI**) and propanamide (**II-LVII**) induced a marked increase of activity: 41±11 % and 95± 3% inhibition, respectively. Unfortunately, this potent amide **II-LVII** elicited toxic effects at 0.4 mM kg^{-1}. Toxicity could be attenuated or suppressed by the introduction of fluorine at C-5 or incorporation of a methoxycarbonylborane moiety in the pyridinyl nucleus, leading to **II-LVIII** and **II-LIX** respectively, with maintenance of a high level of inhibitory activity (86±8% and 84±9%) at 0.1 mM kg^{-1}.

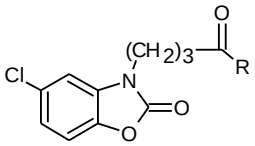
Kalgutkar *et al.*[118] described structure activity relationship studies involved in the transformation of meclofenamic acid into potent and selective COX-2 inhibitors via neutralization of the carboxylate moiety in this nonselective COX-inhibitor. Maximum selectivity of 440 was found with phenoxyethyl amide derivative (**II-LX**).



II-LX



II-LXI



II-LXII

Remmel *et al.*[119] studied metabolism of 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-*N*-phenethyl-acetamide (indomethacin phenethylamide, LM-4108, **II-LXI**) a highly selective COX-2 inhibitor, in rat, mouse, and human liver microsomes. The primary site of oxidation in all species examined was on the methylene carbons of the phenethyl side chain to form the 1'- and 2'-hydroxy and 2'-oxo metabolites as determined by electrospray ionization liquid chromatography-tandem mass spectrometry. Half-lives for the disappearance of 10 µM LM-4108 in rat, human, and mouse liver microsomes (0.15 pmol P450/ml) were 11 min, 21 min, and 51 min, respectively. Indomethacin formation was not observed in incubations with rat, mouse, or human liver microsomes. Both the 2'-hydroxy-LM-4108 and 2'-oxo-LM-4108 metabolites were synthesized and found to be equipotent to the parent compound with regard to COX-2 inhibitory potency and selectivity [2'-hydroxy-LM-4108: IC_{50}(COX-2) = 0.06 µM, IC_{50}(COX-1) >66 µM; 2'-oxo-LM-4108: IC_{50}(COX-2) = 0.05 µM, IC_{50}(COX-1) >66 µM]. *O*-Demethylation was a minor oxidative pathway in contrast to the metabolism of indomethacin. The metabolic pathway has been shown in Figure 2.1.

Gulcan *et al.*[120] prepared amide derivatives (**II-LXII**) of 5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid as potential anti-inflammatory and analgesic compounds. All synthesized compounds exhibited high analgesic and anti-inflammatory activities. None of the compounds except one caused gastric lesions or bleeding in the test animals. None of the compounds active *in vivo* resulted in considerable inhibition at 10µM in *in vitro* COX-2 or COX-1 assays. Therefore it was concluded that these compounds do not exert their analgesic or anti-inflammatory activities through COX-inhibition and that some other mechanism might be involved.

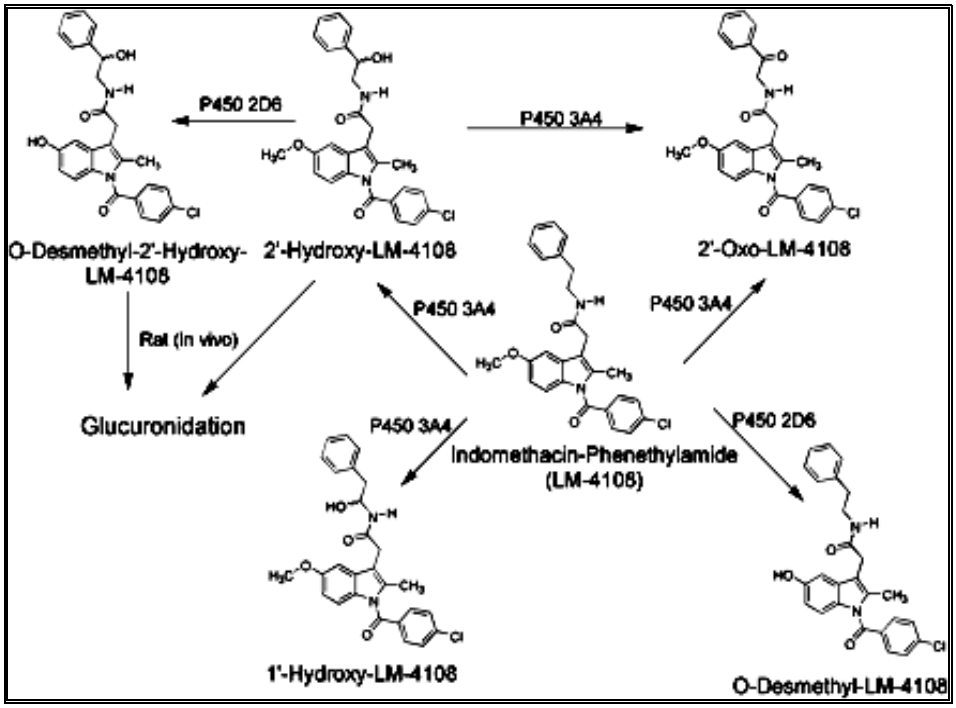


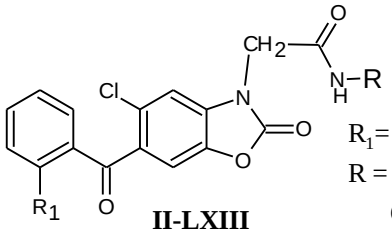
Figure 2.1. Proposed metabolic scheme for indomethacin phenethylamide (**II-LXI**)

Zovko *et al.*[121] synthesized a series of new ketoprofenamides as anti-inflammatory agents. Amide formation was achieved by aminolysis of ketoprofenbenzotriazolide with various amines: primary, secondary, hydroxyl amine and amino acid β-alanine. Ketoprofen amides with heterocyclic residues (2-thiazolinyl, 4-methylpyridyl, 3-hydroxypyridyl, pyridyl, 1,5-dimethyl-2-phenylpyrazolonyl or thiazolyl) showed significant analgesic and anti-inflammatory activities.

Banoglu *et al.*[122] explored the prevention of gastric side effects while maintaining high analgesic and anti-inflammatory activities by the derivatization of the carboxylate moiety into amides (**II-LXIII**) in [5-chloro-6-(2-chloro/flourobenzoyl)-2-benzoxazolidinone-3-yl]acetic acids. Some of the compounds demonstrated selective inhibition of COX-2 to some extent although the inhibitory activity was not very potent.

These authors also prepared new compounds based on the information that 3(2H)-pyridazone derivatives bear potent analgesic and anti-inflammatory activities. In order to explore effect of the presence of acetamide side chain linked to the lactam nitrogen of pyridazinone ring on analgesic and anti-inflammatory activity, amide derivatives (**II-LXIV**) of [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid were synthesized[123]. Some

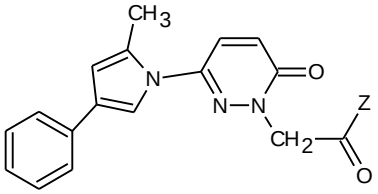
compounds showed good biological activity but it was found that these compounds did not exert their activity through COX-inhibition and other mechanisms might be involved.



II-LXIII

R_1= Cl, F

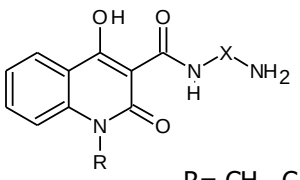
R = Ph(4-OCH_3), Ph(4-Cl), Ph(4-Br), CH_2CH_2Ph, C_8H_{17}, 3-pyridyl



II-LXIV

Detsi *et al.*[124] designed and synthesized a series of N-substituted-quinolinone-3-aminoamides (**II-LXV**) and their hybrids containing the R-lipoic acid functionality (**II-LXVI**) as potential bifunctional agents combining antioxidant and anti-inflammatory activity. In general, the derivatives were found to be potent antioxidant or anti-inflammatory agents. The studies confirmed that the presence of a free hydroxyl group at position 4, a 3,4-double bond, and aminoamide functionality at position 3 were important structural features for the antioxidant/anti-inflammatory activity.

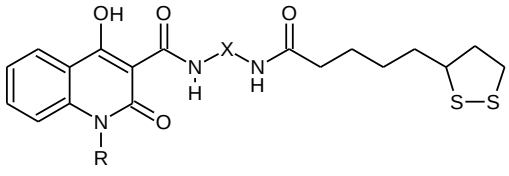
Linomide,[125] (**II-LXVII**) (*N*-phenylmethyl-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinoline carboxamide, a synthetic immunomodulator, is effective against various types of cancers and autoimmune disorders, such as multiple sclerosis (MS), rheumatoid arthritis, systemic lupus erythematosus, and experimental autoimmune encephalomyelitis. This compound was found to be a potent TNF-α inhibitor and was claimed to be useful for the treatment of inflammatory bowel disease and psoriasis[126]. Linomide served as the prototype for the synthesis of a variety of analogues in an effort to optimize this lead compound.



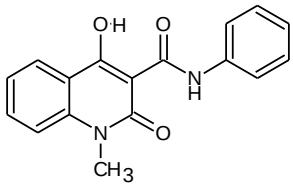
II-LXV

R= CH_3, C_6H_5

X= $(CH_2)_2$, $(CH_2)_6$, o-phenyl, p-phenyl



II-LXVI



II-LXVII

CHAPTER 3

OBJECTIVE, RATIONALE & PLAN OF WORK

3.1. Need for another NSAID: Although many NSAIDs are currently available in the market, there are several reasons why development of new NSAIDs is desirable.

- Patients show considerable variations in their response to NSAID therapy. Only 60% of patient population would respond to any particular NSAID and in treatment of specific disease state one drug may be more effective than another[127]. Thus it is advantageous to the physician to have a wide range of drugs to choose from.

- Development of NSAIDs that will alleviate pain and inflammation without the liability for adverse events caused by COX-1 inhibition.

- Most of the currently available COX-2 inhibitors are sulfur-containing molecules that are intolerable by sulfur-sensitive patients. Therefore, it will be beneficial to have nonsulfur COX-2 inhibitors.

- It has been demonstrated that a number of cancers appear to overexpress the COX-2 enzyme, which may play several roles in carcinogenesis[128]. Research on COX-2 inhibitors in the treatment of cancer will increase our understanding into the general applicability of this new targeted approach for cancer control.

- The molecular and therapeutic mechanisms of Alzheimer's disease (AD) and inflammation have recently been reviewed. Several epidemiological studies have indicated that patients taking NSAIDs for other diseases (e.g., rheumatoid arthritis) have a 50% lower risk of developing AD than those not taking NSAIDs[129]. Recent studies have shown that COX-1 expression is considered important in the pathogenesis of AD and non-selective COX inhibitors have shown positive clinical effects than selective COX-2 inhibitors[43]. This further emphasizes the need for research in the area of NSAIDs and the role of COX in the etiology of AD.

3.2. Objective of the Proposed Research

The proposed research aims at:

1. Investigation on design, synthesis and biological activity profile of variously substituted indan-1-carboxylic and -acetic acids as potential anti-inflammatory agents.
2. Impartation of COX-2 selectivity leading to the development of non-sulfonyl COX-2 inhibitors.
3. Optimisation of structural requirements for the design of better and safer non-steroidal anti-inflammatory drugs.

3.3. Rationale for the synthesis of Indan Analogs as anti-inflammatory agents: Literature survey reveals that indan ring system remains relatively unexplored. Indan moiety was selected for the present work in accordance with the Sternbach principle[130]. This class of compounds is (1) relatively unexplored (2) is readily accessible, (3) gives the possibility of a multitude of variations and transformations, (4) offers some challenging chemical problems, and (5) it could lead to biologically active products. Hence this interesting framework can be exploited for design and synthesis of newer NSAIDs.

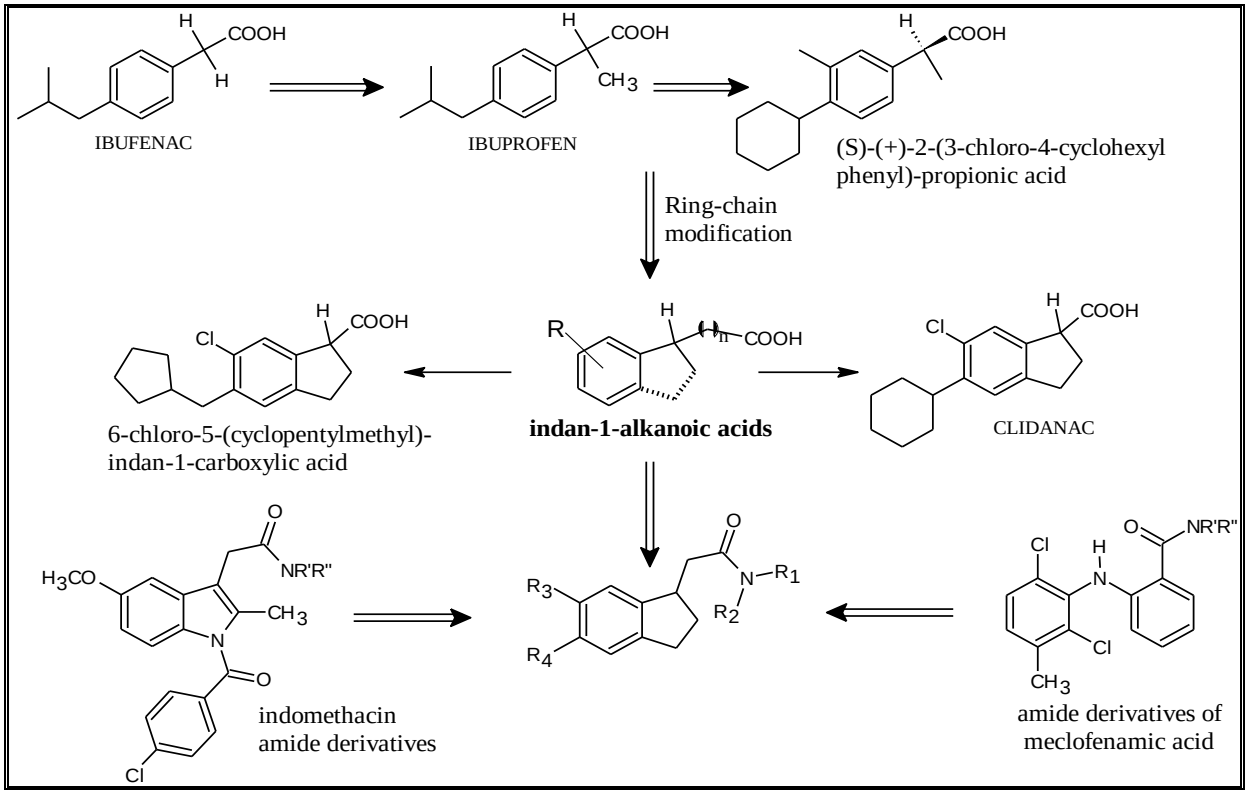


Figure 3.1. Development of anti-inflammatory agents among indans – a schematic representation

3.4. Rationale for amidation: Recently it has been shown that the biochemical differences between the COX isoforms can be exploited to improve upon the selectivity of carboxyl containing NSAIDs as COX-2 inhibitors. Co-crystallization and site-directed mutagenesis have confirmed that ion pairing of the carboxylic acid group in NSAIDs and arachidonate to positively charged R120 residue in COX-1 is the major contributor to both inhibition and catalysis, which is not an important determinant of catalysis by COX-2. Thus appropriate derivatization of carboxyl moiety in NSAIDs would eliminate their ability to inhibit COX-1 without significantly affecting their COX-2 inhibitory properties. As many NSAIDs contain a carboxylic acid group, this would represent a general strategy for the conversion of non-selective NSAIDs into selective COX-2 inhibitors[128]. Indomethacin, an aryl acetic acid NSAID,

has already been transformed into selective COX-2 inhibitor. Structurally diverse indomethacin esters and amides inhibited purified human COX-2 with IC_{50} values in the low nanomolar range but did not inhibit ovine COX-1 activity at concentrations as high as 66μM. Moreover these esters and amides were also found to be stable in human plasma towards hydrolytic enzymatic reactions[131].

Hence, based on the above-established facts we propose to synthesise indan-1-alkanoic acid derivatives as better and safer NSAIDs. The idea behind derivatization is to lower the side effects of gastric irritation and ulceration, which is associated with free carboxyl group. Neutralization of the carboxyl group by esterification and amidation should not only enhance absorption by increasing lipophilicity but should also impart COX-2 selectivity.

3.5. Rationale for the methods adopted for determination of NSAID activities: **Anti-inflammatory activity is associated with other biological activities like analgesic, antipyretic activities because of their similar mechanism of action, i.e., inhibition of prostaglandins. Therefore, the synthesised compounds are evaluated for their anti-inflammatory, analgesic and antipyretic activity. As arthritis is a chronic inflammatory condition, current research for antiarthritic drugs is also concerned with developing better anti-inflammatory compounds. Screening procedures that have been used to assess the anti-inflammatory, analgesic, antipyretic and antirheumatic potentials of drugs are based on an interference with the manifestation of one of the cardinal signs of**

inflammation (swelling, redness, heat, pain and loss of function). The modifications of these syndromes in laboratory animals are believed to represent models for various rheumatoid disease states[76]. The methods adopted were simple and economically feasible.

The anti-inflammatory activity of the compounds is tested by developing swelling upon administration of an edemogen into the plantar tissue of a hind paw of the rat. The percentage inhibition of swelling is correlated to the activity. More is the inhibition better is the activity. The analgesic activity is tested by developing pain in mice. Pain is induced by injection of an irritant into the peritoneal cavity of mice. The animals react with a characteristic stretching behaviour called writhing. This stretching reaction is evaluated to predict the analgesic activity. The evaluation of antipyretic activity is based on measuring heat (temperature) reduction in an acutely inflamed area. Fever is induced in rats by injecting lipopolysaccharides in the peritoneal cavity. A decrease in rectal temperature after administration of test compounds is correlated with the antipyretic activity.

As NSAIDs are associated with the ulcerogenic liabilities (due to inhibition of COX-1) the synthesized compounds were also tested for their ulcerogenecity. Gastric irritation and ulceration are evaluated in fasted rats after oral administration of test compounds. Stomachs are removed after predetermined time intervals and inspected for any lesions.

3.6. Rationale for the biological evaluation of racemic mixtures: The synthesized compounds are chiral compounds and hence exist as enantiomers. It is generally recognized that the S enantiomer of NSAIDs is regarded as the eutomer (the biologically active enantiomer) and the R form as the distomer (the biologically inactive enantiomer) [132]. The absolute configuration, as well as the conformation of S isomer is important for the interactions with the cell receptors, inhibiting prostaglandin synthetase and hence responsible for the therapeutic anti-inflammatory activity. In practice, the NSAIDs are generally administered as racemic mixture. It has been reported that *in vivo*, however, some of the NSAIDs can undergo, to a certain extent, a unidirectional inversion from the R to the S form, leading to an enantiomeric excess of the S form when a racemate of the drug is administered. This unique process was supposed to enhance the effectiveness of NSAIDs racemates as chiral drugs.

The most comprehensive mechanism for this inversion (Figure 3.2), originally proposed by Nakamura *et al.*[133] is a three step process which commences with the enantiospecific enzymatic formation of a thioester between the R enantiomer of the 2-arylpropionic acid and coenzyme A (CoA). This thioester may be hydrolyzed to regenerate the R enantiomer or may undergo epimerization to yield the thioester in which the 2-arylpropionyl moiety has the S configuration. Subsequent hydrolysis of this (S)-CoA thioester completes the inversion process.

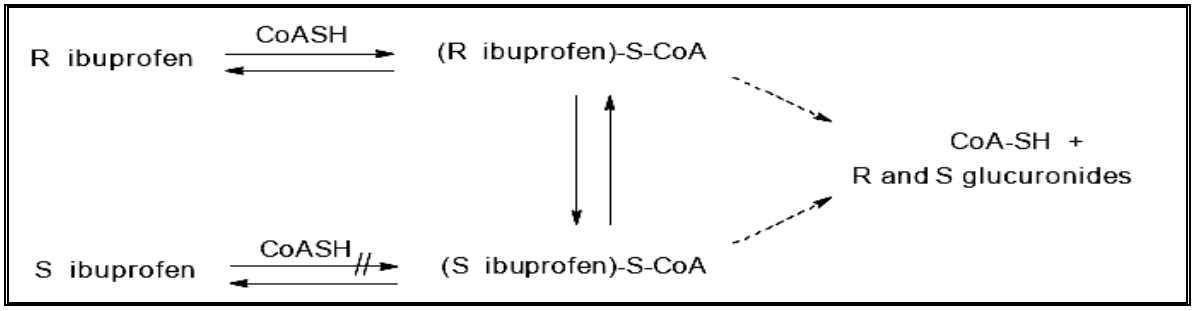


Figure 3.2. Inversion of R isomer to S isomer in ibuprofen

The epimerization step may proceed non-enzymatically, due to the acidic nature of the proton connected to the alpha carbon of the 2-arylpropionic acid substituent of the thioester. This process is clinically important because it generates an active cyclooxygenase inhibitor (S profen) from a relatively inactive precursor (R profen).

Also as there is only one asymmetric carbon atom in each of the synthesized racemic mixtures of the compounds it is therefore expected that half of the administered dose should become available for stereoselective uptake. More over there exist a striking positive correlation between the relative potencies of enantiomers of some clinically used drugs and the average human dose of the drug given as the racemates[134].

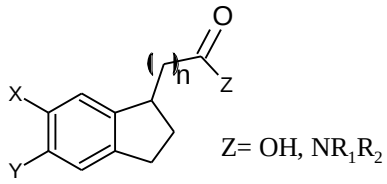
3.7. Rationale for molecular orbital studies: The anti-inflammatory activity of arylalkanoic acids is due to their ability to inhibit cyclooxygenase and thus disrupt prostaglandin biosynthesis. So far attempts at modeling a receptor for this class of anti-inflammatory drugs on the basis of structure activity relationships have failed to establish a truly comprehensive receptor model. Among the models (Gund and Shen, 1977[135]; Appleton and Brown, 1979[136]; Salvetti et al, 1981[137]), the roles ascribed to the aromatic and carboxyl groups in the binding process, and also the relative orientations of these groups differ radically. It is not surprising that SAR for arylalkanoic acids are still poorly defined[138]. Mehler and Gerhards[139] have found interesting correlations between the anti-inflammatory activities of a series of salicylic acids

and the energies and charge density distributions of their highest occupied and lowest unoccupied molecular orbitals, suggesting that drug binding involves charge transfer between the receptor and the aromatic group. Following this line of thought, in this work we looked for correlation between the anti-inflammatory activity and the charge density distribution of their HOMO and LUMO orbitals.

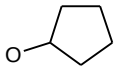
3.8. Plan of work: The plan of work is briefly outlined as follows,

3.8.1. Design and Synthesis

1. Variously substituted indan-1-alkanoic acids were prepared starting from appropriate benzaldehydes
2. Derivatization of these acids (amidation).



Z= OH, NR_1R_2

1. X= OCH_3, Y= OCH_3, n=0,1
2. X= Cl, n=0, 1
3. X= OCH_3, Y=  , n=0,1

3.8.2. Characterization of the Synthesised Compounds

The synthesized compounds were characterized using physicochemical methods. Melting point and thin layer chromatography determinations were done to check the purity of compounds. Structures of the synthesised compounds were confirmed by I.R., ^{1}H NMR, Mass and elemental analysis.

3.8.3. Pharmacological Screening

Synthesized compounds were tested for

1. Anti-inflammatory activity
   - Carrageenan-induced rat paw oedema (acute model)
   - Adjuvant-induced arthritis (chronic model) [for most active compounds]
2. Analgesic Activity

- Acetic Acid-induced writhing

3. Antipyretic Activity
    - LPS-induced pyresis [for some selected active compounds]
4. Gastrointestinal Ulcerogenecity [for most active compounds]

3.8.4. Molecular Modelling Studies: The synthesized compounds were studied for their HOMO and LUMO surface analysis.

CHAPTER 4

MATERIALS AND METHODS

4.1. Chemistry

Melting points were determined in one end open capillaries in a Buchi 530 melting point apparatus and are uncorrected. Identity and purity of the compounds were ascertained by thin layer chromatography (TLC) on silica gel-F-254 (Merck) coated aluminium plates, elemental microanalysis and spectral analysis. Infra-red spectra were recorded with a Jasco IR Report 100 (in KBr using pellet method) or a Shimadzu IR Prestige-21 FT-IR Spectrometer (in KBr using powder diffraction technique). ^{1}H-NMR spectra were recorded on a 400 MHz Brucker Avance II NMR Spectrometer using TMS as the internal standard. Mass Spectra of the compounds were recorded in a Jeol SX 102/DA-6000 spectrometer. 3-Nitrobenzyl alcohol was used as the matrix.

The matrix peaks appear at m/z 136, 137, 154, 289, 307. Elemental microanalysis was done in a Perkin-Elmer 2400 CHN analyzer. Whenever required reactions were carried out under anhydrous conditions and the system was kept protected from the atmospheric moisture. For determining the purity of the compound and the progress of the reaction solvent systems used to check the TLC were ethyl acetate and hexane (10%, 20%, 30%, 40%, 50% ethyl acetate in hexane); chloroform and methanol (1%, 5%, 10%, methanol in chloroform) and ethyl acetate and chloroform (5%, 10%, 20% chloroform in ethyl acetate). Log P values were calculated from www.logp.com

4.2. Generalized Reaction Schemes

4.2.1. Synthesis of Indan-1-acetic acids and their amide derivatives: Scheme 4.1 was followed for the synthesis of indan-1-acetic acids and their amide derivatives. The starting reagent for the preparation of indan-1-acetic acid was benzaldehyde. Appropriately substituted benzaldehyde was used to meet the structural design of the final indan derivatives. This substituted benzaldehyde (**IV-I**) was condensed with ethyl acetoacetate in the presence of an organic base to form the corresponding benzyl bisacetoacetate (**IV-II**). The bisacetoacetate was further hydrolyzed to substituted phenylglutaric acid (**IV-III**). The dicarboxylic acid (**IV-III**) was cyclized by Freidel Craft's intramolecular cyclization to yield the desired oxo compound (**IV-IV**). The keto group was finally reduced to substituted indan-

1-acetic acid (**V-IV**). The acidic group was reacted with various amines under appropriate reaction conditions to form the amides (**IV-VI**).

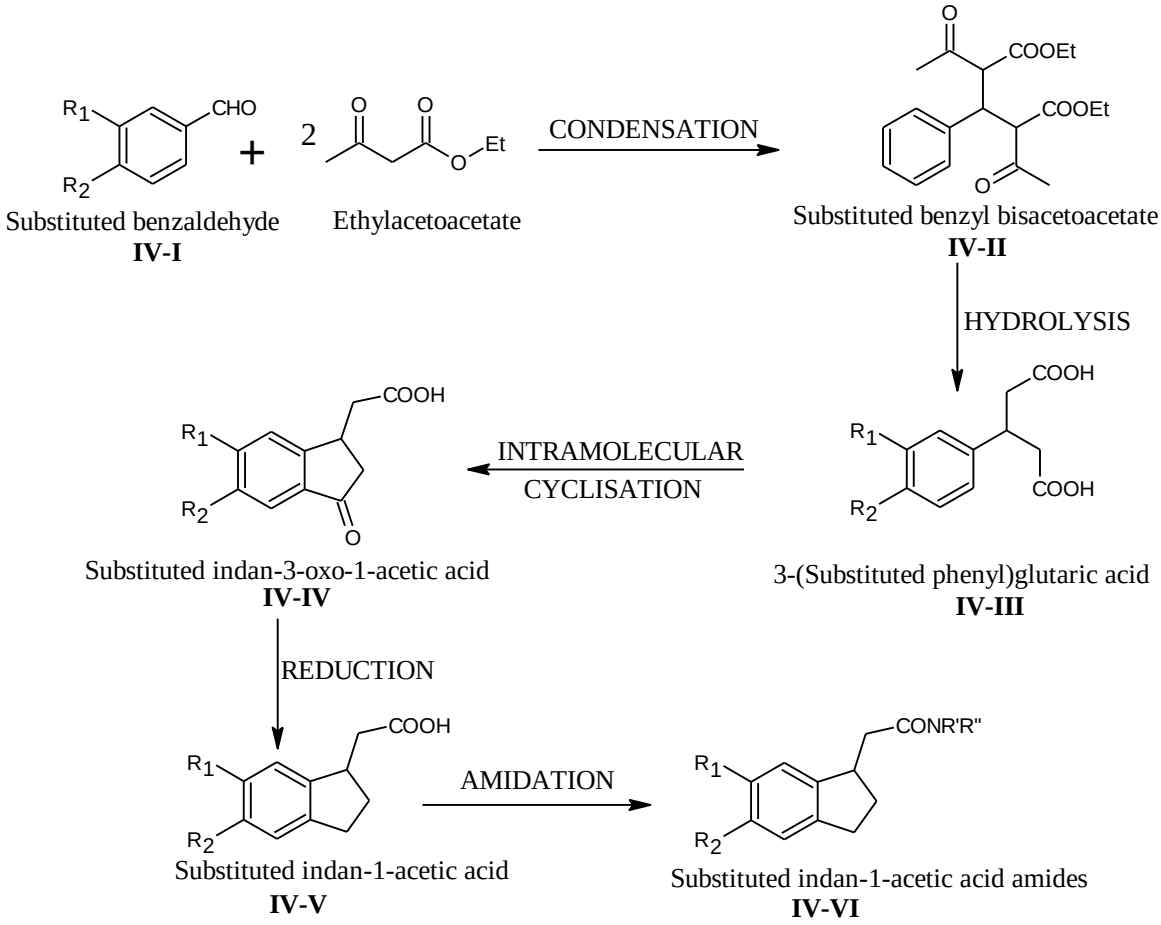


Figure 4.1. Reaction scheme for synthesis of Indan-1-acetic acids and their amide derivatives

4.2.2. **Synthesis of Indan-1-carboxylic acids and their amide derivatives:** Scheme 4.2 was followed for the synthesis of indan-1-carboxylic acids and their amide derivatives. The key intermediate required for this series was 2-phenyl succinic acid. This diacid was obtained via two different routes, the malonate route and the acrylate route. In the malonate route, the required benzaldehyde (**IV-I**) was treated with diethylmalonate. The formed benzylidene malonate (**IV-VII**) was cyanosated to cyanomalonate (**IV-VIII**) which was further hydrolyzed to succinic acid (**IV-IX**). While in the acrylate route the appropriate benzaldehyde (**IV-I**) was reacted with ethylcyanoacetate in the presence of an organic base. The cyanoacrylate (**IV-X**) thus obtained was cyanosated to form dicyanopropanoate (**IV-XI**), which was further hydrolyzed to the required succinic acid (**IV-IX**). The diacid was cyclized by Friedel Craft's intramolecular cyclization to yield the oxo compound (**IV-XII**). The keto group was finally reduced to form the required indan-1-carboxylic acid (**IV-XIII**). The

carboxylic group was reacted with various amines at appropriate reaction conditions to form the amides (**IV-XIV**).

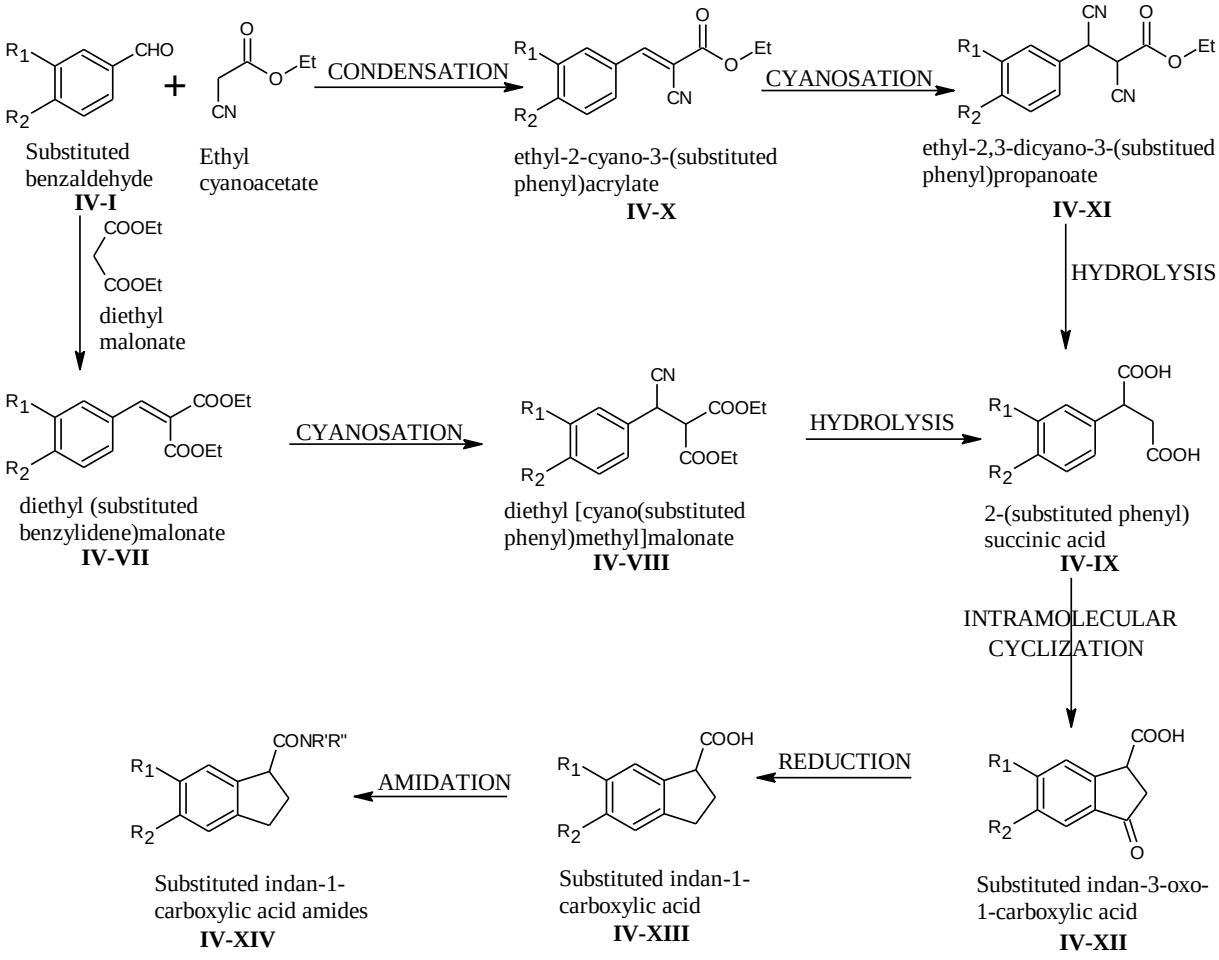


Figure 4.2. Reaction scheme for synthesis of Indan-1-carboxylic acids and their amide derivatives

4.3. Biological Evaluation

All synthesized compounds were evaluated for analgesic and anti-inflammatory activities. Few selected compounds were screened for antipyretic and anti-arthritic activities and also for their ulcerogenic potentials. The test compounds and standard drugs (indomethacin and aspirin) were administered orally as suspensions in 0.5% carboxymethylcellulose sodium in distilled water. Each group consisted of six animals. The animals were maintained at a temperature of 24±2ºC, relative humidity of 45% and kept under a 12 h light and dark cycle. The animals were fasted overnight for analgesic and anti-inflammatory assays and 24 h for

antipyretic and ulcerogenecity studies. During fasting they had free access to water. The protocol [IAEC/RES/7/5] for the animal experiments was approved by the Institutional Animal Ethics Committee (IAEC) as registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

4.3.1. Acute anti-inflammatory activity: The anti-inflammatory activity of the test compounds was determined by carrageenan-induced rat hind paw edema assay as described by Winter *et al*.[140]. Female Wistar rats weighing 160±20g were administered the test compounds and standard indomethacin orally equivalent to 100mg/kg and 10mg/kg respectively. One hour after dosing, 0.1ml of 1% carrageenan was administered into the subplantar region of the right hind paw. The paw volumes were measured using an Ugo Basile plethysmometer at 0, 1, 2, 3, 4, 6 and 24 h after carrageenan injection. The results are expressed as percentage inhibition of edema formation. The percent inhibition of edema in each drug treated group was calculated using the formula given below:

$$\% \text{ inhibition} = 100(1-V_t/V_c)$$

Where V_c and V_t are average edema volumes in control and treated groups respectively.

4.3.2. Analgesic activity: The analgesic activity of the compounds was determined by acetic acid induced writhing test in mice as described by Collier *et al*.[141] with minor modifications. Female Swiss albino mice, 20±5g, were administered the test drugs orally at 100mg/kg and the standard indomethacin and aspirin at 10mg/kg and 100mg/kg respectively. One hour after the oral dosing they were injected intraperitoneally with 0.1ml/10g of 1% v/v acetic acid. Five minutes after the injection the writhing (full extension of hind limbs) were noted for next 15 minutes. Animals giving 20 or more wriths were selected for the study. The percent inhibition by individual drug as well as by the reference drugs were calculated using the following formula,

$$\% \text{ inhibition} = 100\ [1- W_t / W_c]$$

Where W_c represents the average writhing produced by the control group and W_t represents the average writhing produced by the test groups.

4.3.3. Antipyretic activity: The antipyretic activity of the compounds was evaluated by lipopolysaccharide induced pyrexia. The method was that of Dogan *et al*.[142] with minor

modifications. Adult female Wistar rats (170±20g) were used. Phenol extracted LPS from E. coli serotype 0111: B4 (Difco) was used. The experiment was started at 0900h. Test compounds and aspirin were given orally at the dose level of 100mg/kg. Indomethacin was given at a dose level of 10mg/kg. After half an hour of oral administration of the drugs, LPS dissolved in apyrogenic saline was injected intraperitoneally at the dose of 100µg/kg. The rectal temperature was determined using telethermometer probes immediately before and 1, 2, 3, 4, 5, 6, 7 h after LPS administration. For evaluation of antipyretic activity, the temperature index was calculated following the method of Winter *et al*.[143].

4.3.4. Chronic anti-inflammatory activity: Adjuvant-induced arthritis [Prophylactic model]

The method used was essentially that of Newbold[144]. Only three compounds were selected for this study. Female albino rats weighing 160±10 g were chosen for the study. The animals were given the test compounds at the dose level of 100 mg/kg once daily for 14 days starting from the day before the administration of 0.1 ml Freund's complete adjuvant (Genie, Bangalore, India) into the subplantar region of right hind paw of each rat. Indomethacin was taken as the standard drug and was given for 14 days at the dose level of 1 mg/kg p.o. Both hind paw volumes up to a fixed mark at the level of lateral malleolus were measured before and daily after adjuvant administration. The formation of nodules and appearance of erythema in tail, nose and ears were observed and graded as mild, moderate and severe for comparison. Change in body weight of the animals during the test period was also recorded for comparison.

4.3.5. Ulcerogenecity test: Only few amides from the series were selected for ulcerogenic studies. The method used to evaluate the ulcerogenic potential of the test compounds was that of Vela´zquez *et al*.[145] with minor modifications. Twenty four hour fasted Wistar Male rats (200±20g) were used. The test compounds and the standard drug indomethacin were dosed orally at 100mg/kg and 30mg/kg respectively. After six hours of oral dosing the rats were sacrificed using cervical dislocation. The stomachs were taken out and cut along the greater curvature. After washing with saline the stomach mucosa was examined for ulcers using a hand lens. The gastric lesions were counted, and an ulcerative index (UI) for each animal was calculated according to Szelenyl and Thiemer[146];

UI = (n lesion I) + (n lesion II) 2 + (n lesion III) 3

Where:

I = presence of edema, hyperemia and single, submucosal, punctiform hemorrages (petechiae)

II = presence of submucosal, hemorrhagic lesions with small erosions;

III = presence of deep ulcer with erosions and invasive lesions

4.3.6. Metabolism inhibition study using SKF-525A[147]**.** The female Wistar rats weighing 170±20 g were pretreated with SKF 525A (50mg/kg i.p.). One hour later, the test drugs were administered orally at the dose level of 100mg/kg. The remaining protocol followed was the same as that of anti-inflammatory screening by carrageenan induced rat paw edema model.

4.3.7. Statistical analysis

All data was expressed as mean ± standard error of mean (SEM). The data was analyzed using Student's *t* test only when two means were compared (analgesic and metabolism inhibition studies). In the case of anti-inflammatory studies, statistical significance was determined for drug effects by one-way ANOVA, and Bonferroni's post hoc test was used for individual comparisons with control values. In all the pharmacological assays the chosen level of significance was assigned to P less than 0.05. The statistical software package PRISM (Graphpad Software Inc., San Diego, CA) was used for the analyses.

4.4. Molecular Orbital Studies

The quantum mechanical calculations were carried out using Argus Lab 3.1.0 version. The three dimensional structures of all the compounds were drawn and their geometry was optimized using Hamiltonian PM3 (Parameterized Method 3) semi-empirical method. The HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energies and corresponding surfaces were determined using single point energy calculations. The parameters employed for these calculations were PM3 Hamiltonian and RHF (closed shell) [Restricted Hartree-Fock. The eigenvalue (energy) and visualization of the surfaces were then noted corresponding to HOMO and LUMO.

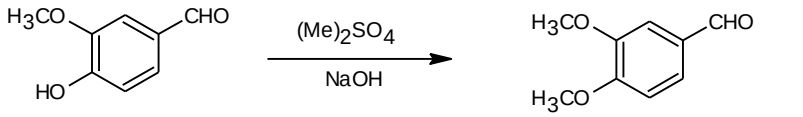
CHAPTER 5

SYNTHESIS

5.1. (5, 6-DIMETHOXY-2, 3-DIHYDRO-1*H*-INDEN-1-YL)ACETIC ACID AMIDES

5.1.1. SYNTHESIS

5.1.1.1. 3,4-Dimethoxybenzaldehyde[148](V-II).

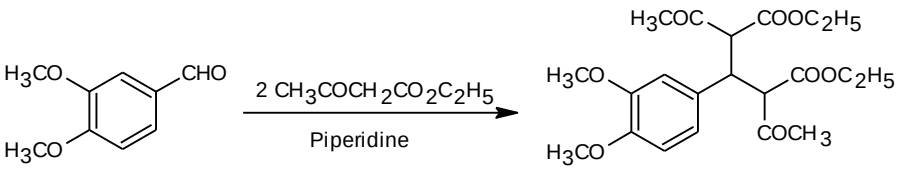


4-hydroxy-3-methoxybenzaldehyde
V-I

3,4-dimethoxybenzaldehyde
V-II

Commercially available vanillin (**V-I**), (15.2g, 0.1 mol), was placed in a 500ml three-necked flask equipped with a magnetic stirrer, two dropping funnels and a reflux condenser. One funnel was charged with potassium hydroxide (8.2 g of KOH in 12 ml of water) and the other funnel with purified dimethyl sulphate (12 ml, 0.104 mol). Vanillin was melted by warming on water bath then KOH was added at the rate of two drops a second. After 20 seconds dimethyl sulphate was also added at the same rate. The heating was stopped after few minutes as the mixture continued to reflux gently from the heat of the reaction. The reaction mixture was vigorously stirred throughout. After all reagents have been added the reaction mixture became turbid and separated into two layers. Pale reddish brown color of the reaction mixture indicating the alkaline condition was maintained throughout the reaction. The yellow reaction mixture was poured into a porcelain basin and was left overnight. The hard crystalline mass thus obtained was ground, washed thoroughly with cold water to make it free of basicity, filtered and dried in a vacuum desiccator to get veratraldehyde (**V-II**) in 70-75% yield, mp 43-44°C.

5.1.1.2. Diethyl 2,4-diacetyl-3-(3,4-dimethoxyphenyl)pentanedionate[149](V-III).



3,4-dimethoxybenzaldehyde

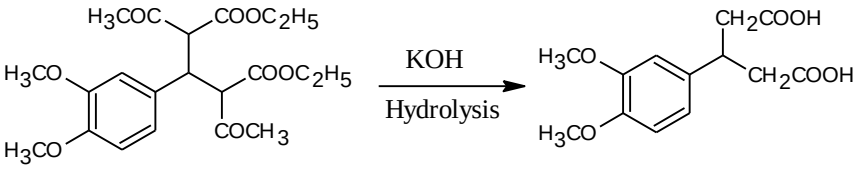
V-II

diethyl-2,4-diacetyl-3-(3,4-dimethoxy phenyl)pentanedionate)

V-III

Veratraldehyde (**V-II**) (33g, 0.2 mol) was dissolved in ethylacetoacetate (56g, 0.43 mol) in a dry conical flask and piperidine (2.5 ml) was added slowly at ambient temperature and then kept for 3 days or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product **V-III** in 70-75% yield[14]. Recrystallization from dil. alcohol gave the analytical product, mp 129-131°C, IR (cm^{-1}) (KBr): 1720 (C=O stretching), 1046, 1253 (C—O stretch of OCH_3), ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 0.82 (t, CH_3, 3H), 0.90 (t, CH_3, 3H), 1.28 (s, CH_3, 3H), 1.34 (s, CH_3, 3H), 2.50, 2.71 (d, CH, H), 3.02, 3.64 (d, CH, H), 3.85 (s, OCH_3, 3H), 3.87 (s, OCH_3, 3H),), 3.93 (qr, CH_2, 2H), 3.96 (qr, CH_2, 2H), 4.03 (m, CH, 1H), 6.59-6.81 (m, ArH, 3H).

5.1.1.3. 3-(3,4-Dimethoxyphenyl)pentanedioic acid[149, 150](V-IV).



diethyl-2,4-diacetyl-3-(3,4-dimethoxy phenyl)pentanedionate)

V-III

3-(3,4-dimethoxyphenyl)pentanedioic acid

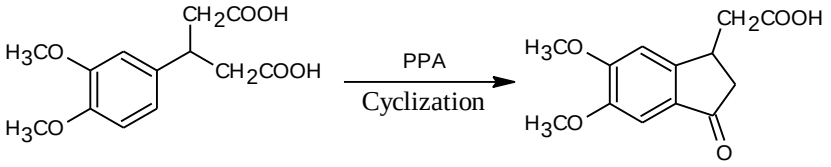
V-IV

Compound **V-III** (40g, 0.089 mol) was dissolved in a freshly made hot solution of KOH (160g in 120 ml of water) and 80 ml of 90% ethanol was added. The hot reaction mixture was refluxed on a water bath for 1 hour. Alcohol was then removed by distillation, and after dilution with water the reaction mixture was cooled and washed with solvent ether. The aqueous phase on acidification with cold conc. HCl with cooling gave crude **V-IV** which was filtered and recrystallized from hot water [14]. Yield 65-70%; mp 189-191°C; IR (cm^{-1}) (KBr): 3300-2400 (br OH stretch), 1710(C=O stretching), 1225 (C—O stretch of OCH_3), 1260 (C—O stretch of COOH) , 1010(C—O stretch of OCH_3);^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS): 2.59,

2.70 (dd, CH_2, 4H), 3.59 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 3.86(s, OCH_3, 3H), 6.78-6.83 (m, ArH, 3H), 10.08 (brs, COOH, 1H), 10.19 (brs, COOH, 1H).

5.1.1.4. (5,6-Dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid[151](V-V).

Cyclization of compound **V-IV** was effected by treating the powdered acid (15g, 0.056 mol) with polyphosphoric acid (PPA) (225g) on a steam bath for 4 hours with stirring. After decomposition of the hot reaction mixture with crushed ice, the keto acid (**V-V**) was isolated by extraction with chloroform. The solvent was distilled off to get the crude keto acid.



3-(3,4-dimethoxyphenyl) pentanedioic acid

V-IV

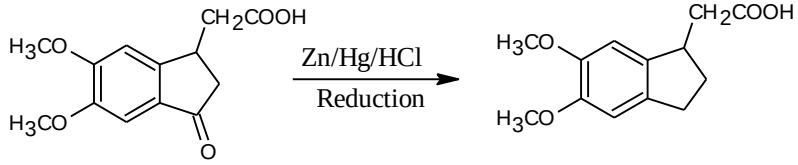
(5,6-dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-V

The crude product was finally recrystallized from acetone to get the pure compound. Yield 75-80%; mp 177-178°C; IR (cm^{-1}) (KBr): 3400-2400(br OH stretch), 1680, 1724(C=O stretching), 1045, 1255 (C—O stretch of OCH_3), ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 2.46, 2.57 (dd, CH_2, 2H), 2.79, 2.91 (dd, CH_2, 2H), 3.72 (qn, CH, 1H), 3.91 (s, OCH_3, 3H), 3.98 (s, OCH_3, 3H), 7.00 (s, ArH, 1H), 7.15 (s, ArH, 1H), 10.12 (brs, COOH, 1H).

Note: Polyphosphoric acid was prepared from equivalent weights of phosphorous pentoxide and phosphoric acid.

5.1.1.5. (5,6-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid[152, 153](V-VI).



(5,6-dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-V

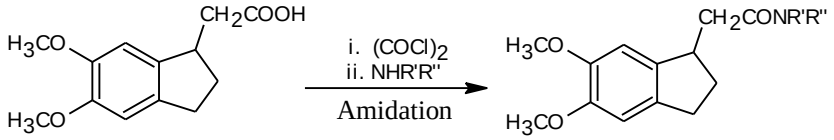
(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-VI

Compound **V-V** was reduced to **V-VI** using Clemmensen's reduction. Compound **V-V** (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200 ml of benzene was added as a co-solvent. The reaction mixture was refluxed on a steam

bath for about 8 hrs (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was then washed with water to make it free of acidity, dried over anhydrous sodium sulfate and finally distilled off to get the reduced acid **V-VI**. The analytical product was obtained on recrystallization from benzene. Yield 75-80%; mp 152-153^{o}C; IR (cm^{-1}) (KBr): 3400-2800 (br OH stretch), 1712(C=O stretching), 1040, 1256 (C—O stretch of OCH_3); ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS): 1.81 (m, CH_2, 1H), 2.43 (m, CH_2, 1H), 2.49 (dd, CH_2, 1H), 2.80 (dd, CH_2, 1H), 2.89 (m, CH_2, 2H), 3.55 (qn, CH, 1H), 3.86 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 6.76 (s, ArH, 1H), 6.78 (s, ArH, 1H), 10.11 (brs, COOH, 1H), M.S 236(M^{+}).

5.1.1.6. General methods for the synthesis of amide derivatives[151] (DA-1 – DA-34).



(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-VI

Amide Derivatives
DA-1 - DA-34

A solution of compound **V-VI** was made in dry dichloromethane and catalytic amount of dimethylformamide was added. This solution was treated with oxalyl chloride in 1:2.5 molar ratios under ice cold conditions. The reaction mixture was protected with a calcium chloride guard tube to protect it from moisture. The solution was allowed to stand for 24 hours at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation with dry benzene under reduced pressure. The acyl halide thus obtained was not isolated or characterized and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1 mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 hours and was protected with a calcium chloride guard tube. The resulting reaction mixture was then extracted with 0.1N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled to obtain the title compounds (**DA-1 – DA-34**).

All the synthesized amide derivatives were recrystallized from appropriate solvents and their melting points were determined. Their nomenclature and physical data are given in Table 5.1

and Table 5.2 respectively. For few selected compounds the spectral (^{1}H-NMR and Mass) and the elemental analyses were done and are shown in Table 5.3.

5.1.2. NOMENCLATURE AND PHYSICAL DATA OF (5,6-DIMETHOXY-2,3-DIHYDRO -1*H*-INDEN-1- YL)ACETIC ACID AMIDES

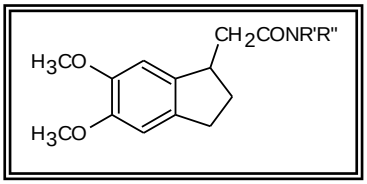


Table 5.1. Nomenclature of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	R'	R''	NOMENCLATURE (IUPAC)
DA-1	H	H	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-2	H	Me	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-methyl acetamide
DA-3	Me	Me	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*,*N*-dimethylacetamide
DA-4	H	Et	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylacetamide
DA-5	Et	Et	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*,*N*-diethylacetamide
DA-6	H	n-Pr	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylacetamide
DA-7	H	i-Pr	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-isopropylacetamide
DA-8	H	n-Bu	*N*-butyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-9	H	tr-Bu	*N*-tert-butyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-10	H	n-Amyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pentylacetamide
DA-11	H	n-Hexyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide
DA-12	H	Cyclopropyl	*N*-cyclopropyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-13	H	Cyclopentyl	*N*-cyclopentyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide

DA-14	H	Cyclohexyl	*N*-cyclohexyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-15	--Pyrrolidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]pyrrolidine
DA-16	--Piperidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]piperidine
DA-17	--Piperazino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]piperazine
DA-18	--Morpholino--		4-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]morpholine
DA-19	H	CH_2CH_2OH	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)acetamide
DA-20	H	Benzyl	*N*-benzyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-21	H	Phenyl (C_6H_5)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylacetamide
DA-22	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-23	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-24	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-25	H	C_6H_4(o-CH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-methylphenyl)acetamide
DA-26	H	C_6H_4(m-CH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)acetamide

Contd….

Table 5.1 Contd.

DA-27	H	C_6H_4(p-CH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*n*-(4-methylphenyl)acetamide
DA-28	H	C_6H_4(o-OCH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-methoxyphenyl)acetamide
DA-29	H	C_6H_4(p-OCH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methoxyphenyl)acetamide
DA-30	H	C_6H_4(p-NHAc)	*N*-[4-(acetylamino)phenyl]-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)aa*
DA-31	H	C_6H_4(p-NO_2)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)acetamide
DA-32	H	2-Pyridyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-2-ylacetamide
DA-33	H	3-Pyridyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylacetamide
DA-34	H	4-Pyridyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylacetamide

*aa- acetamide

Table 5.2. Physical data of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	RECRYSTALLIZATION SOLVENT	YIELD (%)	M.P (°C)	MOLECULAR FORMULA	MOLECULAR WEIGHT	Calc. Log P
DA-1	water	54.0	166-168	$C_{13}H_{17}NO_3$	235	1.45
DA-2	water	59.6	137-138	$C_{14}H_{19}NO_3$	249	1.92
DA-3	dilute alcohol	60.4	117-118	$C_{15}H_{21}NO_3$	263	2.29
DA-4	water	61.8	104-105	$C_{15}H_{21}NO_3$	263	2.44
DA-5	dilute alcohol	64.9	62-63	$C_{17}H_{25}NO_3$	291	3.21
DA-6	dilute alcohol	66.7	107-108	$C_{16}H_{23}NO_3$	277	2.90

DA-7	benzene	66.6	140-141	$C_{16}H_{23}NO_3$	277	2.72
DA-8	cyclohexane	59.2	103-104	$C_{17}H_{25}NO_3$	291	3.36
DA-9	cyclohexane	65.2	156-157	$C_{17}H_{25}NO_3$	291	3.24
DA-10	cyclohexane	65.1	102-103	$C_{18}H_{27}NO_3$	305	3.83
DA-11	cyclohexane	57.9	94-95	$C_{19}H_{29}NO_3$	319	4.31
DA-12	ethyl acetate	62.7	128-129	$C_{16}H_{21}NO_3$	275	2.63
DA-13	cyclohexane	72.5	136-138	$C_{18}H_{25}NO_3$	303	3.34
DA-14	cyclohexane	70.5	152-153	$C_{19}H_{27}NO_3$	317	3.83
DA-15	EtOAc/hexane	64.3	114-115	$C_{17}H_{23}NO_3$	289	2.75
DA-16	chloroform	57.5	101-102	$C_{18}H_{25}NO_3$	303	3.28
DA-17	EtOAc/hexane	63.7	212-213	$C_{17}H_{24}N_2O_3$	304	1.54
DA-18	dilute alcohol	61.6	137-138	$C_{17}H_{23}NO_4$	305	1.90
DA-19	benzene	64.4	72-74	$C_{15}H_{21}NO_4$	279	0.88
DA-20	dilute alcohol	77.4	146-147	$C_{20}H_{23}NO_3$	325	2.95
DA-21	dilute alcohol	72.8	148-149	$C_{19}H_{21}NO_3$	311	2.98

Contd….

Table 5.2 Contd.

DA-22	dilute alcohol	65.6	104-106	$C_{19}H_{20}ClNO_3$	345	3.84
DA-23	dilute alcohol	70.6	118-120	$C_{19}H_{20}ClNO_3$	345	3.79
DA-24	dilute alcohol	65.2	151-152	$C_{19}H_{20}BrNO_3$	390	3.82
DA-25	dilute alcohol	69.5	132-134	$C_{20}H_{23}NO_3$	325	3.26
DA-26	dilute alcohol	72.6	146-148	$C_{20}H_{23}NO_3$	325	3.40
DA-27	dilute alcohol	75.7	128-129	$C_{20}H_{23}NO_3$	325	3.49
DA-28	dilute alcohol	71.5	118-120	$C_{20}H_{23}NO_4$	341	2.97
DA-29	dilute alcohol	60.1	180-181	$C_{20}H_{23}NO_4$	341	3.06
DA-30	EtOAc/hexane	60.7	206-208	$C_{21}H_{24}N_2O_4$	368	2.38
DA-31	EtOAc/hexane	57.5	164-166	$C_{19}H_{20}N_2O_5$	356	3.08
DA-32	dilute alcohol	54.6	112-114	$C_{18}H_{20}N_2O_3$	312	2.19
DA-33	dilute alcohol	55.7	120-122	$C_{18}H_{20}N_2O_3$	312	2.16
DA-34	EtOAc/hexane	58.6	104-106	$C_{18}H_{20}N_2O_3$	312	2.19

Calc. Log P stands for calculated log P (from www. logp.com)

Table 5.3. Spectral and elemental analyses data of (5, 6-dimethoxy-2, 3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMP		**^{1}H-NMR**	**ELEMENTAL ANALYSES**	**MASS**

OUND	IR (cm^{-1}; KBr)	(δ ppm, $CDCl_3$, TMS)	(CALCULATED/FOUND)			(m/z)
			C%	H%	N%	M^+ Peak
DA-1	3391, 3216 (NH str), 1640 (C=O str), 1268, 1087 (OCH_3)	1.77, 2.42 (m, CH_2, 2H), 2.35, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.62 (qn, CH, 1H), 3.83 (s, CH_3, 3H), 3.84 (s, CH_3, 3H), 5.49 (s, NH, 2H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	66.36 66.15	7.28 7.25	5.95 5.93	235
DA-7	3285 (NH), 1644 (C=O), 1265, 1082 (OCH_3)	1.45 (d, CH_3, 6H), 1.81, 2.33 (m, CH_2, 2H), 2.21, 2.47 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.58 (qn, CH, 1H), 3.83 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 3.94 (m, CH, 1H) 5.22 (s, NH, 1H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	69.29 69.35	8.36 8.35	5.05 5.04	277
DA-11	3323 (NH), 1648 (C=O), 1267, 1083 (OCH_3)	0.98 (t, CH_3, 3H), 1.31 (m, CH_2, 6H), 1.53 (qn, CH_2, 2H), 1.78, 2.24 (m, CH_2, 2H), 2.26, 2.52 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.20 (t, CH_2, 2H), 3.60 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 5.39 (s, NH, 1H), 6.75 (s, ArH, 1H), 6.76 (s, ArH, 1H)	71.44 71.60	9.12 9.14	4.38 4.40	319
DA-14	3327 (NH), 1645 (C=O), 1269, 1086 (OCH_3)	1.11 (qn, CH_2, 2H), 1.35 (m, CH_2, 4H), 1.71(m, CH_2, 4H), 1.79, 2.30 (m, CH_2, 2H), 2.19, 2.46 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.58 (qn, CH, 1H), 3.65 (qn, CH, 1H), 3.82 (s, OCH_3, 3H), 3.84 (s, OCH_3,3H), 5.29 (s, NH,1H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	71.89 71.86	8.57 8.58	4.41 4.42	317

Contd….

Table 5.3 Contd.

DA-18	1648 (C=O), 1270, 1081 (OCH_3)	1.74, 2.43 (m, CH_2, 2H), 2.68, 2.45 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.47 (t, CH_2, 4H), 3.60 (qn, CH, 1H), 3.69 (t, CH_2, 4H) 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	66.86 66.69	7.59 7.56	4.59 4.60	305
DA-20	3333 (NH), 1643 (C=O), 1272, 1084 (OCH_3)	1.80, 2.48 (m, CH_2, 2H), 2.36, 2.57 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.63 (qn, CH, 1H), 3.81 (s, OCH_3, 3H), 3.83 (s, OCH_3, 3H), 4.46 (s, Bnz CH_2, 2H), 5.71 (s, NH, 1H), 6.74 (s, ArH, 1H), 6.75 (s, ArH, 1H), 7.18-7.33 (m, ArH, 5H**)**	73.82 73.91.	7.12 7.14	4.30 4.31	325
DA-21	3307 (NH), 1645 (C=O), 1265, 1081 (OCH_3)	1.82, 2.41 (m, CH_2, 2H), 2.50, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.64 (qn, CH, 1H), 3.72 (s, OCH_3, 3H), 3.84 (s, OCH_3, 3H), 6.74 (s, ArH, 1H), 6.76 (s, ArH, 1H), 7.09 (s, NH, 1H), 7.26-7.49 (m, ArH, 5H)	73.29 73.08	6.80 6.82	4.50 4.51	311
DA-23	3324 (NH), 1651 (C=O), 1269, 1087	1.82, 2.43 (m, CH_2, 2H), 2.51, 2.66 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.68 (qn,	65.99 65.74	5.83 5.82	4.05 4.06	345

	(OCH_3)	CH, 1H), 3.76 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 7.19 (s, NH, 1H), 7.24 (d, ArH, 2H), 7.53 (d, ArH, 2H)				
DA-26	3294 (NH), 1643 (C=O), 1263, 1078 (OCH_3)	1.82, 2.42 (m, CH_2, 2H), 2.32(s, CH_3, 3H) 2.49, 2.69 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.67 (qn, CH, 1H), 3.74 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 6.91 (d, ArH, 1H), 7.10 (s, NH, 1H), 7.16-7.35 (m, ArH, 3H)	73.82 74.09	7.12 7.14	4.30 4.31	325
DA-29	3317 (NH), 1641 (C=O), 1259, 1077 (OCH_3)	1.81, 2.41 (m, CH_2, 2H), 2.49, 2.61 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.68 (qn, CH, 1H), 3.74 (s, OCH_3, 3H), 3.78 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 6.84 (d, ArH, 2H), 7.14 (s, NH, 1H), 7.38 (d, ArH, 2H)	70.36 70.58	6.79 6.81	4.10 4.11	341
DA-33	3337 (NH), 1649 (C=O), 1268, 1087 (OCH_3)	1.82, 2.42 (m, CH_2, 2H), 2.49, 2.65 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.66 (qn, CH, 1H), 3.75 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 7.23 (d, ArH, 1H), 7.29 (s, NH, 1H), 8.11-8.43 (m, ArH, 3H)	69.21 68.99	6.45 6.43	8.97 8.94	312

5.1.3. RESULTS AND DISCUSSION

The starting reagent for the preparation of the key intermediate, 5,6-Dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)acetic acid (**V-VI**) was vanillin (**V-I**). The hydroxyl group of vanillin was methylated by dimethyl sulphate under alkaline conditions to get the methylated product veratraldehyde (**V-II**). This method avoids the use of the more expensive alkyl halides required in the Williamson synthesis. The formation of phenyl alkyl ether is an example of electrophilic substitution reaction where the proton is substituted with the methyl group.

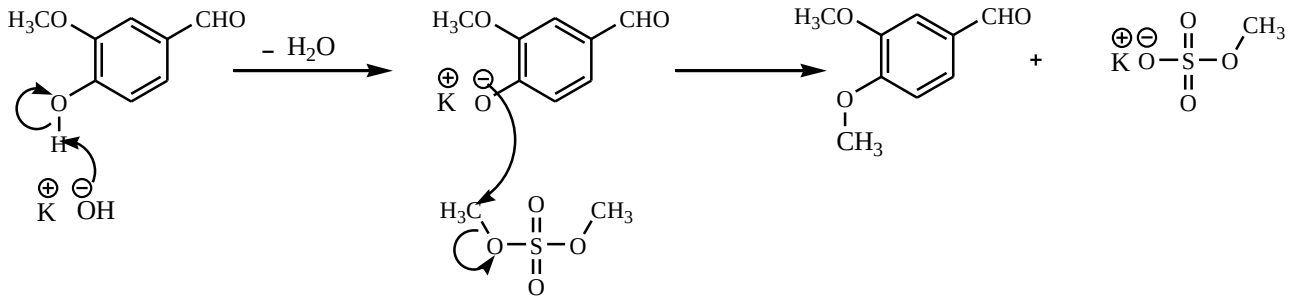


Figure 5.1. Possible reaction mechanism for the synthesis of **V-II**

Veratraldehyde (**V-II**) was reacted with two moles of ethylacetoacetate (EAA) in the presence of catalytic amount of piperidine at room temperature to obtain the benzylic bisacetoacetate **V-VIII.**

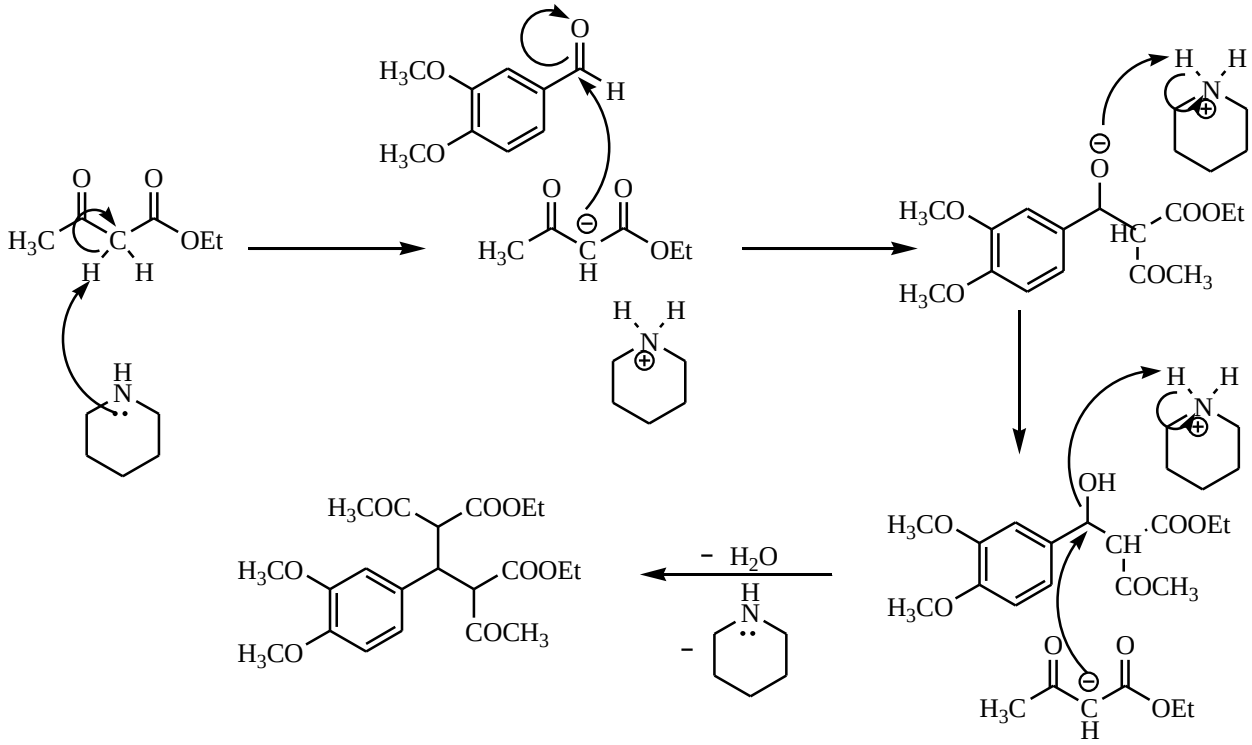


Figure 5.2. Possible reaction mechanism for the synthesis of **V-III**

Piperidine serves as the catalytic base and it abstracts the acidic proton from ethylacetoacetate. The negative charge on the active methylene group attacks the partially positively charged carbonyl carbon of aldehyde group. Similarly another molecule of ethylacetoacetate attacks through its active methylene carbon on the electron deficient carbon to form the benzylic bisacetoacetate (Figure 5.2). The IR spectrum of the compound **V-III** shows the carbonyl C=O stretching at 1720 cm^{-1}

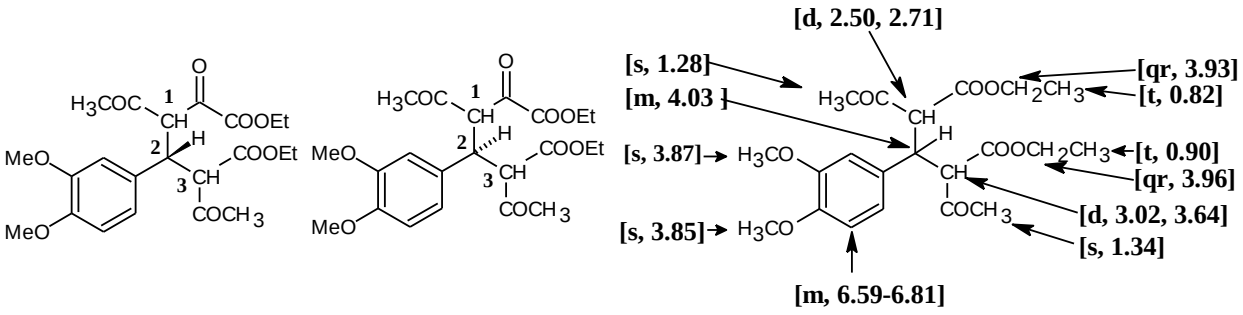


Figure 5.3. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-III**

The ^{1}H-NMR signal from proton present on carbon 1 and 3 are spilt into two doublets at 2.50, 2.71 and 3.02, 3.64 respectively by the neighboring proton of carbon 2 as shown above in Figure 5.3. This is because the proton present on carbon 2 is having two different configurations as shown above in Figure 5.3, causing the same proton to behave as present in two different environments. Similarly the signal from the proton on carbon 2 is split into a multiplet because of different configurational arrangements of proton on carbon 1 and 3.

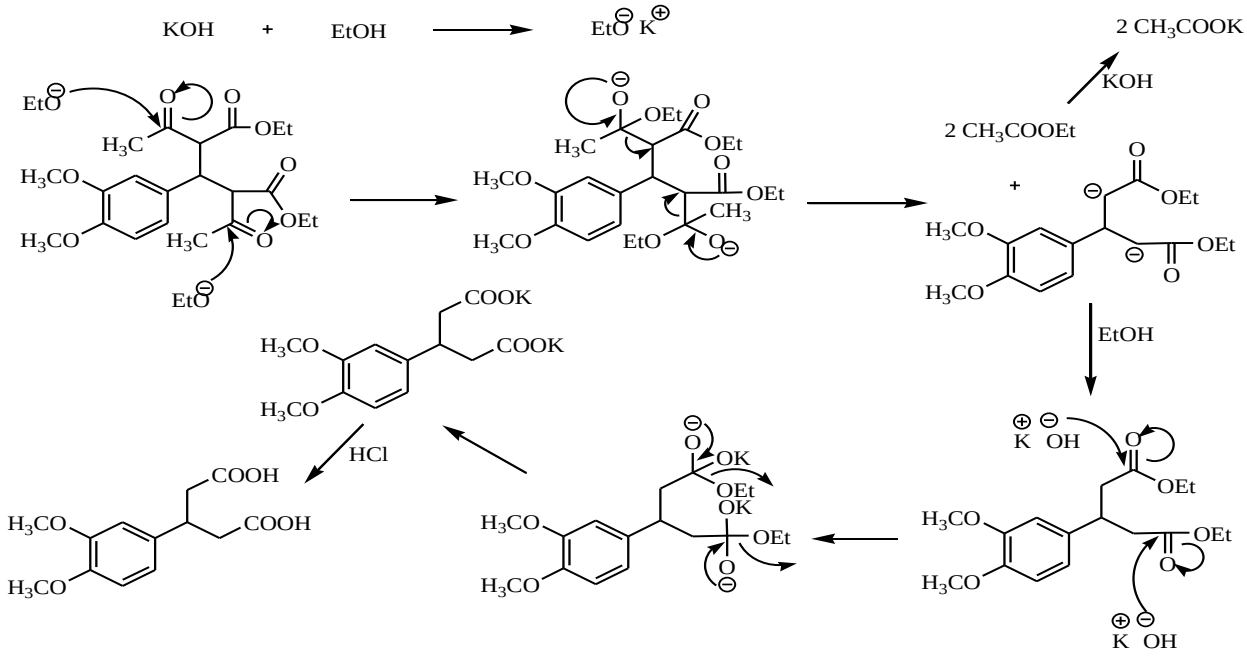


Figure 5.4. Possible reaction mechanism for the synthesis of **V-IV**

Alkaline hydrolysis of ester groups as well as ketonic hydrolysis of acetyl groups of the bisacetoacetate compound **V-III** was carried out with 6N potassium hydroxide (KOH) in 90% ethanol (Figure 5.4). The diacid product obtained was in the form of potassium salt which was liberated in free form by treatment with dilute hydrochloric acid under cold conditions. The IR spectrum of the compound **V-IV** showed broad OH stretch at 3300-2400 cm^{-1} and C=O stretch at 1710 cm^{-1}.

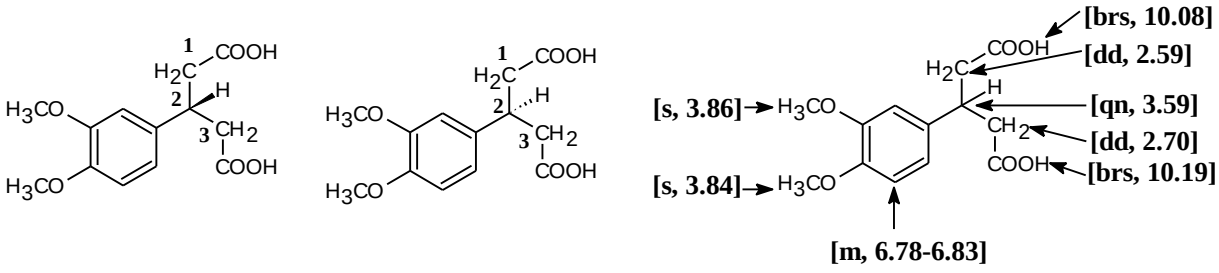


Figure 5.5. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-IV**

^{1}H-NMR of the diacid (**V-IV)** showed singlets at δ 3.84 and 3.86 ppm corresponding to methoxyl protons, doublet of doublet at 2.59 and 2.70 ppm as well as quintet at 3.59 ppm for CH_2 and CH of glutaric acid side chain respectively, multiplet at δ 6.78-6.83 ppm for aromatic protons and small broad singlets at δ 10.08 ppm and δ 10.19 ppm indicating the presence of carboxyl groups. Doublet of doublet was obtained for the protons on carbon 1 and 3 because of the different configurational arrangement of proton on carbon 2 as shown in Figure 5.5. This single proton on carbon 2 present in either equatorial or axial positions behaves in two different environments equivalent to two protons.

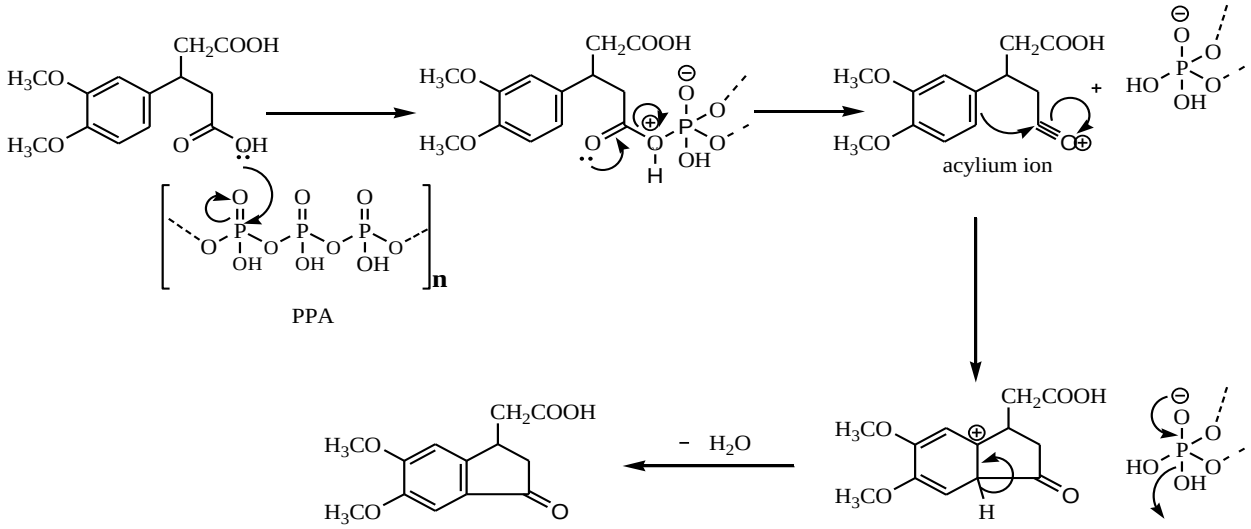


Figure 5.6. Possible reaction mechanism for the synthesis of **V-V**

The diacid compound **V-IV** was treated with polyphosphoric acid (PPA) when intramolecular Friedel-Craft's cyclization and hence ring closure took place to give the cyclic ketonic product. The lone pair of electrons on oxygen of carboxylic group attacks the partially electron deficient polyphosphoric acid and leads to the formation of acylium ion, a highly reactive electrophile. The aromatic electrons attack electron deficient carbon of acylium ion leading to ring closure and formation of the keto compound **V-V** (Figure 5.6).

The IR spectrum of keto acid **V-V** showed a broad OH stretch at 3400-2400 cm^{-1}. Two C=O stretching peaks were obtained at 1680 and 1724 cm^{-1} corresponding to oxo group and to carbonyl of acid group respectively.

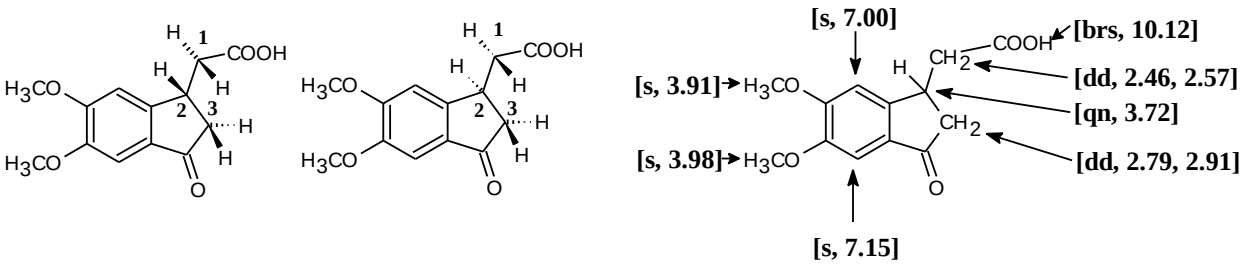


Figure 5.7. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-V**

Presence of doublet of doublet at 2.46 and 2.57 ppm and doublet of doublet at 2.79 and 2.91 ppm corresponding to CH_2 of side chain and CH_2 of the indan ring respectively as well as the presence of quintet at 3.72 ppm in the ^{1}H-NMR spectrum of **V-V** indicates ring closure (Figure 5.7). Doublet of doublets for protons on carbons 1 and 3 were obtained because of different configurational arrangements of the single proton present on carbon 2. The presence of methoxyl protons (singlets at 3.91 and at 3.98 ppm), aromatic protons (singlets at 7.00 and 7.15 ppm) and acid protons (broad singlet at 10.12 ppm) further supports the proposed structure of keto acid (**V-V**).

The ketone group of **V-V** was finally removed using Clemmensen's reduction to give **V-VI**, 5,6-dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)acetic acid. Clemmensen's reduction is a suitable approach for reduction of acid stable ketone compounds. Compound **V-V** is acid stable and hence can be deoxygenated to the corresponding hydrocarbon under the reaction conditions employed for Clemmensen's reduction. The reaction involves heating a carbonyl compound with amalgamated zinc in a hydroxylic solvent (often an aqueous mixture) containing a mineral acid such as HCl. The mercury alloyed with the zinc does not participate in the reaction; it serves only to provide a clean active metal surface. The reaction mechanism involves some sort of zinc ketone complex and is shown in Figure 5.8 [155].

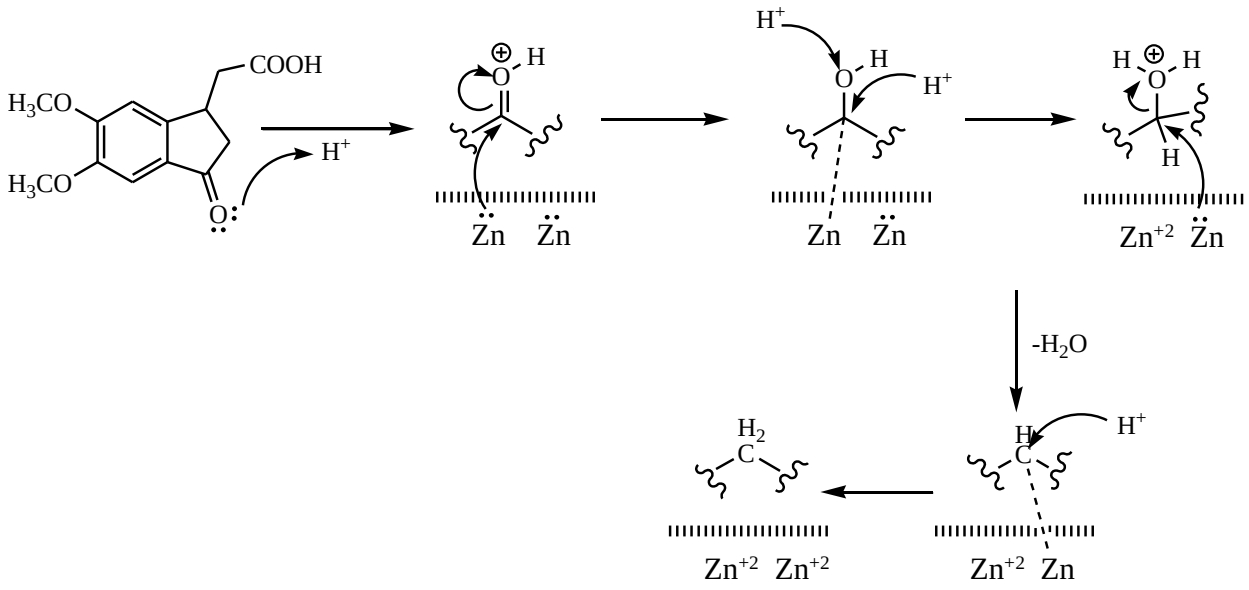


Figure. 5.8. The reaction mechanism of Clemmensen's reduction

Because of the different conformational arrangement of proton present on CH of indan ring both neighbouring CH_2 peaks got split into two peaks (two doublets of doublet at 2.49, 2.80 ppm and two multiplets at 1.81, 2.43 ppm) in the ^{1}H-NMR spectrum of the compound **V-VI** (Figure 5.9). The presence of multiplet at 2.89 ppm equivalent to two protons in addition to peaks obtained for compound **V-V** indicates the reduction of carbonyl group and formation of the indan ring system. The presence of aromatic protons (singlets at 6.76 and 6.78 ppm) and acid protons (broad singlet at 10.11 ppm) further supports the proposed structure of compound **V-VI**.

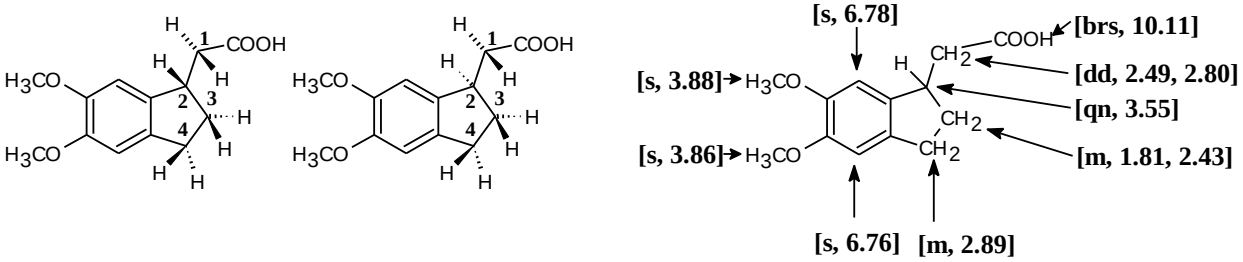


Figure 5.9. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of the compound **V-VI**

Compound **V-VI** was treated with oxalyl chloride in presence of catalytic amount of dimethylformamide to convert the carboxyl group of **V-VI** into acyl halide. The acyl halide thus obtained was not isolated or characterized. It was used directly in the next step. The acyl halide was reacted with various primary and secondary amines under Schotten- Baumann

conditions for the formation of desired amides. The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides. Also disappearance of broad OH stretch at 3400-2400 cm^{-1} indicates the formation of amide bond. The formation of amides was further confirmed by presence of a small singlet at 5-6 ppm for aliphatic amides and 7-8 ppm for the aromatic amides in ^{1}H-NMR. In most of the cases there was an overlap of two peaks at 2.2-2.4 ppm corresponding to doublet of doublet and multiplet; also in case of aromatic amides there was an overlap of peak corresponding to NH of amide group and the peaks corresponding to the aromatic group.

A modification of thionyl chloride method for making acyl halide uses oxalyl chloride plus catalytic amount of DMF. Oxalyl chloride reacts with DMF to produce a highly electrophilic cationic intermediate, plus carbon dioxide and carbon monoxide. As with thionyl chloride these by products are all gases. The first two steps are the nucleophilic substitution of chloride ion at the carbonyl group. The reactive intermediate is highly electrophilic and reacts rapidly with the carboxylic acid producing another intermediate which intercepts chloride ion to give the acyl halide and regenerate DMF (Figure 5.10)[156].

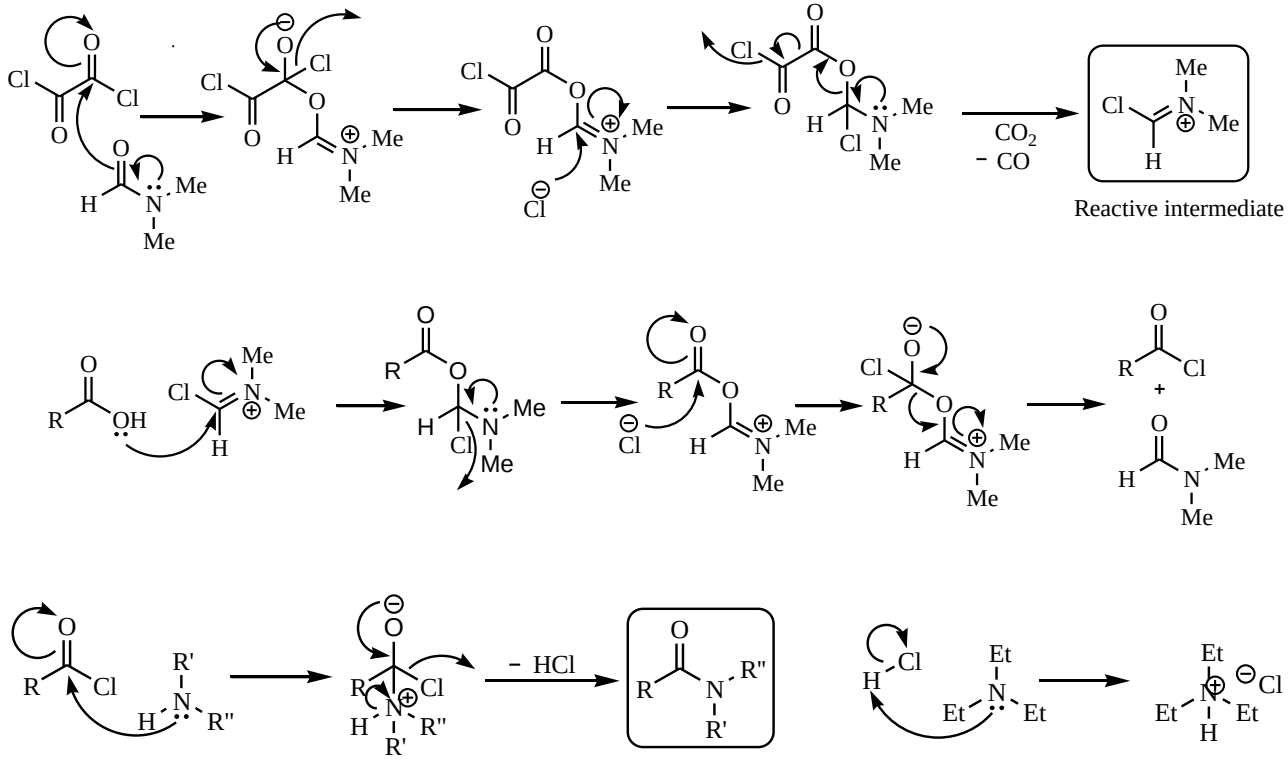


Figure 5.10. The reaction mechanism for formation of amide derivatives from compound **V-VI**

The acyl halide formation was also tried with thionyl chloride, but better yields of amides were obtained when the acyl halide was formed with oxalyl chloride even though the thionyl chloride used was distilled and dried. As heating was involved in the thionyl chloride method, the chances of highly reactive acyl halide's decomposition were more. Also the formation of highly reactive intermediate in oxalyl chloride provides much stronger electrophile to be attacked by the acid. These factors may be the likely reason for the better yields of amides obtained when acyl halide is made by reaction with oxalyl chloride.

In Mass spectroscopy of the compound **V-VI**, 5,6-dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)acetic acid, the M^+ peak was observed as the base peak. The fragmentation pattern has been shown in Figure 5.11. In most of the amide derivatives M^+ peak was observed as the base peak.

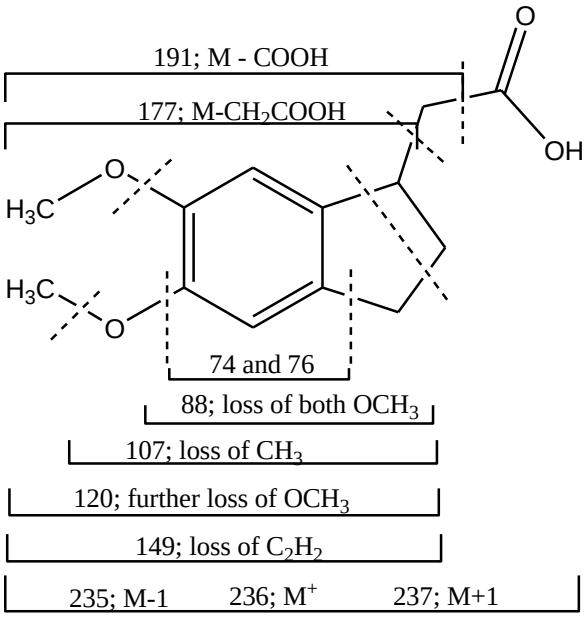
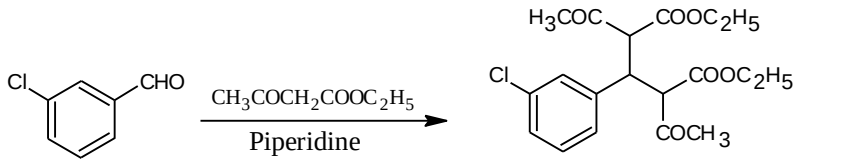


Figure 5.11. Mass spectrum of **V-VI**

5.2. (6-CHLORO-2, 3-DIHYDRO-1*H*-INDEN-1- YL)ACETIC ACID AMIDES

5.2.1. SYNTHESIS

5.2.1.1. Diethyl 2,4-diacetyl-3-(3-chlorophenyl)pentanedionate (V-VIII).



3-chlorobenzaldehyde

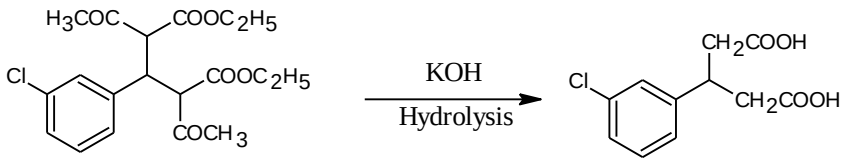
V-VII

diethyl 2,4-diacetyl-3-(3-chloro phenyl)pentanedioate

V-VIII

3-Chlorobenzaldehyde (**V-VII**) (28g, 0.2 mol) was dissolved in ethylacetoacetate (56g, 0.43 mol) in a dry conical flask and piperidine (2.5 ml) was added slowly at ambient temperature and then kept for 3 days or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product **V-VIII** in 70-75% yield. Recrystallization from dil. alcohol gave the analytical product, mp 120-121°C, IR (cm^{-1}) (KBr): 1722, 1682 (C=O stretching), 657 (C-Cl stretch), NMR: δ (ppm) ($CDCl_3$, TMS): 1.23 (t, CH_3, 3H), 1.31 (t, CH_3, 3H), 1.86 (s, CH_3, 3H), 1.93 (s, CH_3, 3H), 2.91, 3.26 (d, CH, H), 3.38, 3.85 (d, CH, H), 4.03 (qr, CH_2, 2H), 4.06 (qr, CH_2, 2H), 4.33 (m, CH, 1H), 6.74-7.17 (m, ArH, 4H).

5.2.1.2. 3-(3-chlorophenyl)pentanedioic acid (V-IX).

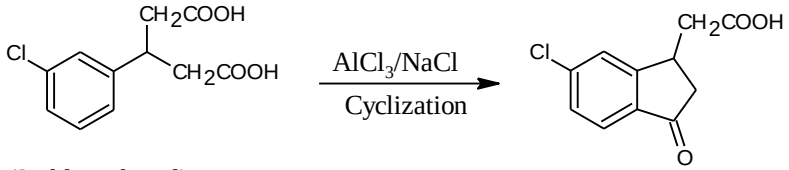


diethyl 2,4-diacetyl-3-(3-chloro phenyl)pentanedioate
V-VIII

3-(3-chlorophenyl) pentanedioic acid
V-IX

Compound **V-VIII** (40g, 0.1 mol) was dissolved in a hot solution of KOH (160g in 120 ml of water) and 160 ml of 50% ethanol was added. The hot reaction mixture was refluxed on a water bath for 1 hour. Alcohol was then removed by distillation, and after dilution with water it was cooled and washed with solvent ether. The aqueous layer on acidification with cold conc. HCl with cooling gave crude **V-IX** which was filtered and recrystallized from hot water. Yield 80-85%; mp 147-148°C; IR (cm^{-1}) (KBr): 3400-2200 (br OH stretch), 1710 (C=O stretching), 663 (C—Cl stretch); ^{1}H NMR:δ (ppm) ($CDCl_3$, TMS): 2.58, 2.70 (dd, CH_2, 4H), 3.60 (qn, CH, 1H), 7.15-7.26 (m, ArH, 4H), 12.01 (brs, COOH, 1H), 12.13 (brs, COOH, 1H)

5.2.1.3. (6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid[157, 158](V-X).

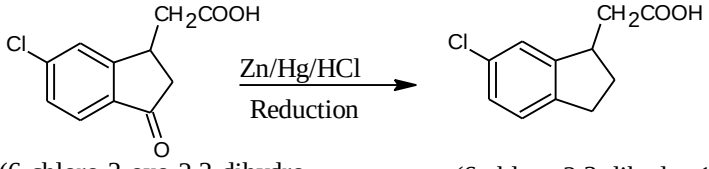


3-(3-chlorophenyl) pentanedioic acid
V-IX

(6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-X

Cyclization of compound **V-IX** was carried by treating this acid (14g, 0.058 mol) with aluminium chloride (49g, 0.37 mol) in presence of sodium chloride (4.47g, 0.08 mol) on an oil bath maintained at 180°C for 30 minutes after cessation of liberation of HCl gas from the reaction mixture, with stirring. After decomposition of the hot reaction mixture with crushed ice, the crude keto acid, **V-X,** thus obtained was filtered. The crude product was finally recrystallized from dilute acetic acid to get the pure compound. Yield 60-65%; mp 188-190°C; IR (cm^{-1}) (KBr): 3400-2400 (br OH stretch), 1680, 1713 (C=O stretching), 657 (C—Cl stretch), ^{1}H NMR: δ (ppm) ($CDCl_3$, TMS): 2.46, 2.58 (dd, CH_2, 2H), 2.84, 2.99 (dd, CH_2, 2H), 3.79 (qn, CH, 1H), 7.38 (d, ArH, 1H), 7.59 (s, ArH, 1H), 7.65 (d, ArH, 1H), 11.18 (brs, COOH, 1H).

5.2.1.4. (6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid (V-XI).



(6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid

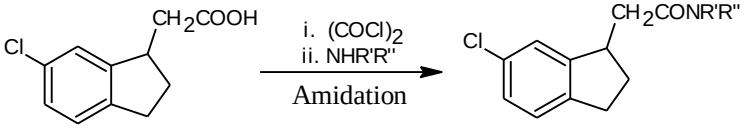
V-X

(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-XI

Compound **V-X** was subjected to Clemmensen's reduction. Compound V-X (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on steam bath for about 24 hrs (until the reaction mixture became keto-negative). After every 8 hrs around 10ml of conc. HCl was added to the reaction mixture. The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free of acidity and then dried over anhydrous sodium sulfate and was finally distilled off to get the reduced acid (**V-XI**). The reduced acid so obtained was a liquid and was subjected to vacuum distillation to get the pure compound. Yield 80-85%; distilled at 148 ºC / 0.3 mm Hg.; IR (cm^{-1}) (thin film): 3400-2400 (br OH stretch), 1700 (C=O stretching), 657 (C—Cl stretch); ^{1}H NMR: δ (ppm) ($CDCl_3$, TMS): 1.78, 2.42 (m, CH_2, 2H), 2.48, 2.81 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.56 (qn, CH, 1H), 7.11-7.16 (m, ArH, 3H), 10.91 (brs, COOH, 1H), M.S 211 (M^+).

5.2.1.5. General methods for the synthesis of amide derivatives (CA-1 - CA-25)



(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-XI

Amide derivatives

CA-1 - CA-25

A solution of compound **V-XI** was made in dry dichloromethane and catalytic amount of dimethylformamide was added. This solution was treated with oxalyl chloride in 1:2.5 molar ratio under ice cold conditions. The reaction flask was attached with a calcium chloride guard tube to protect it from moisture. The solution was allowed to stand for 24 hours at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation

with dry benzene under reduced pressure. The acyl halide thus obtained was not isolated or characterized and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 hours and was kept covered with a calcium chloride guard tube to protect it from moisture. The resulting reaction mixture was then extracted with 0.1N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled to obtain the title compounds (**CA-1 – CA-25**).

All the synthesized amide derivatives were recrystallized from appropriate solvents and their melting points were determined. Their nomenclature and physical data are given in Table 5.4 and Table 5.5 respectively. For few selected compounds the spectral (^{1}H-NMR and Mass) and the elemental analyses were done and are shown in Table 5.6.

5.2.2. NOMENCLATURE AND PHYSICAL DATA OF (6-CHLORO-2,3-DIHYDRO-1*H*-INDEN-1- YL)ACETIC ACID AMIDES

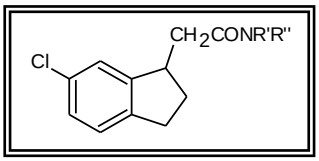


Table 5.4. Nomenclature of (6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	R’	R’’	NOMENCLATURE (IUPAC)
CA-1	H	H	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-2	H	Me	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-methyl acetamide
CA-3	H	Et	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylacetamide
CA-4	H	n-Pr	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylacetamide
CA-5	H	i-Pr	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-isopropylacetamide
CA-6	H	n-Bu	*N*-butyl-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-7	H	n-Amyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide
CA-8	H	n-Hexyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide
CA-9	H	Cyclopentyl	*N*-cyclopentyl-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide

CA-10	H	Cyclohexyl	*N*-cyclohexyl-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-11	--Piperidino--		1-[(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetyl]piperidine
CA-12	--Piperazino--		1-[(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetyl]piperazine
CA-13	H	CH_2CH_2OH	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)acetamide
CA-14	H	$CH_2C_6H_5$	*N*-benzyl-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-15	H	C_6H_5	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylacetamide
CA-16	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-17	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-18	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-19	H	C_6H_4(m-CH_3)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)acetamide
CA-20	H	C_6H_4(p-CH_3)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methylphenyl)acetamide
CA-21	H	C_6H_4(p-OCH_3)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methoxyphenyl)aa*
CA-22	H	C_6H_4(p-NHAc)	*N*-[4-(acetylamino)phenyl]-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)aa*
CA-23	H	C_6H_4(p-NO_2)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)acetamide
CA-24	H	3-Pyridyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylacetamide
CA-25	H	4-Pyridyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylacetamide

*aa- acetamide

Table. 5.5. Physical data of (6-chloro-2, 3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	RECRYSTALLIZATION SOLVENT	YIELD (%)	M.P (°C)	MOLECULAR FORMULA	FORMULA WEIGHT	Calc. Log P
CA-1	water	57.8	109-110	$C_{11}H_{12}ClNO$	210	1.92
CA-2	dilute alcohol	58.9	102-103	$C_{12}H_{14}ClNO$	224	2.31
CA-3	dilute alcohol	60.4	98-100	$C_{13}H_{16}ClNO$	238	2.80
CA-4	dilute alcohol	63.6	74-76	$C_{14}H_{18}ClNO$	252	3.27
CA-5	benzene	62.5	104-106	$C_{14}H_{18}ClNO$	252	3.07
CA-6	-	66.8	ss	$C_{15}H_{20}ClNO$	266	3.75
CA-7	-	65.1	ss	$C_{16}H_{22}ClNO$	280	4.24
CA-8	-	67.9	ss	$C_{17}H_{24}ClNO$	294	4.75
CA-9	cyclohexane	66.8	136-138	$C_{16}H_{20}ClNO$	274	3.74
CA-10	cyclohexane	64.3	145-147	$C_{17}H_{22}ClNO$	292	4.24
CA-11	dilute alcohol	69.1	108-110	$C_{16}H_{20}ClNO$	278	3.82
CA-12	dilute alcohol	57.8	215-216	$C_{15}H_{19}ClN_2O$	279	2.00
CA-13	benzene	60.3	60-62	$C_{13}H_{16}ClNO_2$	254	1.57
CA-14	cyclohexane	69.7	106-108	$C_{18}H_{18}ClNO$	300	3.54
CA-15	dilute alcohol	73.7	146-148	$C_{17}H_{16}ClNO$	286	3.59

CA-16	dilute alcohol	72.8	138-140	$C_{17}H_{15}Cl_2NO$	320	4.53
CA-17	dilute alcohol	74.6	145-146	$C_{17}H_{15}Cl_2NO$	320	4.37
CA-18	dilute alcohol	65.8	127-129	$C_{17}H_{15}BrClNO$	365	4.41
CA-19	dilute alcohol	71.3	109-111	$C_{18}H_{18}ClNO$	300	3.90
CA-20	dilute alcohol	70.4	194-195	$C_{18}H_{18}ClNO$	300	4.00
CA-21	dilute alcohol	69.1	176-178	$C_{18}H_{18}ClNO_2$	316	3.76
CA-22	dilute alcohol	67.6	156-158	$C_{19}H_{19}ClN_2O_2$	343	3.14
CA-23	dilute alcohol	65.8	137-139	$C_{17}H_{15}ClN_2O_3$	331	3.83
CA-24	dilute alcohol	60.3	196-198	$C_{16}H_{15}ClN_2O$	287	2.74
CA-25	dilute alcohol	58.6	157-159	$C_{16}H_{15}ClN_2O$	287	2.78

ss: semisolid
Calc. Log P is the calculated log P (from www. logp. com)

Table.5.6. Spectral and elemental analyses data of (6-chloro-2, 3-dihydro-1*H*-inden-1-yl) acetic acid amides

COMPOUND	**IR (cm^{-1}; KBr)**	**^{1}H-NMR (δ ppm, $CDCl_3$, TMS)**	**ELEMENTAL ANALYSES (CALCULATED/FOUND)**			**MASS (m/z) M^+ Peak**
			C%	**H%**	**N%**	
CA-1	3374, 3229 (NH), 1638 (C=O), 657 (C-Cl)	1.77, 2.43 (m, CH_2, 2H), 2.35, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.62 (qn, CH, 1H), 5.49 (s, NH, 2H), 7.10-7.18 (m, ArH, 3H)	63.01 62.86	5.77 5.76	6.68 6.70	210
CA-5	3294 (NH), 1631 (C=O), 658 (C-Cl)	1.28 (d, CH_3, 6H), 1.80, 2.40 (m, CH_2, 2H), 2.33, 2.61 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.87 (m, CH, 1H), 5.26 (s, NH, 1H), 7.10-7.18 (m, ArH, 3H)	66.79 66.58	7.21 7.23	5.56 5.57	252
CA-7	3281 (NH), 1640 (C=O), 658 (C-Cl)	0.97 (t, CH_3, 3H), 1.31 (m, CH_2, 4H), 1.52 (qn, CH_2, 2H), 1.79, 2.45(m, CH_2, 2H), 2.37, 2.66 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.19 (t, CH_2, 2H), 3.64 (qn, CH, 1H), 5.40 (s, NH, 1H), 7.09-7.17 (m, ArH, 3H)	68.68 68.90	7.93 7.91	5.01 4.99	280

CA-9	3310 (NH), 1640 (C=O), 658 (C-Cl) cm^{-1} MS (m/z): 278	1.52 (m, CH_2, 4H) 1.86 (m, CH_2, 4H), 1.79, 2.42 (m, CH_2, 2H), 2.33, 2.64 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.68 (qn, CH, 1H), 5.32 (s, NH, 1H), 7.11-7.18 (m, ArH, 3H)	69.18 69.41	7.26 7.29	5.04 5.06	278
CA-11	1624 (C=O), 657 (C-Cl) 663 cm^{-1}	1.65 (m, CH_2, 6H),1.75, 2.45 (m, CH_2, 2H), 2.72, 2.46 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.37 (t, CH_2, 4H), 3.66 (qn, CH, 1H), 7.10-7.17 (m, ArH, 3H)	69.18 69.31	7.26 7.29	5.04 5.06	278
CA-14	3306 (NH), 1642 (C=O), 658 (C-Cl) cm^{-1}	1.77, 2.40 (m, CH_2, 2H), 2.32, 2.57 (dd, CH_2, 2H), 2.84 (m, CH_2, 2H), 3.65 (qn, CH, 1H), 4.46 (s, Bnz CH_2, 2H), 5.81 (s, NH, 1H), 7.09-7.17 (m, ArH, 3H), 7.31 (m, ArH, 5H)	72.11 72.33	6.05 6.06	4.67 4.69	300
CA-15	3282 (NH), 1648 (C=O), 663 (C-Cl) cm^{-1}	(t, 1.81, 2.40 (m, CH_2, 2H), 2.47, 2.75 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.24 (s, NH, 1H), 7.10-7.21 (m, ArH, 4H), 7.31-7.48 (m, ArH, 4H)	71.45 71.67	5.64 5.66	4.90 4.92	286
CA-16	3306 (NH), 1642 (C=O), 662 (C-Cl)	1.80, 2.44 (m, CH_2, 2H), 2.47, 2.76 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.17 (s, NH, 1H), 7.08-7.33 (m, ArH, 6H), 7.64 (s, ArH, 1H)	63.76 63.89	4.72 4.74	4.37 4.39	320

Contd….

Table 5.6 Contd.

CA-20	3320 (NH), 1648 (C=O), 659 (C-Cl) cm^{-1}	1.84, 2.39 (m, CH_2, 2H), 2.30 (s, CH_3, 3H), 2.47, 2.74 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.66 (qn, CH, 1H), 7.10 (s, NH, 1H), 7.05-7.16 (m, ArH, 5H), 7.45 (d, ArH, 2H)	72.11 72.31	6.05 6.03	4.67 4.69	300
CA-24	3321 (NH), 1650 (C=O), 658 (C-Cl) cm^{-1};	1.83, 2.46 (m, CH_2, 2H), 2.53, 2.81 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.31 (s, NH, 1H), 7.10-7.30 (m, ArH, 4H), 8.17-8.51 (m, ArH, 3H)	67.02 67.23	5.27 5.25	9.77 9.73	287
CA-25	3330 (NH), 1648 (C=O), 660 (C-Cl) cm^{-1}	1.80, 2.42 (m, CH_2, 2H), 2.49, 2.79 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.28 (s, NH, 1H), 7.11-7.19 (m, ArH, 3H), 7.65-8.34 (m, ArH, 4H)	67.02 66.86	5.27 5.29	9.77 9.80	287

5.2.3. RESULTS AND DISCUSSION

The starting reagent for the preparation of the key intermediate, 6-chloro-2,3-dihydro-1*H*-inden(-1-yl)acetic acid **(V-XI)** was 3-chlorobenzaldehyde **(V-VII)**. Compound **V-VII** was reacted with two moles of ethylacetoacetate (EAA) in presence of catalytic amount of piperidine at room temperature to obtain the bisacetoacetate **V-VIII**. The reaction mechanism is same as described in Figure 5.2. The IR spectrum of this bisacetoacetate shows C=O stretches at 1722 and 1682cm^{-1}. In the ^{1}H-NMR spectrum of compound **V-VIII** (Figure 5.12) because of the different configurational arrangement of benzylic proton (as shown for dimethoxy series in Figure 5.3) the signal from the neighboring CH groups are split into two doublets each. The two equivalent side chains of bisacetoacetate showed two different peaks; the chain closer to the chloro group appeared at a slightly higher ppm (more deshielding effect) as compared to the one facing away from the chloro group.

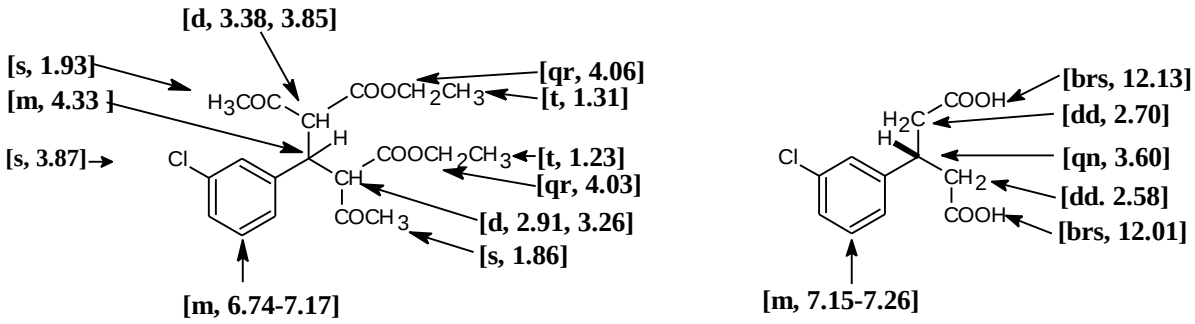


Figure 5.12 ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-VIII** and **V-IX**

Alkaline hydrolysis of **V-VIII** was carried out with 6N potassium hydroxide (KOH) in 50% ethanol to get the diacid **V-IX**. The reaction mechanism is same as shown in Figure 5.4. The IR spectrum of **V-IX** showed broad OH stretch at 3400-2200 cm^{-1} and C=O stretch at 1710 cm^{-1}. ^{1}H-NMR of **V-IX** (Figure 5.12) showed doublet of doublet at δ 2.58 and 2.70 ppm as well as quintet at 3.60 ppm for CH_2 and CH of glutaric acid side chain respectively, multiplet at δ 7.15-7.26 ppm for aromatic protons and small broad singlets at δ 12.13 and 12.01 ppm indicating the presence of carboxyl group. The doublet of doublet for CH2 of glutaric acid was obtained due to different configurational arrangement of the proton present on neighboring CH group. Both CH_2 groups are in different environment and hence behave differently therefore slight difference in δ values of the signals (dd) are observed (as shown in figure 5.12).

Compound **V-IX** was treated with aluminium chloride in presence of catalytic amount of sodium chloride when intramolecular Friedel-Craft's acylation and hence ring closure took

place to give the ketonic product **V-X**. The cyclization was also tried with polyphosphoric acid (PPA) but it gave poorer yield of the keto product with high impurity content. Various reaction conditions were tried to get the better yield of the cyclic oxo compound (Table 5.7 and 5.8). The compound **V-IX** gets deactivated towards the cyclisation by the presence of chloro group at the meta position. Hence aluminium chloride being a stonger lewis acid gave better yields in carrying the cyclization of **V-IX** in comparison to PPA.

Table 5.7. Cyclization of 3-(3-chlorophenylglutaric acid) using $AlCl_3$/ NaCl

S.No.	Temperature(°C)	Time*(min)	Yield (%)
1.	130	0	38.5
2.	160	15	50.54
3.	160	30	56.8
4.	160	60	40.57
5.	180	15	60.85
6.	180	30	63.88
7.	180	60	56.80
8.	190	15	56.80
9.	190	30	50.71
10.	200	15	56.8
11.	200	30	48.74

* Time after cessation of liberation of HCl gas from the reaction mixture
Shaded area shows the most optimum reaction conditions
The oxo compound after single recrystallisation from dilute acetic acid gave melting point of 188-190°C.

Table 5.8. Cyclization of 3-(3-chlorophenylglutaric acid) using Polyphosphoric acid (PPA)

S.No.	3-ClGA : PPA (w /w)	Time (min)	Temperature(°C)	Yield (%)
1.	1 : 25	8hrs	100	26.3
2.	1 : 15	10	120	30.45
3.	1 : 15	10	130	42.6
4.	1 : 15	10	140	25.6

Contd…

Table 5.8. Contd.

5.	1 : 15	10	150	17.05
6.	1 : 15	5	130	56.8
7.	1 : 15	15	130	40.9
8.	1 : 15	30	130	29.8
9.	1 : 15	3	130	44.7
10.	1 : 10	5	130	46.9
11.	1 : 20	5	130	51.5
12.	1 : 25	5	130	40.5

Shaded area shows the most optimum reaction conditions
The oxo compound after single recrystallization from dilute acetic acid gave broad melting point range (176-184°C)

In this reaction sodium chloride is added to aluminium chloride to lower the melting point of the latter and hence to lower the reaction temperature and also the reaction time[159]. Organic reactions have been performed in the binary alkali-metal chloride-aluminium chloride salts, which are lower melting ionic liquids[160]. It has been reported that at higher temperatures the aluminium chloride can cause intramolecular cyclization of the acids to oxo compounds without formation of the acyl halide.

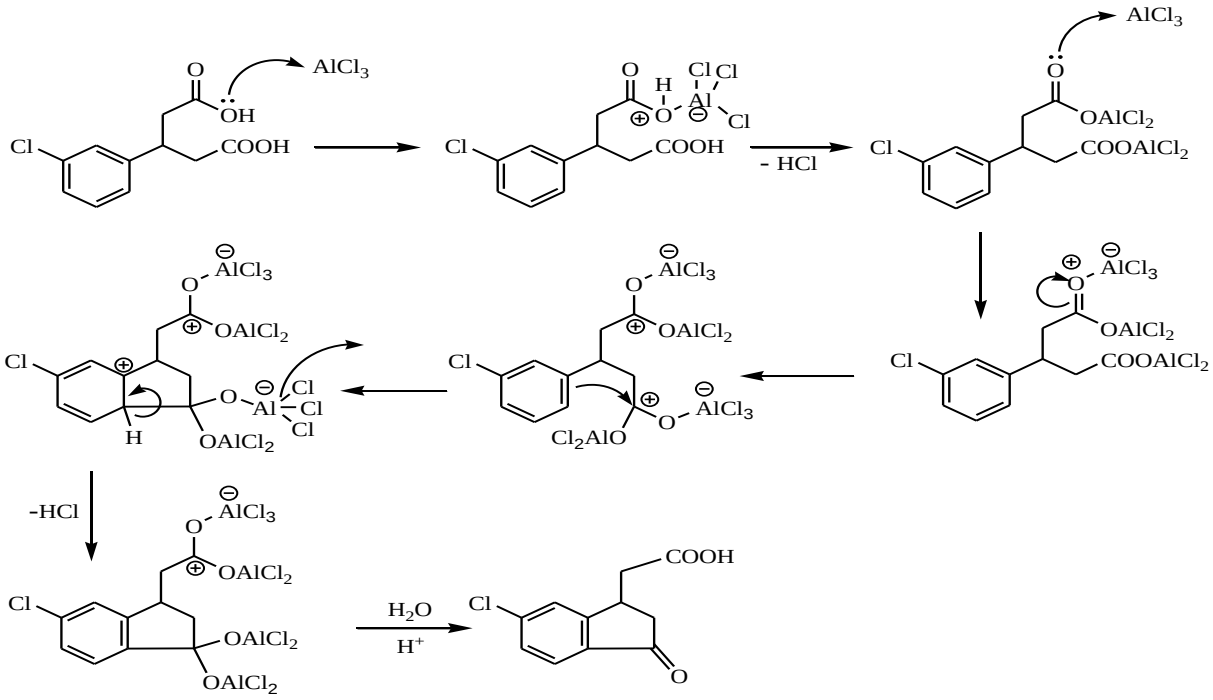


Figure 5.13. Possible reaction mechanism for cyclization of compound **V-IX**[161].

The IR spectrum of compound **V-X** shows broad OH stretch at 3400-2400 cm^{-1}, two carbonyl stretching at 1680, 1713 cm^{-1} and C—Cl stretch at 657 cm^{-1} . Presence of doublet of doublet at 2.46 and 2.58 ppm and doublet of doublet at 2.84 and 2.99 corresponding to CH_2 of side chain and CH_2 of the indan ring respectively as well as the presence of quintet at 3.79 ppm indicates the ring closure and hence formation of compound **V-X** (Figure 5.14). The presence of aromatic protons (doublets at 7.38 and 7.65 ppm and singlet at 7.59) and acid protons (broad singlet at 10.91 ppm) further supports the proposed structure of compound **V-X**.

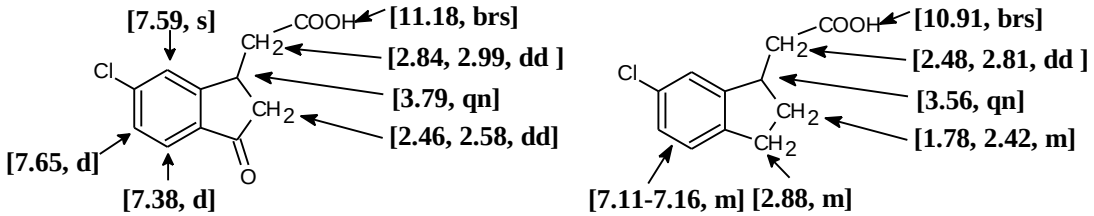


Figure 5.14. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-X** and **V-XI**

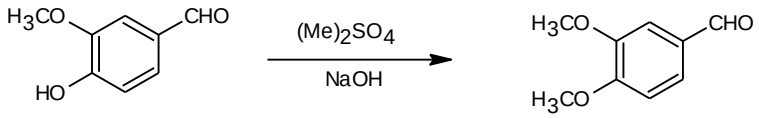
The ketone group of compound **V-X** was removed using Clemmensen's reduction to give 6-chloro-2,3-dihydro-1*H*-inden(-1-yl)acetic acid (**V-XI)**. The mechanism is the same as shown in Figure 5.8. The IR spectrum shows broad OH stretch at 3400-2400, C=O stretch at 1700 and C—Cl stretch 657cm-^{1}. Because of the different conformational arrangement of proton present on CH of indan ring both neighbouring CH_2 peaks got split into two peaks (two doublets of doublet at 2.48 and 2.81 ppm and two multiplets at 1.78 and 2.42 ppm). The presence of multiplet at 2.88 ppm equivalent to two protons in addition to peaks obtained for compound **V-XI** indicates the reduction of carbonyl group and formation of indan ring system. The presence of aromatic protons (multiplet at 7.11-7.16 ppm) and proton of COOH group (small broad singlet at 11.18 ppm) further supports the structure of compound **V-XI**.

Compound **V-XI** was treated with oxalyl chloride in presence of catalytic amount of dimethylformamide to convert carboxyl group of **V-XI** into acyl chloride. The acyl halide thus obtained was not isolated or characterized. It was used directly in the next step. The acyl halide was reacted with various primary and secondary amines under Schotten- Baumann conditions for the formation of desired amides (**CA-1 – CA-25**). The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides respectively. Also disappearance of broad OH stretch at 3400-2400 cm^{-1} indicates the formation of amide bond. The formation of amides was further confirmed by presence of a small singlet at 5-8 ppm in the 1-H NMR spectrum. In most of the cases there was an overlap of two peaks at around 2.3-2.5 corresponding to doublet of doublet and multiplet. The amide peak overlapped with the aromatic peaks around 7.1- 7.3 in case of many aromatic amides. In the mass spectrum of compound **V-XI,** M^+ peak was observed as the base peak at 210 and M+ 2 peak at 212. In most of the amides M^+ peak was observed as the base peak and the corresponding M+2 peaks were also observed.

5.3. (5, 6-DIMETHOXY-2, 3-DIHYDRO-1*H*-INDEN-1- YL)CARBOXLIC ACID AMIDES

5.3.1. SYNTHESIS

5.3.1.1. 3, 4-Dimethoxybenzaldehyde (V-II).

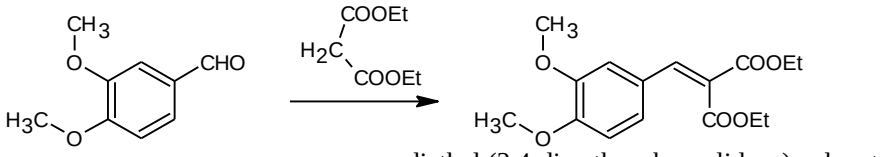


4-hydroxy-3-methoxybenzaldehyde
V-I

3,4-dimethoxybenzaldehyde
V-II

Commercially available vanillin (**V-I**) (15.2g, 0.1 mol) was placed in a 500ml three-necked flask equipped with a magnetic stirrer, two dropping funnels and a reflux condenser. One funnel was charged with potassium hydroxide (8.2g in 12ml) and the other funnel with purified dimethyl sulphate (12 ml, 0.104 mol). Vanillin was melted by warming on water bath, and then KOH was added at the rate of two drops a second. After 20 seconds dimethyl sulphate was also added at the same rate. The heating was stopped after few minutes as the mixture continued to reflux gently from the heat of reaction. The reaction mixture was vigorously stirred throughout. After all reagents have been added the reaction mixture became turbid and separated into two layers. Pale reddish brown color of the reaction mixture indicating the alkaline condition was maintained throughout the reaction. The yellow reaction mixture was poured into a porcelain basin and was left overnight. The hard crystalline mass thus obtained was ground, washed thoroughly with cold water to make it free of basicity, filtered and dried in a vacuum desiccator to get veratraldehyde (**V-II**) in 70-75% yield, mp 43-44°C.

5.3.1.2. Diethyl (3,4-dimethoxybenzylidene)malonate[162] (V-XII).



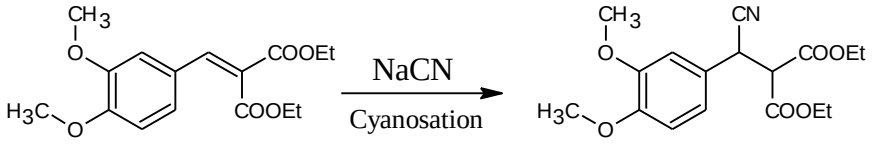
3,4-dimethoxybenzaldehyde
V-II

diethyl (3,4-dimethoxybenzylidene)malonate
V-XII

To the veratraldehyde (**V-II**) (16.6g, 0.1mol) in dry round bottomed flask diethylmalonate (20g, 0.104 mol) was added. To this reaction mixture 1ml of piperidine, 1ml of glacial acetic acid and 100ml of dry benzene was added. The reaction mixture was refluxed in a Dean Stark's apparatus. The reaction was carried until no more water collects in the Dean Stark's apparatus (about 18 hrs). The reaction mixture was washed with water and dried over anhydrous sodium sulphate. Benzene was then removed from the reaction mixture under reduced pressure. The remaining brownish oily liquid was set for vacuum distillation.

Benzylidene malonate, **V-XII,** was distilled at 205-210 °C at 6-7mm Hg as a clear liquid. The overall yield was 85-90%. IR (cm^{-1}) (thin film): 1718 (C=O stretching), 1043, 1260 (C—O stretch of OCH_3); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.26 (t, CH_3, 3H), 1.29 (t, CH_3, 3H), 3.83 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 4.24 (qr, CH_2, 2H), 4.30 (qr, CH_2, 2H), 4.80 (s, CH, 1H), 6.89-7.11 (m, ArH, 3H)

5.3.1.3. Diethyl [cyano(3,4-dimethoxyphenyl)methyl]malonate[162] (V-XIII).

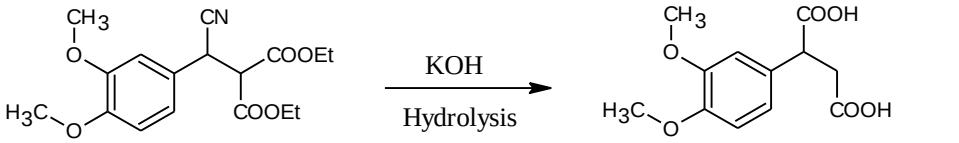


diethyl (3,4-dimethoxy benzylidene)malonate
V-XII

diethyl [cyano(3,4-dimethoxy phenyl)methyl]malonate
V-XIII

To 30.9 g of benzylidene malonate (**V-XII**) (0.1mol) in dry round bottomed flask 5g of sodium cyanide (0.102 mol) dissolved in 90% alcohol was added. The reaction mixture was refluxed for 18 hrs at 100 °C and then alcohol was distilled at reduced pressure. The formed cyanomalonate, **V-XIII,** was not purified and was used directly for the next step.

5.3.1.4. 2-(3, 4-dimethoxyphenyl)succinic acid (V-XIV).

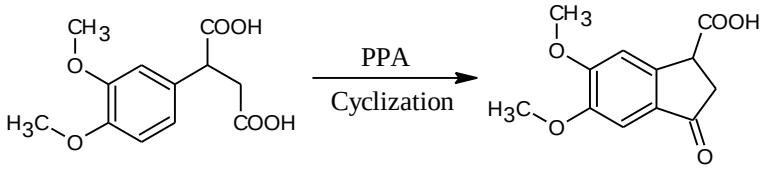


diethyl [cyano(3,4-dimethoxy phenyl)methyl]malonate
V-XIII

2-(3,4-dimethoxyphenyl)succinic acid
V-XIV

To the above reaction mixture 25.2g of KOH dissolved in 75ml of water (6N, 0.45mol) was added. The reaction mixture was refluxed for 16 hrs and then was allowed to cool. The cooled reaction mixture was slowly poured on to ice-sulphuric acid mixture with stirring when the diacid (**V-XIV**) precipitated out. The crude product obtained was filtered, dried and recrystallized from hot water to get the desired succinic acid. Yield 75-80%; mp 171-173°C; IR (cm^{-1}) (KBr): 3400-2400 (OH stretch) 1700 (C=O stretching), 1038, 1256 (C—O stretch of OCH_3); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 2.60 (dd, CH_2, 1H), 3.10 (dd, CH_2, 1H) 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 3.96 (dd, CH, 1H), 6.81-6.88 (m, ArH, 3H), 11.19 (brs, COOH, 1H), 11.26 (brs, COOH, 1H).

5.3.1.5. (5,6-Dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XV)



2-(3,4-dimethoxyphenyl)succinic acid

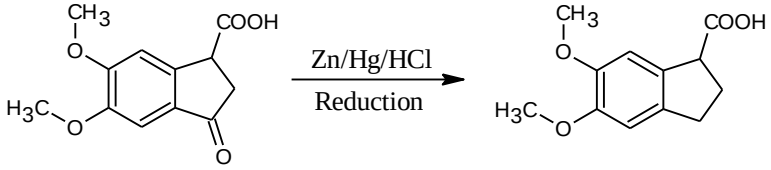
V-XIV

(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XV

Cyclization of compound **V-XIV** was effected by treating the powdered acid (15g, 0.059 mol) with 375 g of polyphosphoric acid (PPA) [1:25 ratio, w/w] on a steam bath for 4 hours with occasional stirring. After decomposition of the hot reaction mixture with crushed ice, the keto acid (**V-XV**) was isolated by extraction with chloroform. The solvent was distilled off to get the crude keto acid. The crude product was finally recrystallized from hot water to get the pure compound. Yield 70-75%; mp 190-191°C; IR (cm^{-1}) (KBr): 3400-2200(br OH stretch), 1726, 1650 (C=O stretching), 1042, 1225 (C—O stretch of OCH_3), ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 2.84 (dd, CH_2, 1H), 3.10 (dd, CH_2, 1H), 4.17 (dd, CH, 1H), 3.91 (s, OCH_3, 3H), 3.98 (s, OCH_3, 3H), 7.21 (s, ArH, 1H), 7.16 (s, ArH, 1H), 10.34 (brs, COOH, 1H).

5.3.1.6. (5,6-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XVI).



(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XV

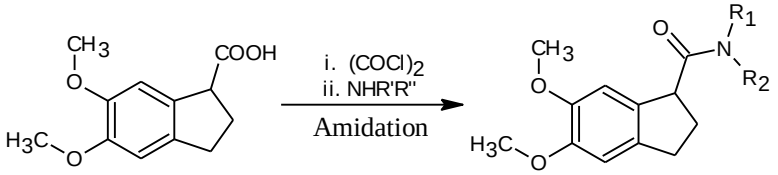
(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XVI

Compound **V-XV** was subjected to Clemmensen's reduction to get the reduced product **V-XVI.** The keto acid, **V-XV,** (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on steam bath until the reaction mixture became keto-negative (about 8 hrs). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free of acidity and then dried over anhydrous sodium sulfate and was finally distilled off to get the reduced acid. The analytical product was obtained on recrystallization from benzene. Yield 70-75%; mp 114-115^{o}C ; IR (cm^{-1}) (KBr): 3400-2400 (br OH stretch), 1700(C=O stretching), 1037, 1220 (C—O stretch of OCH_3); ^{1}H-NMR:δ (ppm) ($CDCl_3$, TMS): 2.36 (m, CH_2, 1H),

2.45 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 3.06 (m, CH_2, 1H), 4.02 (dd, CH, 1H), 3.86 (s, OCH_3, 3H), 3.87 (s, OCH_3, 3H), 6.77 (s, ArH, 1H), 6.94 (s, ArH,1H),10.26 (brs,COOH,1H)

5.3.1.7. General Method for the synthesis of amide derivatives (DC-1 – DC-22).



(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XVI

Amide Derivatives

DC-1 - DC-22

A solution of compound **V-XVI** was made in dry dichloromethane and catalytic amount of dimethylformamide was added. This solution was treated with oxalyl chloride in 1:2.5 molar ratios under ice cold conditions. The reaction flask was fitted with a calcium chloride guard tube to protect the reaction mixture from moisture. The solution was allowed to stand for 24 hours at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation with dry benzene under reduced pressure. The acyl halide thus obtained was not isolated or characterized and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 hours and was kept protected from moisture using a calcium chloride guard tube. The resulting reaction mixture was then extracted with 0.1N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled off to obtain the title compounds (**DC-1 – DC-22**).

All the synthesized amide derivatives were recrystallized from appropriate solvents and their melting points were determined. Their nomenclature and physical data are given in Table 5.9 and Table 5.10 respectively. For few selected compounds the spectral (^{1}H-NMR and Mass) and the elemental analyses were done and are shown in Table 5.11.

5.3.2. NOMENCLATURE AND PHYSICAL DATA OF (5,6-DIMETHOXY-2,3-DIHYDRO-1*H*-INDEN-1- YL)CARBOXYLIC ACID AMIDES

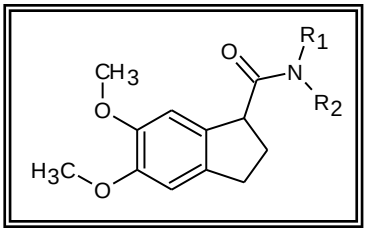


Table 5.9. Nomenclature of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

COMPOUND	R'	R''	NOMENCLATURE (IUPAC)
DC-1	H	H	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -2	H	Me	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-methylcarboxamide
DC -3	H	Et	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylcarboxamide
DC -4	H	n-Pr	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylcarboxamide
DC -5	H	n-Bu	*N*-butyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -6	H	n-amyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pentylcarboxamide
DC -7	H	n-hexyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylcarboxamide
DC -8	H	cyclopentyl	*N*-cyclopentyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -9	H	cyclohexyl	*N*-cyclohexyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -10	--piperidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carbonyl]piperidine
DC -11	--piperazine--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carbonyl]piperazine
DC -12	H	Benzyl	*N*-benzyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -13	H	Phenyl (C_6H_5)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylcarboxamide
DC -14	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -15	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -16	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -17	H	C_6H_4(m-CH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)carboxamide
DC -18	H	C_6H_4(p-CH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methylphenyl)carboxamide
DC -19	H	C_6H_4(p-OCH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)carboxamide
DC -20	H	C_6H_4(p-NO_2)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)carboxamide
DC -21	H	3-pyridyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylcarboxamide
DC -22	H	4-pyridyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylcarboxamide

Table 5.10. Physical data of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

COMPOUND	RECRYSTALLIZATION SOLVENT	YIELD (%)	M.P (°C)	MOLECULAR FORMULA	FORMULA WEIGHT	Calc. Log P
DC-1	dilute alcohol	58.0	150-152	$C_{12}H_{15}NO_3$	221	1.23
DC -2	dilute alcohol	59.1	110-112	$C_{13}H_{17}NO_3$	235	1.76
DC -3	dilute alcohol	61.3	103-105	$C_{14}H_{19}NO_3$	249	2.25
DC -4	EtOAc/hexane	62.5	92-94	$C_{15}H_{21}NO_3$	263	2.71
DC -5	EtOAc/hexane	61.8	104-106	$C_{16}H_{23}NO_3$	277	3.16
DC -6	dilute alcohol	63.4	96-98	$C_{17}H_{25}NO_3$	291	3.62
DC -7	dilute alcohol	65.3	96-97	$C_{18}H_{27}NO_3$	305	4.09
DC -8	alcohol	62.7	169-170	$C_{17}H_{23}NO_3$	289	3.12
DC -9	alcohol	61.6	174-176	$C_{18}H_{25}NO_3$	303	3.60
DC -10	EtOAc/hexane	59.4	132-134	$C_{17}H_{23}NO_3$	289	3.00
DC -11	benzene	60.8	228-230	$C_{16}H_{22}N_2O_3$	290	1.43
DC -12	alcohol	64.8	134-136	$C_{19}H_{21}NO_3$	311	2.80
DC -13	dilute alcohol	71.7	138-139	$C_{18}H_{19}NO_3$	297	2.85
DC -14	dilute alcohol	69.3	131-132	$C_{18}H_{18}NO_3Cl$	332	3.70
DC -15	alcohol	70.9	191-192	$C_{18}H_{18}NO_3Cl$	332	3.65
DC -16	alcohol	68.4	196-198	$C_{18}H_{18}NO_3Br$	376	3.67
DC -17	alcohol	70.6	122-123	$C_{19}H_{21}NO_3$	311	3.25
DC -18	alcohol	71.5	163-164	$C_{19}H_{21}NO_3$	311	3.35
DC -19	alcohol	69.2	162-164	$C_{19}H_{21}NO_4$	327	2.98
DC -20	alcohol	60.4	133-135	$C_{18}H_{18}N_2O_5$	342	2.95
DC -21	dilute alcohol	62.7	174-176	$C_{17}H_{18}N_2O_3$	298	2.06
DC -22	dilute alcohol	64.8	120-122	$C_{17}H_{18}N_2O_3$	298	2.08

Calc. Log P is the calculated log P (from www. logp. com)

Table 5.11. Spectral and elemental analyses data of (5, 6-dimethoxy-2, 3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

			ELEMENTAL ANALYSES (CALCULATED/FOUND)	MASS

COMPOUND	IR (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, $CDCl_3$, TMS)	C%	H%	N%	(m/z) M^+ Peak
DC-6	3314 (NH str), 1648 (C=O str), 1263, 1059 (OCH_3)	0.87 (t, CH_3, 3H), 1.27 (m, CH_2, 4H), 1.44 (qn, CH_2, 2H), 2.28 (m, CH_2, 1H) 2.48 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 2.96 (m, CH_2, 1H), 3.24 (t, CH_2, 2H), 3.86 (s, OCH_3, 3H), 3.88(s, OCH_3, 3H), 3.92 (dd, CH, 1H), 5.42 (s, NH, 1H), 6.79 (s, ArH, 1H), 6.81 (s, ArH, 1H)	70.07 69.85	8.65 8.62	4.81 4.82	291

Contd….

Table 5.11 Contd.

DC-8	3321 (NH), 1643 (C=O), 1269, 1074 (OCH_3)	1.49 (m, CH_2, 4H), 1.62 (m, CH_2, 2H), 1.84 (m, CH_2, 2H), 2.35 (m, CH_2, 1H) 2.47 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 2.98 (m, CH_2, 1H), 3.56 (qn, CH_2, 1H), 3.85 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 4.01 (dd, CH, 1H), 5.34 (s, NH, 1H), 6.78 (s, ArH, 1H), 6.80 (s, ArH, 1H)	70.56 70.79	8.01 8.04	4.84 4.83	289
DC-11	3341 (NH of piperazine), 1638 (C=O), 1256, 1059 (OCH_3)	2.09 (s, NH of piperazine, 1H), 2.30 (m, CH_2, 1H) 2.49 (m, CH_2, 1H), 2.79 (t, CH_2, 4H), 2.89 (m, CH_2, 1H), 3.01 (m, CH_2, 1H), 3.31 (t, CH_2, 4H), 3.85 (s, OCH_3, 3H), 3.87 (s, OCH_3, 3H), 3.99 (dd, CH, 1H), 6.80 (s, ArH, 1H), 6.81 (s, ArH, 1H)	66.18 66.38	7.64 7.66	9.65 9.62	290
DC-12	3327 (NH), 1646 (C=O), 1273, 1085 (OCH_3)	2.34 (m, CH_2, 1H) 2.49 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 2.96 (m, CH_2, 1H), 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 4.00 (dd, CH, 1H), 4.42 (s, CH_2, 2H), 5.66 (s, NH, 1H), 6.79 (s, ArH, 1H), 6.81 (s, ArH, 1H), 7.06-7.15 (m, ArH, 5H)	73.29 73.01	6.80 6.78	4.50 4.51	311
DC-14	3311 (NH), 1645 (C=O), 1259, 1061 (OCH_3)	2.44 (m, CH_2, 1H) 2.58 (m, CH_2, 1H), 2.93 (m, CH_2, 1H), 3.07 (m, CH_2, 1H), 3.86 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 4.13 (dd, CH, 1H), 6.85 (s, ArH, 1H), 6.87 (s, ArH, 1H), 7.01 (d, ArH, 1H) 7.10 (s, NH, 1H), 7.21 (t, ArH, 1H), 7.49 (d, ArH, 1H), 7.64 (s, ArH, 1H)	65.16 65.41	5.47 5.49	4.22 4.23	332
DC-18	3318 (NH), 1651 (C=O), 1260, 1058 (OCH_3)	1.57 (s, CH_3, 3H), 2.40 (m, CH_2, 1H) 2.57 (m, CH_2, 1H), 2.91 (m, CH_2, 1H), 3.05 (m, CH_2, 1H), 3.85 (s, OCH_3, 3H), 3.90 (s, OCH_3, 3H), 4.01 (dd, CH, 1H), 6.85 (s, ArH, 1H), 6.86 (s, ArH, 1H), 7.09 (s, NH, 1H), 7.10 (d, ArH, 2H), 7.33 (d, ArH, 2H),	73.29 73.51	6.80 6.82	4.50 4.49	311
DC-20	3324 (NH), 1656 (C=O),	2.42 (m, CH_2, 1H) 2.58 (m, CH_2, 1H), 2.93 (m, CH_2, 1H), 3.07 (m, CH_2, 1H),	63.15 62.94	5.30 5.28	8.18 8.21	342

	1264, 1077 (OCH_3)	3.87 (s, OCH_3, 3H), 3.90 (s, OCH_3, 3H), 4.07 (dd, CH, 1H), 6.78 (s, ArH, 1H), 6.80 (s, ArH, 1H), 7.13 (s, NH, 1H), 7.86 (d, ArH, 2H), 8.09 (d, ArH, 2H),				
DC-22	3321 (NH), 1653 (C=O), 1269, 1072 (OCH_3)	2.41 (m, CH_2, 1H) 2.56 (m, CH_2, 1H), 2.88 (m, CH_2, 1H), 3.05 (m, CH_2, 1H), 3.85 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 4.05 (dd, CH, 1H), 6.77 (s, ArH, 1H), 6.79 (s, ArH, 1H), 7.08 (s, NH, 1H), 7.65 (d, ArH, 2H), 8.38 (d, ArH, 2H)	68.44 68.63	6.08 6.10	9.39 9.43	298

5.2.3. RESULTS AND DISCUSSION:

The starting reagent for the preparation of the key intermediate, 5,6-dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)carboxylic acid was vanillin. The hydroxyl group of vanillin was methylated by dimethyl sulphate under alkaline conditions to get the methylated product veratraldehyde as detailed earlier. The veratraldehyde obtained was reacted with diethylmalonate in presence of catalytic amount of piperidine, an organic base. The base, piperidine, abstracts the acidic methylene proton from the diethylmalonate. The nucleophile thus generated attacks the carbonyl carbon leading to the formation of benzylidene malonate (**V-XII**) after elimination of one molecule of water (Figure 5.15).

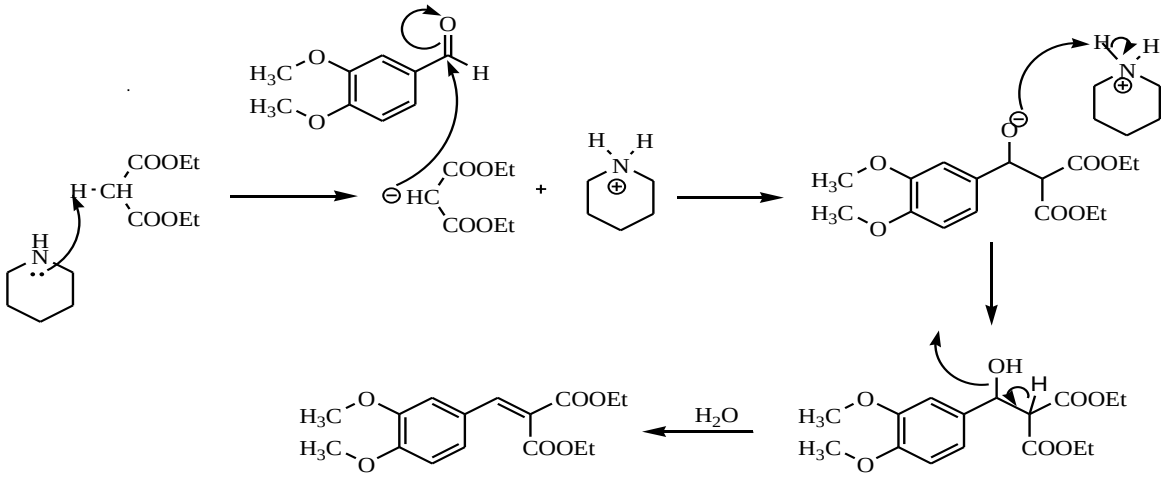


Figure 5.15. Possible reaction mechanism for the synthesis of compound **V-XII**

The IR spectrum of compound V-XII shows the C=O stretching at 1718 cm^{-1} indicating the presence carbonyl group. In ^{1}H-NMR the benzylidene proton is observed far downfield at 4.80 ppm, methoxyl protons at 3.83 and 3.85 ppm and aromatic protons are observed at 6.89-

7.11 ppm. For the side chain of diethyl malonate two triplets (1.26 & 1.29) and two quartet (4.24 & 4.30) are observed at slightly different δ value due to the presence of same protons in slightly different environment as shown in Figure 5.16

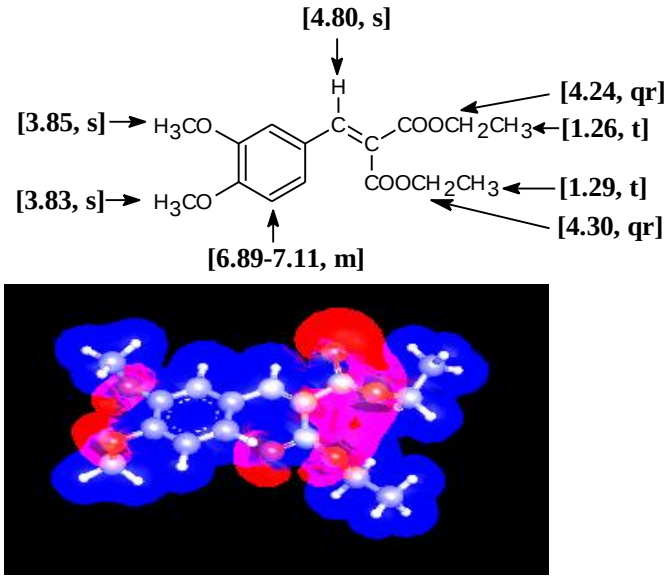


Figure 5.16. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) and electrostatic potential map of compound **V-XII**

The benzylidene malonate (**V-XII**) obtained was reacted with sodium cyanide when cyanosation took place at the double bond to give compound **V-XIII**. The mechanism of reaction is shown in Figure 5.17.

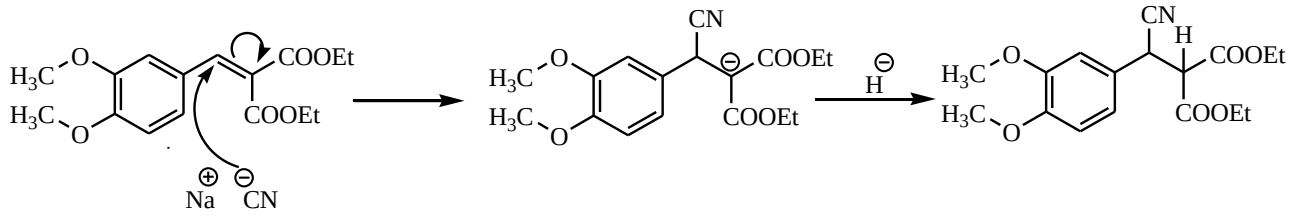


Figure 5.17. Possible reaction mechanism for the synthesis of compound **V-XIII**

The cyano group and the two ester groups were further hydrolyzed with 6N potassium hydroxide solution. The diacid thus obtained got converted into potassium salt due to excess potassium hydroxide in the reaction mixture. This potassium salt was neutralized with dilute hydrochloric acid under ice cold conditions to liberate the free diacid **V-XIV** (Figure 5.18).

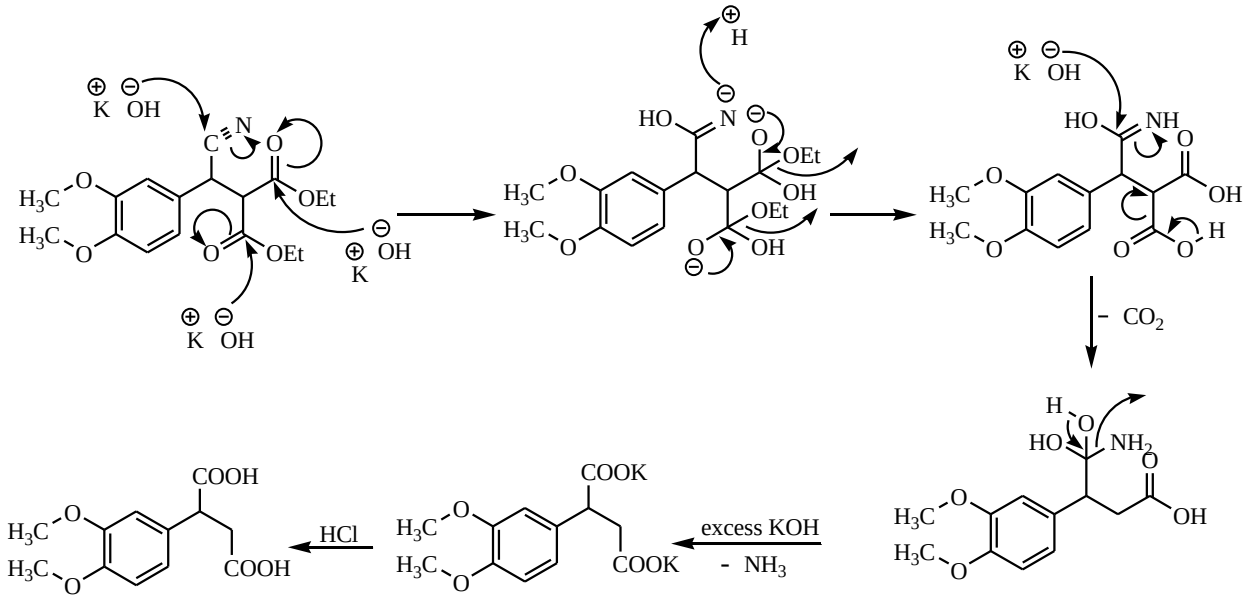


Figure 5.18. Possible reaction mechanism for the synthesis of compound **V-XIV**

The broad OH stretch at 3400-2400 cm^{-1} and C=O stretch at 1700 cm^{-1} indicated the presence of carboxylic group. The proton NMR showed broad singlet at 11.19 and 11.26 ppm indicating the presence of two carboxylic groups. Due to different configurational arrangements of protons on carbon 1 and 2 (as shown in figure) two doublets of doublets and single doublets of doublets were obtained for CH_2 and CH of succinic acid side chain respectively (Figure 5.19).

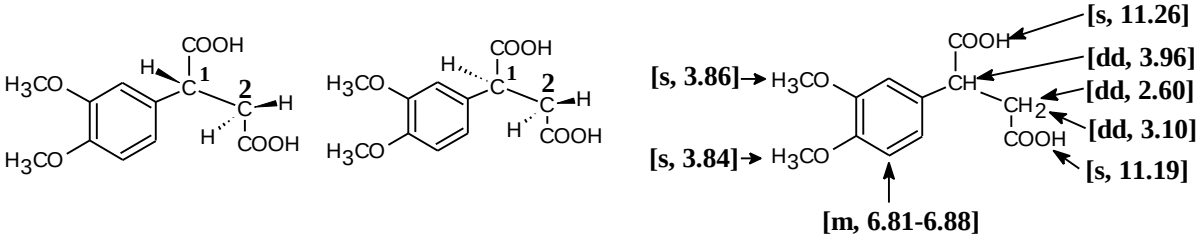


Figure 5.19. ^1H- NMR:δ (ppm) ($CDCl_3$, TMS) of compound **V-XIV**

The compound V-XIV was also synthesized through acrylate route[163] where **V-II** was reacted with ethylcyanoacetate in the presence of an organic base. The cyanoacrylate thus obtained was cyanosated to form dicyanopropanoate, which was further hydrolyzed to the required succinic acid **V-XIV.** However**,** the yields obtained by the malonate route were much better than the acrylate route.

The diacid (**V-XIV**) was treated with polyphosphoric acid when intramolecular Friedel Craft's cyclization took place to form a keto acid. The reaction mixture is poured onto ice water to decompose the excess of polyphosphoric acid. The reaction mechanism is similar to as shown in Figure 5.6. In case of synthesis of keto acid (**V-XV**) more PPA was required as compared to keto acid (**V-V**) because of lesser reactivity of succinic acid (**V-XIV**) towards cyclization. The compound **V-XIV** has only one COOH group available whereas in case of **V-V** both COOH groups are separated from the central ring by two carbons and hence are available for cyclization.

The IR spectrum of compound **V-XV** shows broad OH stretch at 3400-2200 and C=O stretching at 1726 and 1650cm^{-1} indicating the presence of carboxylic group, carbonyl of acid and carbonyl of keto functionalities respectively. The proton NMR showed two doublets of doublets for CH_2 group and a doublet of doublet for CH group. As shown in Figure 5.20 this appearance of signals at slightly different δ values was due to different arrangements of protons on carbon 1 and 2. The broad singlet at 10.34 indicated the presence of acidic proton of carboxylic acid group. The two aromatic protons showed singlets at δ values of 7.16 ppm and 7.21 ppm

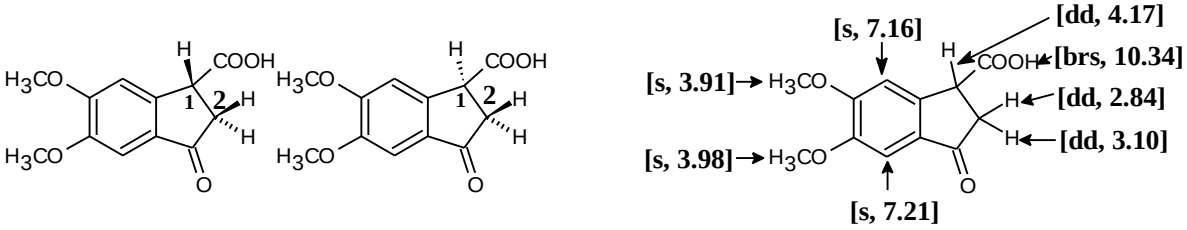


Figure 5.20. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XV**

The keto group was finally removed by Clemmenson's reduction to obtain the final 5,6-dimethoxy indan(-1-yl)carboxylic acid. The IR spectrum of this compound showed broad OH stretch at 3400-2200 and C=O stretching at 1700 indicating the presence of carboxylic group. The proton NMR showed two singlets at 3.86 and 3.87 equivalent to six protons indicating the presence of two methoxyl groups. The broad singlet equivalent to one proton indicated the presence of carboxylic group. Two multipets were obtained for each of the two CH_2 groups (carbon 2 and 3) and a doublet of doublet for CH (carbon 1) of the indan ring system (Figure 5.21). Such splitting pattern was observed due to different configurational arrangement of protons making the protons present on the same carbon to behave differently (in different environments)

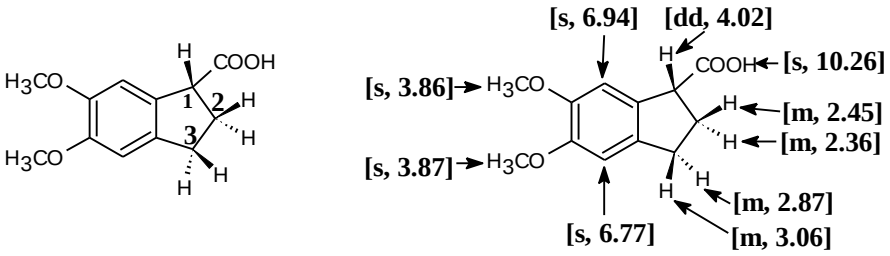


Figure 5.21. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XVI**

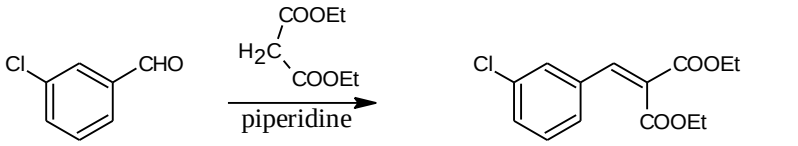
The acid obtained was converted to amides via acyl halide under Schotten- Baumann conditions.

The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides respectively. Also disappearance of broad OH stretch at 3400-2400 cm^{-1} indicates the formation of amide bond. The formation of amides was further confirmed by presence of a small singlet at 5-6 ppm (aliphatic amide derivatives) and at 7-8 ppm (aromatic amides) in ^{1}H-NMR spectrum. In case of many aromatic amides the amide peak was overlapped with the aromatic peaks around 7.1- 7.3 ppm. In the mass spectrum of compound **V-XVI**, base peak was observed at 177 and M^+ peak at 222. However in amides M^+ peak was observed as the base peak.

5.4. (6-CHLORO-2, 3-DIHYDRO-1H-INDEN-1- YL)CARBOXYLIC ACID

5.4.1. SYNTHESIS

5.4.1.1. Diethyl (3-chlorobenzylidene)malonate (V-XVII).

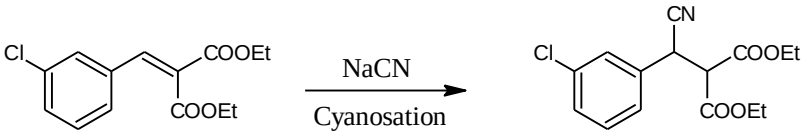


3-chlorobenzaldehyde
V-VII

diethyl (3-chlorobenzylidene)malonate
V-XVII

To 14.05g of m-chlorobenzaldehyde (0.1mol) in a dry round bottomed flask 20g of diethylmalonate (0.104 mol) was added. To this reaction mixture 1ml of piperidine, 1ml of glacial acetic acid and 100ml of dry benzene was added. The reaction mixture was refluxed in a Dean Stark's apparatus (for nearly 18hrs) until no more water collects. The reaction mixture was washed with water to remove the water soluble reactants and then it was dried over anhydrous sodium sulphate. The solvent (benzene) was removed from the reaction mixture under reduced pressure. The remaining brownish oily liquid was set for vacuum distillation. The product benzylidene malonate was distilled at 172-175 °C at 2mm Hg as a clear liquid. The overall yield was 80-85%. The IR spectrum (thin film) showed C-Cl stretch at 661cm^{-1} and C=O stretch at 1713cm^{-1}.

5.4.1.2. Diethyl [cyano(3-chlorophenyl)methyl]malonate (V-XVIII).

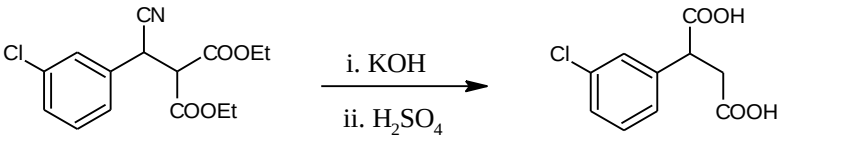


diethyl (3-chlorobenzyl idene)malonate
V-XVII

diethyl [cyano(3-chloro phenyl)methyl]malonate
V-XVIII

To 22.86 g of benzylidene malonate (0.1mol) in a dry round bottomed flask 5g of sodium cyanide (0.102 mol) dissolved in 90% alcohol was added. The reaction mixture was refluxed for 18 hrs at 100 °C on water bath and then alcohol was distilled out at reduced pressure. The formed cyanomalonate was not purified and was used directly for the next step.

5.4.1.3. 2-(3-chlorophenyl)succinic acid (V-XIX).



diethyl [cyano(3-chloro phenyl)methyl]malonate

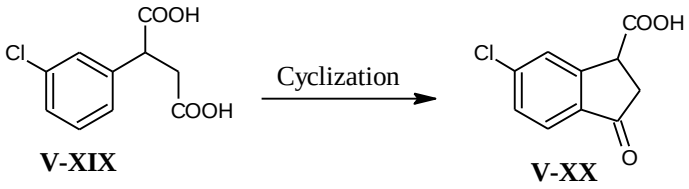
V-XVIII

2-(3-chlorophenyl)succinic acid

V-XIX

To the above reaction mixture 25.2g of KOH dissolved in 75ml of water (6N, 0.45mol) was added. The reaction mixture was refluxed for 16 hrs and then was allowed to cool. The cooled reaction mixture was slowly poured onto ice-sulphuric acid mixture with stirring when the diacid precipitated out. The crude product obtained was filtered, dried and recrystallized from hot water to get the desired succinic acid derivative. Yield 70-75%; mp 163-164°C; IR (cm^{-1}): 3400-2400(br OH stretch), 1703(C=O stretching), 641 (C-Cl stretch)

5.4.1.4. (6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XX)



V-XIX **V-XX**

This reaction was tried using various reagents under varying reaction conditions. Following are the different cyclization reagents used for carrying out this intramolecular cyclization.

1. Phosphorous pentoxide
2. Methanesulphonic acid
3. Mixture of phosphorous pentoxide and methanesulphonic acid
4. Polyphosphoric acid(1:1)
5. Polyphosphoric acid(1:1.8)
6. Anhydrous aluminium chloride / sodium chloride
7. Acyl halide / anhydrous aluminium chloride in nitrobenzene as a solvent
8. Acyl halide / anhydrous aluminium chloride in dichloromethane as a solvent
9. Sulphuric acid
10. Heteropolyacid (Phosphotungstic acid)

These reactions were tried at various temperatures for varying durations of time. In most of the cases the starting acid (**V-XIX**) was recovered back and if drastic reaction conditions

(higher temperatures) were applied it resulted in charring of the compound. Taking into consideration the reaction mechanism for cyclization it can be seen that the electron withdrawing chloro group at meta position deactivates the ring system towards the intramolecular cyclisation (electrophilic substitution) as shown in Figure 5.22. Also in comparison to glutaric acid side chain the chances of cyclization are halved in case of succinic acid side chain.

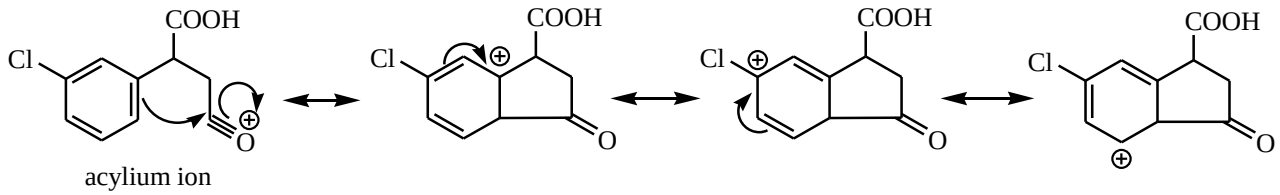


Figure 5.22. Mechanism for hindered cyclization of compound **V-IX**

It has been reported that ring closure of β-m- and - β-p-nitrophenylglutaric acid failed to undergo ring closure with (a) aluminium chloride in carbon disulphide or (b) aluminium chloride in nitrobenzene at 150°C. This free acid also failed to undergo ring closure with anhydrous hydrogen fluoride[149]. It has been reported that for cyclization of phenylsuccinic acid, the corresponding acyl halide was treated with aluminium chloride in nitrobenzene to yield (60%) indan-3-one-1-carboxylic acid. However the cyclization of phenylsuccinic acid was achieved in 22% yield by use of concentrated sulfuric acid[164].

5.5. 5-(CYCLOPENTYLOXY)-6-METHOXY-INDAN-1-ALKANOIC ACIDS.

Literature survey reveals that (17) 6-chloro-5-(cyclopentylmethyl)-indan-1-carboxylic acid, an isomer of 6-chloro-5-cyclohexylindan-1-carboxylic acid (clidanac), was found to be non-ulcerogenic after acute and chronic treatment in rats and monkeys. This compound did not show any mucosal damage in the rat stomach after single oral application up to highest tested dose of 400mg/kg p.o. whereas clidanac has been shown to be ulcerogenic in anti-inflammatory active doses (≤ 3mg/kg). Also this cyclopentyl methyl compound did not exhibit any gastrointestinal toxicity in rats and monkeys during chronic daily drug treatment upto 400 mg / kg p.o. for 4 weeks. This prompted us to synthesize and evaluate the activity of 5-(cyclopentyloxy)-6-methoxy-indan-1-alkanoic acid (an isostere of 6-chloro-5-

(cyclopentylmethyl)-indan-1-carboxylic acid) and its amide derivatives as potential nonsteroidal anti-inflammatory compounds with lesser ulcerogenecity.

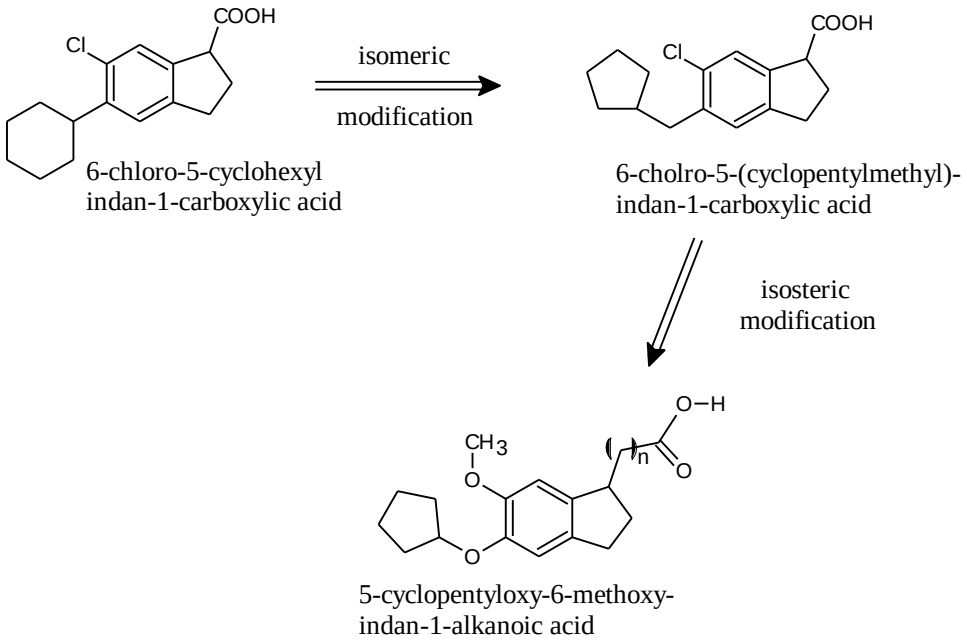
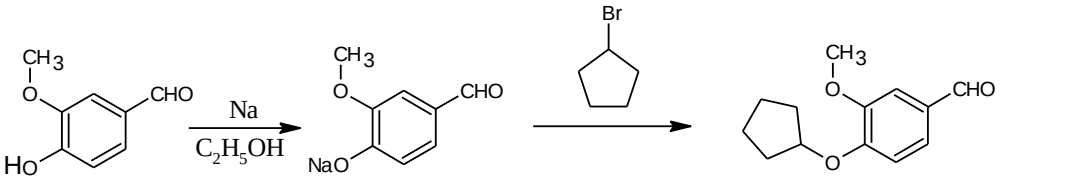


Figure 5.23. Design of 5-cyclopentyloxy-6-methoxy-indan-1-alkanoic acid

5.5.1 SYNTHESIS OF (5-CYCLOPENTYLOXY -6-METHOXY-2, 3-DIHYDRO-1*H*-INDEN-1- YL) ACETIC ACID

5.5.1.1. 5-cyclopentyloxy-6-methoxybezaldehyde[165] (V-XXII).



4-hydroxy-3-methoxybenzaldehyde
I

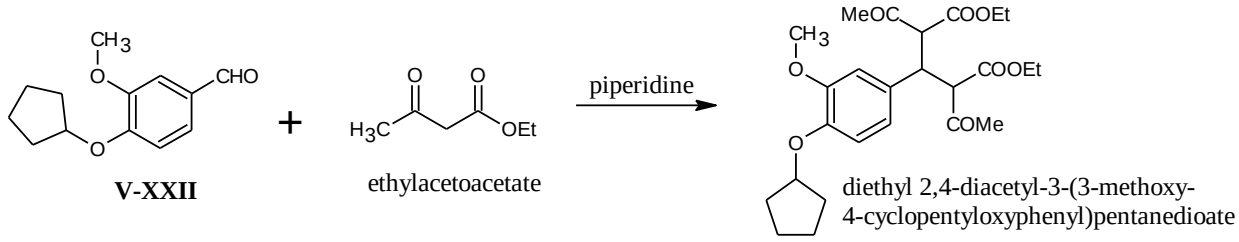
Sodium vanillate
V-XXI

4-(cyclopentyloxy)-3-methoxybenzaldehyde
V-XXII

Clean sodium (1.55g, 0.067mol) was placed in a 250ml of round bottomed flask equipped with a reflux condenser. To this 40ml of absolute alcohol was added. Once the vigorous reaction has subsided, the flask was warmed on water bath until the whole amount of sodium went into solution. The flask was cooled and to this 10g (0.066mol) of vanillin (**I**) dissolved

in alcohol was added. After refluxing the reaction mixture on water bath for 30min sodium vanillate (**V-XXI**) separated out. Then alcohol was distilled out and a sufficient quantity of dimethylformamide was added to sodium vanillate until a clear solution was obtained. To this solution cyclopentyl bromide (0.1mol) was slowly added under ice cold conditions. The reaction mixture was heated on water bath for six hours and finally poured onto 150ml of water. The system was kept protected with a calcium chloride guard tube through out the reaction. It was extracted with two 100ml portions of benzene. The combined benzene extracts was washed with three portions of 10% sodium hydroxide solution. Benzene and the unreacted cyclopentyl bromide was removed under reduced pressure and the residue was further distilled at 138-140 °C at 0.4mm Hg to get 5-cyclopentyloxy-6-methoxy benzaldehyde (**V-XXI**) in 45-50% yield, IR (cm^{-1}) (thin film): 1680 (C=O stretching) and 1037, 1264 (C-O stretch of ether linkage).

5.5.1.2. Diethyl 2,4-diacetyl-3-(5-cyclopentyloxy-6-methoxy)pentanedionate (V-XXIII)



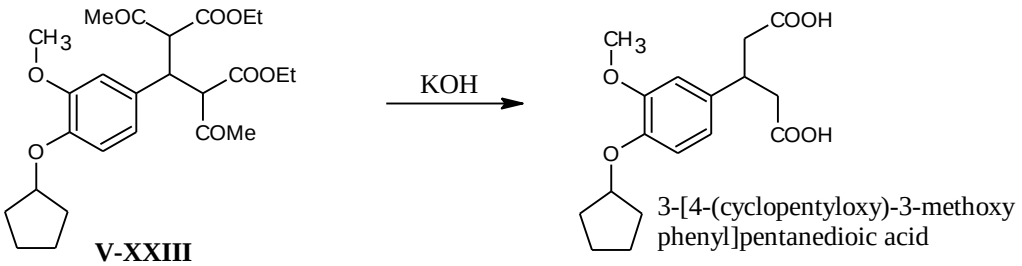
V-XXII ethylacetoacetate

diethyl 2,4-diacetyl-3-(3-methoxy-4-cyclopentyloxyphenyl)pentanedioate

V-XXIII

5-cyclopentyloxy-6-methoxy benzaldehyde (11g, 0.05 mol) was dissolved in ethylacetoacetate (15.6g, 0.12mol) in a dry conical flask and piperidine (0.85 ml) was added slowly at ambient temperature and then kept for 3 days or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product in 50-55% yield[14]. Recrystallization from dil. alcohol gave the analytical product, mp 142-144 °C IR (cm^{-1}) (KBr): 1720 (C=O stretching), 1036, 1260 (C-O stretch of ether linkage).

5.5.1.3. 3-(5-cyclopentyloxy-6-methoxy)pentanedioic acid (V-XXIV).



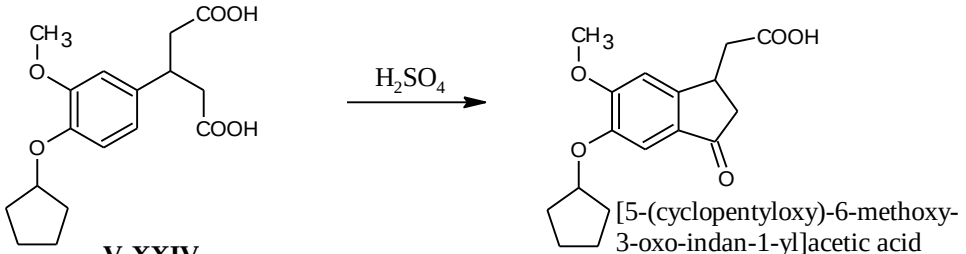
V-XXIII

3-[4-(cyclopentyloxy)-3-methoxy phenyl]pentanedioic acid

V-XXIV

Compound **V-XXIII** (9.24g, 0.02mol) was dissolved in a hot solution of KOH (37g in 37 ml of water) and 74ml of absolute alcohol was added. The hot reaction mixture was refluxed on a water bath for 1 hour. Alcohol was then removed by distillation, and after dilution with water the reaction mixture was cooled and washed with solvent ether. The aqueous layer on acidification with cold conc. HCl with cooling gave crude diacid which was filtered and recrystallized from hot water [14]. Yield 70-75 %, mp 131-132°C, IR (cm^{-1}) (KBr): 3400-2600 (O-H stretch), 1730 (C=O stretching), 1261, 1022 (C-O stretch of ether linkage). ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.62 (m, CH_2, 2H), 1.85 (m, CH_2, 6H), 2.69 (dd, CH_2, 2H), 2.73 (dd, CH_2, 2H), 3.67 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 4.73 (m, CH, 1H), 6.71-6.83 (m, ArH, 3H), 10.11 (brs, COOH, 1H), 10.25 (brs, COOH, 1H)

5.5.1.4. (5-cyclopentyloxy-6-methoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid[166](V-XXV).



V-XXIV

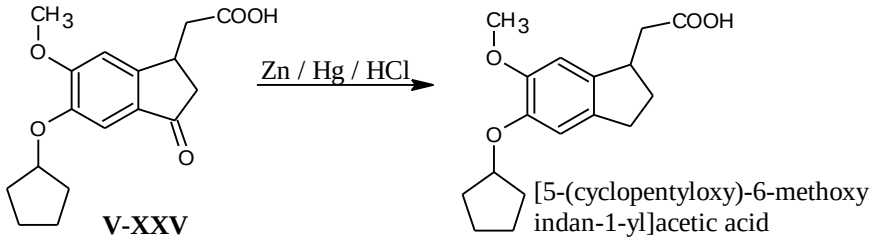
[5-(cyclopentyloxy)-6-methoxy-3-oxo-indan-1-yl]acetic acid

V-XXV

The substituted glutaric acid **V-XXIV** (5g, 0.0155 mol) was taken in a round bottomed flask. To this 12.4 ml of concentrated sulphuric acid (15times) was added. The flask was fitted with a calcium chloride guard tube and was left overnight at room temperature. The solution was poured onto ice and extracted with ether for 2-3 times. The ethereal extract was washed with water to remove any sulphonated products and finally dried and distilled to yield the crude keto product (V-XXV). Yield 55-60%, mp 232-234 °C, IR (cm^{-1}) (KBr): 3400-2400 (O-H stretch), 1721, 1640 (C=O stretching), 1245, 1033 (C-O stretch of ether linkage); ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS): 1.63 (m, CH_2, 2H), 1.87 (m, CH_2, 6H), 2.43, 2.67 (dd, CH_2, 2H), 2.73,

2.99 (dd, CH_2, 2H), 3.71(qn, CH, 1H), 3.84 (s, OCH_3, 3H), 4.78 (m, CH, 1H), 6.89 (s, ArH, 1H), 7.31 (s, ArH, 1H), 10.28 (brs, COOH, 1H).

5.5.1.5. (5-cyclopentyloxy-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid (V-XXVI).



V-XXV

[5-(cyclopentyloxy)-6-methoxy indan-1-yl]acetic acid

V-XXVI

The keto compound, **V-XXV,** was subjected to Clemmensen's reduction to obtain the title compound. The keto acid (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on a steam bath for about 8 hrs (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free from acidity. After drying over anhydrous sodium sulfate it was finally distilled to get the reduced acid **V-XXVI**. The analytical product was obtained upon recrystallization from benzene. Yield 65-70 %; mp 114-115^oC; IR (cm^{-1}): 3400-2400 (O-H stretch), 1724 (C=O stretching), 1251, 1036 (C-O stretch of ether linkage). ^1H- NMR: δ (ppm) ($CDCl_3$, TMS): 1.59 (m, CH_2, 2H), 1.81 (m, CH_2, 6H), 1.75, 2.33 (m, CH_2, 2H), 2.39, 2.63 (dd, CH_2, 2H), 2.81 (m, CH_2, 2H), 3.67 (qn, CH, 1H), 3.85 (s, OCH_3, 3H), 4.67 (m, CH, H), 6.81 (s, ArH, 1H), 6.85 (s, ArH, 1H), 10.21 (brs, COOH, 1H).

5.5.2. RESULTS AND DISCUSSION

The compound **I** was converted to compound **V-XXII** by Williamson's synthesis. The reaction is a nucleophilic substitution reaction where the phenolic oxygen attacks the electron deficient carbon of cyclopentyl bromide, leading to formation of **V-XXIII** by elimination of bromine as sodium bromide (Figure 5.24). As the alkyl halide involved in this reaction was secondary in nature the yield obtained for the compound **V-XXIII** was comparatively less. The reaction was tried for longer durations (maximum of 36 hrs), under varying reaction

conditions including microwave and phase transfer catalysis, but the yields could not be improved further.

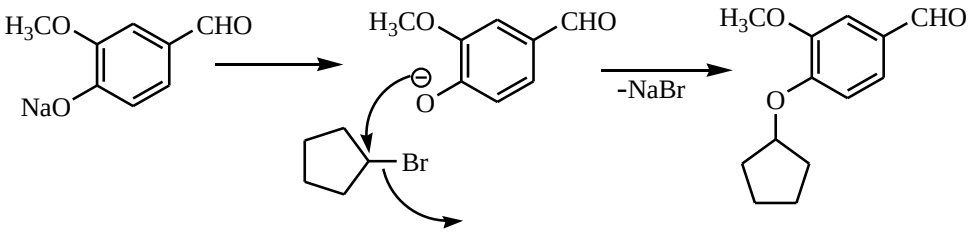


Figure 5.24. The reaction mechanism for the synthesis of compound **V-XXII**

The IR spectrum showed C=O stretching 1680 cm^{-1} indicating the presence of carbonyl group and also C-O stretch of ether linkage 1037 and 1264 cm^{-1}. Absence of broad O-H stretch also supported the structure of **V-XXIII.**

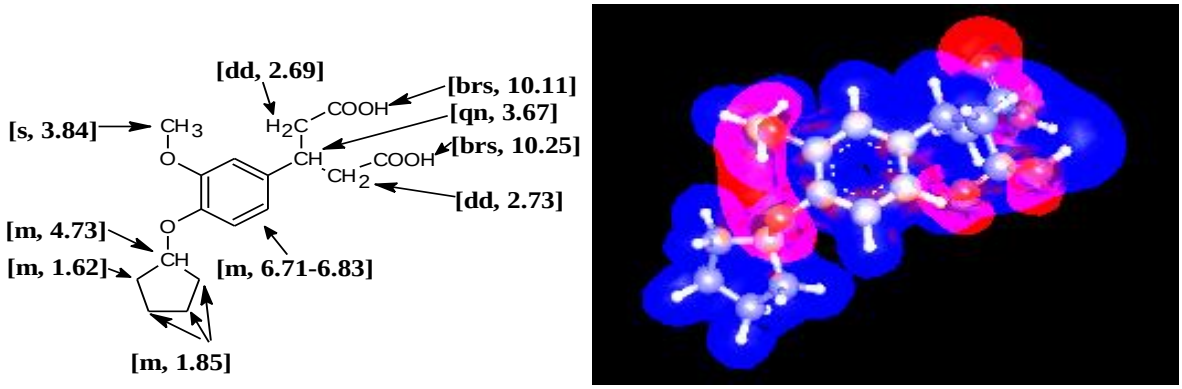


Figure 5.25. ^{1}H- NMR:δ (ppm) ($CDCl_3$, TMS) and electrostatic potential map of compound **V-XXV**

The compound **V-XXIII** was converted into the benzylic bisacetoacetate **V-XXIV** under the reaction conditions as explained earlier. The IR spectra showed C=O stretching at 1720 cm^{-1}corresponding to carbonyl of an ester group and C-O stretch of ether linkage at 1036 and 1260 cm^{-1}. The hydrolysis of compound **V-XXIV** gave the glutaric acid derivative **V-XXV.** The IR spectrum shows a broad O-H stretch at 3400-2600cm^{-1} indicating the formation of carboxylic group. The ^{1}H-NMR spectrum of compound shows small broad singlets at 10.11 and 10.25 δ values indicating the presence two carboxylic acid protons. The two CH_2 groups in the glutaric acid side chain of the compound **V-XXV** give two doublet of doublet at slightly different δ values. This splitting pattern was observed due to equatorial and axial arrangement of proton on CH of glutaric acid side chain of the compound **V-XXV.** The CH of

cyclopentyloxy ring gives multiplet at higher δ value. Because of the existence of axial and equatorial arrangements of protons in a rigid cyclopentane ring, the multiplets are observed for all the protons as shown in Figure 5.25.

The compound **V-XXV** was cyclized to from the keto compound **V-XXVI** by treating it with sulfuric acid. Sulfuric acid being a dehydrating agent has been reported as cyclizing agent for phenylglutaric acid. The yields were comparatively low because of the probability of formation of sulfonated by products. This reaction was also tried with polyphosphoric acid and aluminium chloride at different temperatures and varying duration of time. However the cyclization could not be achieved by these methods.

The mechanism of the reaction (Figure 5.26) may be as follows:

(a) Sulfuric acid leads to the formation of anhydride.

(b) The anhydride adds on sulfuric acid.

(c) Ring formation occurs owing to the production of sulfuric acid from appropriate nuclear hydrogen atom

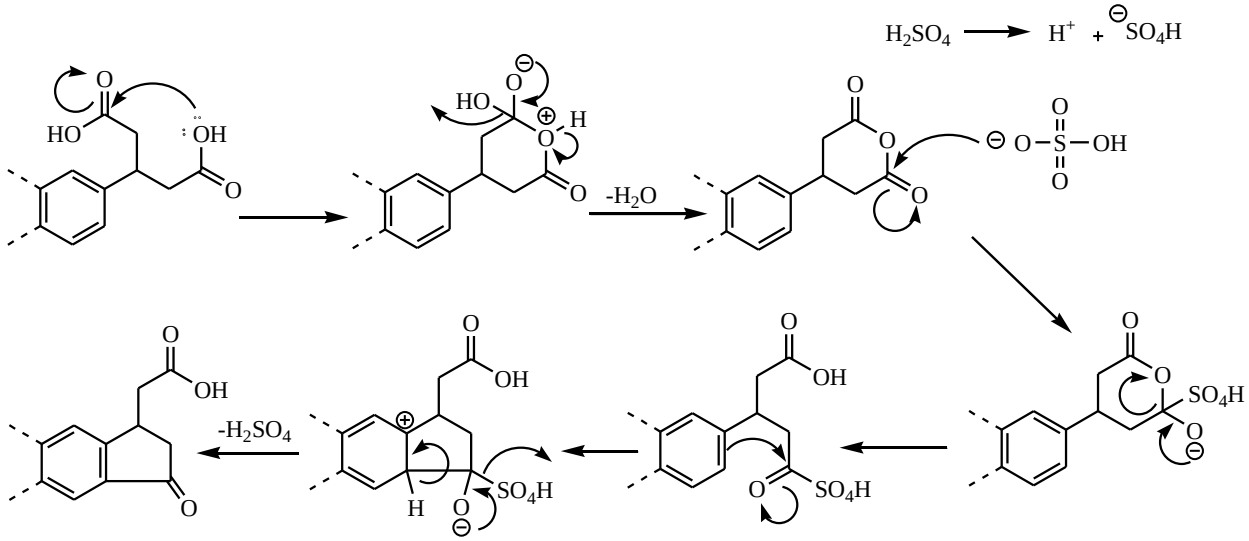


Figure 5.26. Possible reaction mechanism for synthesis of **V-XXVI**

The IR spectra showed broad O-H stretch at 3400-2400 cm^{-1} indicating the presence of free carboxylic group. The C=O stretch was obtained at 1721 and 1640 corresponding to carbonyl of acid group and the keto goup introduced after cyclization respectively. Presence of quintet for CH group and doublet of doublet for the neighbouring CH_2 groups indicated the ring closure as shown in the Figure 5.27.

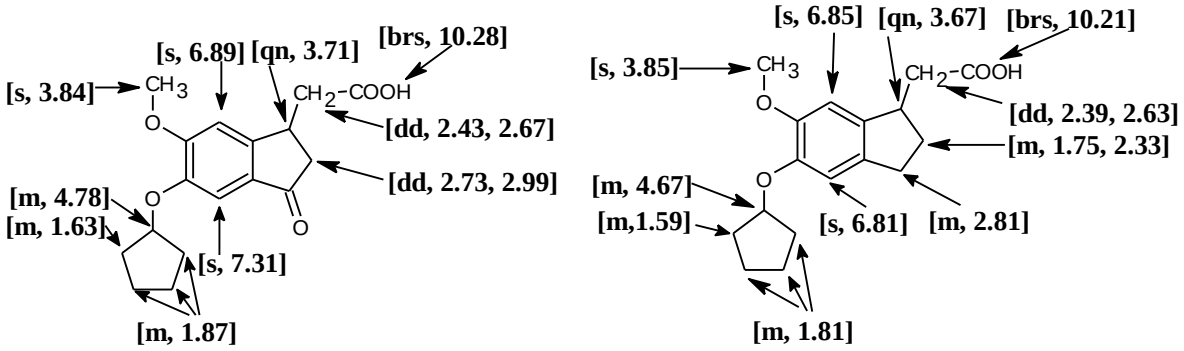
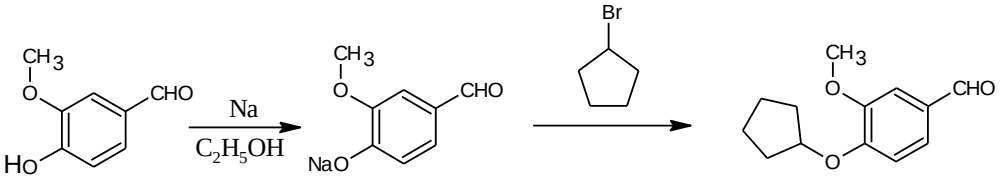


Figure 5.27. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XXVI and V-XXVII**

The keto group of compound **V-XXVI** was reduced to methylene by Clemmensen's reduction; converting indanone to indan **V-XXVII**. The IR spectra showed broad O-H stretch and C=O stretch in confirmation of the structure. The NMR spectra as shown in Figure 5.27 further support the proposed structure of V-**XXVII.**

5.5.3. SYNTHESIS OF (5-CYCLOPENTYLOXY -6-METHOXY-2, 3-DIHYDRO-1*H*-INDEN-1- YL) CARBOXYLIC ACID

5.5.3.1. 5-cyclopentyloxy-6-methoxybezaldehyde (V-XXII).



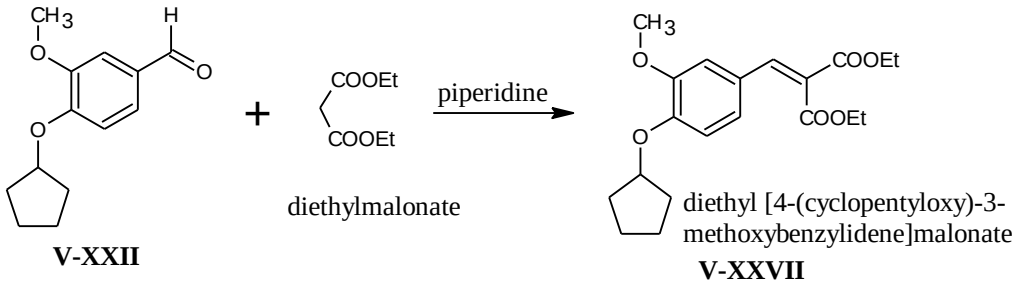
4-hydroxy-3-methoxybenzaldehyde **I**

Sodium vanillate **V-XXI**

4-(cyclopentyloxy)-3-methoxybenzaldehyde **V-XXII**

The methodology adopted for synthesis of this compound has been described in section 5.5.1.1.

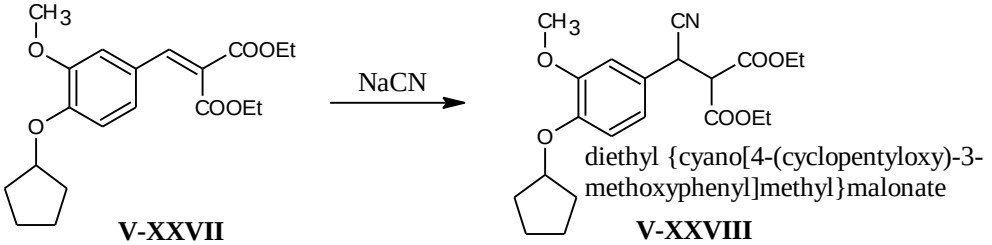
5.5.3.2. Diethyl (4-cyclopentyloxy-3-methoxy benzylidene)malonate (V-XXVII).



V-XXII diethylmalonate diethyl [4-(cyclopentyloxy)-3-methoxybenzylidene]malonate **V-XXVII**

To 11 g (0.05mol) of 5-cyclopentyloxy-6-methoxy benzaldehyde, **V-XXII,** in a dry round bottomed flask 8.5 g (0.053 mol) of diethylmalonate was added. To this reaction mixture 0.5ml of piperidine, 0.5ml of glacial acetic acid and 50ml of dry benzene was added. The reaction mixture was refluxed for about 18 hrs in Dean Stark's apparatus. The reaction was carried until no more water collects in the Dean Stark's apparatus. The reaction mixture was washed with water to remove the added piperidine and acetic acid. After drying over anhydrous sodium sulfate benzene was removed from the reaction mixture under reduced pressure. The remaining brownish oily liquid was set for vacuum distillation. The benzylidene malonate, **V-XXVII,** was distilled at 215-220 °C at 0.5mm Hg as a clear liquid. The overall yield was 85-90%, IR (cm^{-1}) (thin film): 1710 (C=O stretching), 1038, 1252 (C-O stretch of ether linkage), 1620 (C=C stretch); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.31 (t, CH_3, 3H), 1.34 (t, CH_3, 3H), 1.62 (m, CH_2, 2H), 1.89 (m, CH_2, 6H), 3.83 (s, OCH_3, 3H), 4.29 (qr, CH_2, 2H), 4.35 (qr, CH_2, 2H), 4.80 (m, CH, 1H), 6.85 (d, ArH, 1H), 7.02(s, ArH, 1H), 7.05(d, ArH, 1H), 7.64 (s, CH, 1H).

5.5.3.3. Diethyl [cyano(4-cyclopentyloxy-3-methoxyphenyl) methyl] malonate (V-XXVIII).

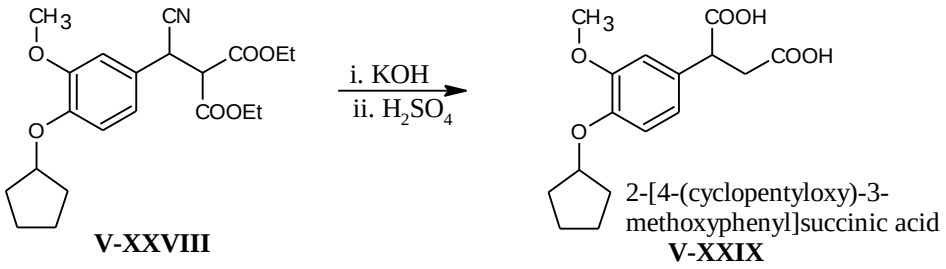


V-XXVII diethyl {cyano[4-(cyclopentyloxy)-3-methoxyphenyl]methyl}malonate **V-XXVIII**

To benzylidene malonate, **V-XXVII,** (9 g, 0.025mol) in a dry round bottomed flask 1.3g (0.026 mol) of sodium cyanide dissolved in 90% alcohol was added. The reaction mixture

was refluxed for 18 hrs at 100 °C and then alcohol was distilled out at reduced pressure. The formed cyanomalonate **V-XXVIII** was not purified and was used directly in the next step.

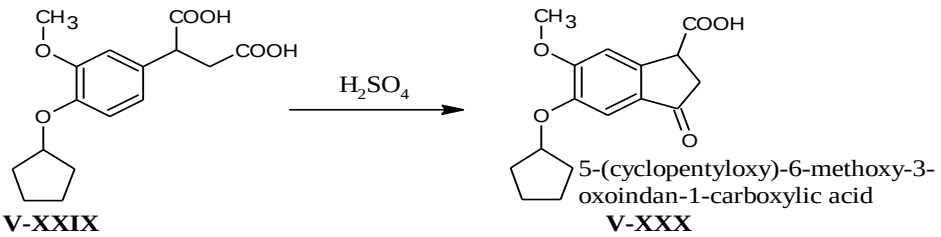
5.5.3.4. 2-(4-cyclopentyloxy-3-methoxy phenyl)succinic acid (V-XXIX).



V-XXVIII **V-XXIX**

To the above reaction mixture (vide 5.5.2.3) 7.4g of KOH dissolved in 22ml of water (6N, 0.45mol) was added. The reaction mixture was refluxed for 16 hrs and then allowed to cool. The cooled reaction mixture was slowly poured on to calculated amount of ice-sulphuric acid mixture with stirring when the diacid precipitated out. The crude product obtained was filtered, dried and recrystallised from hot water to get the desired succinic acid. Yield 75-80%; mp 171-173°C; IR (cm^{-1}) (KBr): 3400-2400 (br OH stretch), 1700(C=O stretching), 1040, 1248 (C—O stretch ether linkage); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.60 (m, CH_2, 2H), 1.79 (m, CH_2, 4H), 1.88 (m, CH_2, 2H), 2.54 (dd, CH_2, 1H), 3.01 (dd, CH_2, 1H), 3.79 (s, OCH_3, 3H), 3.86 (dd, CH, 1H), 4.73 (m, CH, 1H), 6.79-6.83 (m, ArH, 3H), 10.81 (brs, COOH, 1H), 10.92 (brs, COOH, 1H).

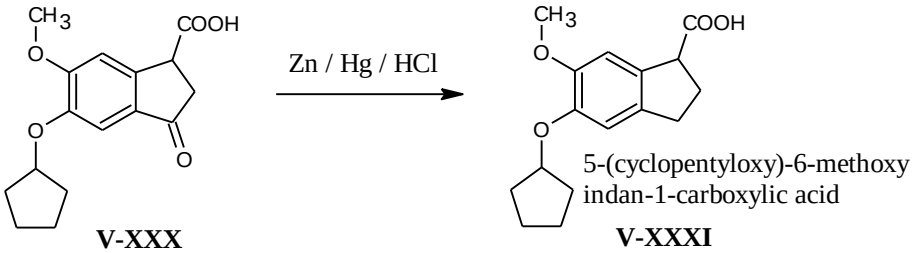
5.5.3.5. (5-cyclopentyloxy-6-methoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XXX).



V-XXIX **V-XXX**

The appropriate succinic acid **V-XXIV** (5g, 0.0162 mol) was taken in a round bottomed flask. To this 21.6 ml of concentrated sulphuric acid (25times) was added. The flask was fitted with a calcium chloride guard tube and was left overnight at room temperature. The solution was poured onto ice and extracted with ether for 2-3 times. The ethereal extract was washed with water to remove any sulphonated product and inorganic acid and finally dried and distilled to yield the crude keto product **V-XXX** which was recrystallized from dilute alcohol. Yield 65-70% mp 125-127 ^{o}C, IR (cm^{-1}) (KBr): 3500-2400 (br OH stretch), 1703, 1668 (C=O stretching), 1042, 1263 (C—O stretch ether linkage); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.61 (m, CH_2, 2H), 1.84 (m, CH_2, 6H), 2.91, 3.23 (dd, CH_2, 2H), 4.18 (dd, CH, 1H), 3.83 (s, OCH_3, 3H), 4.77 (m, CH, 1H), 7.10 (s, ArH, 1H), 7.19 (s, ArH, 1H), 10.31 (brs, COOH, 1H).

5.5.3.6. (5-cyclopentyloxy-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XXXI).



V-XXX **V-XXXI**

Clemmensen's reduction was employed to obtain the title compound. The keto acid **V-XXX** (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on a steam bath for about 8 hrs (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free of acidity. After drying over anhydrous sodium sulfate it was distilled to get the reduced acid V-XXXI. The analytical product was obtained on recrystallization from benzene. Yield 70-75%; mp 94-96^{o}C (benzene); IR (cm^{-1}) (KBr): 3600-2400 (br OH stretch), 1720 (C=O stretching), 1047, 1262 (C—O stretch ether linkage) ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.62 (m, CH_2, 2H), 1.85 (m, CH_2, 6H), 2.33, 2.51 (m, CH_2, 2H), 2.91 (m, CH_2, 2H), 3.84 (s, OCH_3, 3H), 3.94 (dd, CH, 1H), 4.65 (m, CH, 1H), 6.79 (s, ArH, 1H), 6.91 (s, ArH, 1H), 10.28 (brs, COOH, 1H).

5.5.4. RESULTS AND DISCUSSION

The hydroxyl group of vanillin was changed to cyclopentyloxy group by Williamson's synthesis. The aldehyde group of compound **V-XXII** was reacted with diethyl malonate in the presence of base to form benzylidene malonate **V-XXVII**. The reaction mechanism is the same as described in the Figure 5.23. The IR and the NMR spectra also confirm the structure of compound **V-XXVII**. The protons on CH of benzylidene part of **V-XXVII** being most desheilded give signal far downfield at δ value of 7.64 ppm as shown in the Figure 5.28.

The malonate **V-XXVII** was cynosated at the double bond and then hydrolyzed to succinic acid derivative **V-XXIX**. The broad O-H signal in IR and two small broad singlets far downfield (Figure 5.28) indicated the presence of two carboxylic groups in **V-XXIX**.

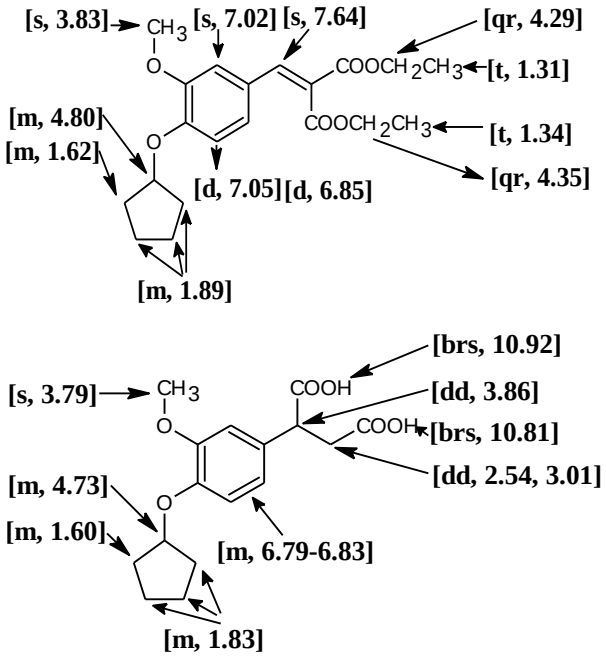


Figure 5.28. ^{1}H- NMR:δ (ppm) ($CDCl_3$, TMS) of compounds **V-XXVII** and **V-XXIX**

Compound V-XXIX was cyclized to indanone derivative V-XXX by treatment with sulfuric acid. This reaction was also tried with polyphosphoric acid and aluminium chloride at different temperatures and durations of time. However the cyclization could not be achieved by these methods. The doublet of doublet for CH and two doublet of doublet for CH_2 on indanone ring confirmed the ring closure and formation of compound **V-XXX** (Figure 5.29).

The keto group of compound **V-XXX** was reduced to CH_2 to form the indan derivative **V-XXXI**. The IR spectra showed broad O-H stretch at 3600-2400 $cm^{-1}$and C=O stretch at 1720 cm^{-1}. The doublet of doublet at 3.94 ppm corresponding to CH, two multiplets at δ 2.33 ppm

and 2.51 ppm corresponding to CH_2 and another multiplet at 2.91 ppm corresponding to CH_2 indicates the presence of indan ring system in **V-XXXI** (Figure 5.29). Other signals obtained in ^{1}H-NMR are also in consonance with the proposed structure of the compound **V-XXXI**.

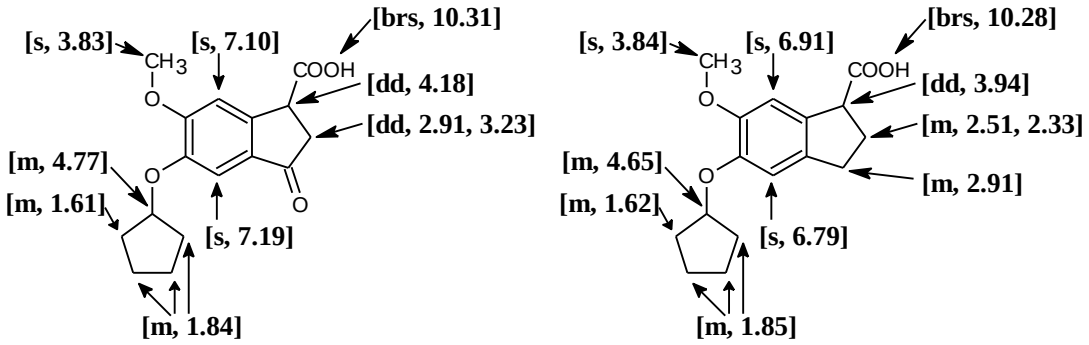


Figure 5.29. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XXX** and **V-XXXI**

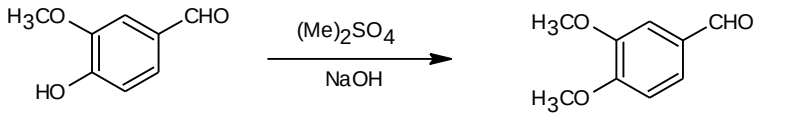
CHAPTER 5

SYNTHESIS

5.1. (5, 6-DIMETHOXY-2, 3-DIHYDRO-1*H*-INDEN-1-YL)ACETIC ACID AMIDES

5.1.1. SYNTHESIS

5.1.1.1. 3,4-Dimethoxybenzaldehyde[148](V-II).

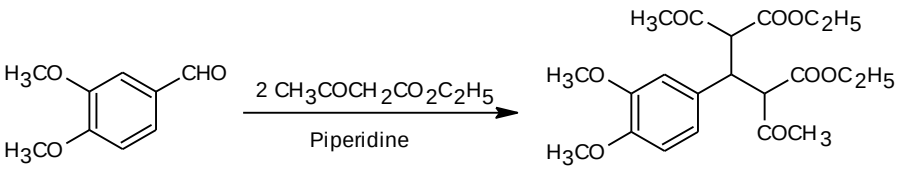


4-hydroxy-3-methoxybenzaldehyde
V-I

3,4-dimethoxybenzaldehyde
V-II

Commercially available vanillin (**V-I**), (15.2g, 0.1 mol), was placed in a 500ml three-necked flask equipped with a magnetic stirrer, two dropping funnels and a reflux condenser. One funnel was charged with potassium hydroxide (8.2 g of KOH in 12 ml of water) and the other funnel with purified dimethyl sulphate (12 ml, 0.104 mol). Vanillin was melted by warming on water bath then KOH was added at the rate of two drops a second. After 20 seconds dimethyl sulphate was also added at the same rate. The heating was stopped after few minutes as the mixture continued to reflux gently from the heat of the reaction. The reaction mixture was vigorously stirred throughout. After all reagents have been added the reaction mixture became turbid and separated into two layers. Pale reddish brown color of the reaction mixture indicating the alkaline condition was maintained throughout the reaction. The yellow reaction mixture was poured into a porcelain basin and was left overnight. The hard crystalline mass thus obtained was ground, washed thoroughly with cold water to make it free of basicity, filtered and dried in a vacuum desiccator to get veratraldehyde (**V-II**) in 70-75% yield, mp 43-44°C.

5.1.1.3. Diethyl 2,4-diacetyl-3-(3,4-dimethoxyphenyl)pentanedionate[149](V-III).



3,4-dimethoxybenzaldehyde

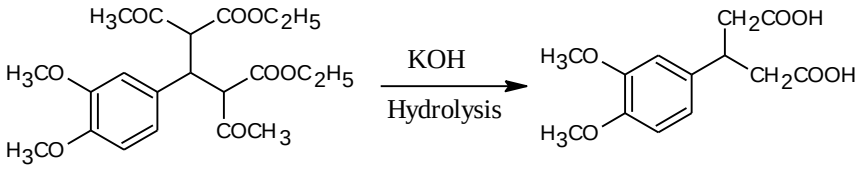
V-II

diethyl-2,4-diacetyl-3-(3,4-dimethoxy phenyl)pentanedionate)

V-III

Veratraldehyde (**V-II**) (33g, 0.2 mol) was dissolved in ethylacetoacetate (56g, 0.43 mol) in a dry conical flask and piperidine (2.5 ml) was added slowly at ambient temperature and then kept for 3 days or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product **V-III** in 70-75% yield[14]. Recrystallization from dil. alcohol gave the analytical product, mp 129-131°C, IR (cm^{-1}) (KBr): 1720 (C=O stretching), 1046, 1253 (C—O stretch of OCH_3), ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 0.82 (t, CH_3, 3H), 0.90 (t, CH_3, 3H), 1.28 (s, CH_3, 3H), 1.34 (s, CH_3, 3H), 2.50, 2.71 (d, CH, H), 3.02, 3.64 (d, CH, H), 3.85 (s, OCH_3, 3H), 3.87 (s, OCH_3, 3H),), 3.93 (qr, CH_2, 2H), 3.96 (qr, CH_2, 2H), 4.03 (m, CH, 1H), 6.59-6.81 (m, ArH, 3H).

5.1.1.3. 3-(3,4-Dimethoxyphenyl)pentanedioic acid[149, 150](V-IV).



diethyl-2,4-diacetyl-3-(3,4-dimethoxy phenyl)pentanedionate)

V-III

3-(3,4-dimethoxyphenyl)pentanedioic acid

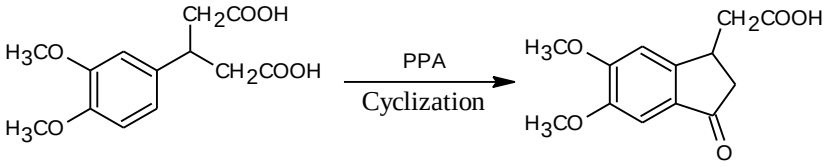
V-IV

Compound **V-III** (40g, 0.089 mol) was dissolved in a freshly made hot solution of KOH (160g in 120 ml of water) and 80 ml of 90% ethanol was added. The hot reaction mixture was refluxed on a water bath for 1 hour. Alcohol was then removed by distillation, and after dilution with water the reaction mixture was cooled and washed with solvent ether. The aqueous phase on acidification with cold conc. HCl with cooling gave crude **V-IV** which was filtered and recrystallized from hot water [14]. Yield 65-70%; mp 189-191°C; IR (cm^{-1}) (KBr): 3300-2400 (br OH stretch), 1710(C=O stretching), 1225 (C—O stretch of OCH_3), 1260 (C—O stretch of COOH) , 1010(C—O stretch of OCH_3);^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS): 2.59,

2.70 (dd, CH_2, 4H), 3.59 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 3.86(s, OCH_3, 3H), 6.78-6.83 (m, ArH, 3H), 10.08 (brs, COOH, 1H), 10.19 (brs, COOH, 1H).

5.1.1.5. (5,6-Dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid[151](V-V).

Cyclization of compound **V-IV** was effected by treating the powdered acid (15g, 0.056 mol) with polyphosphoric acid (PPA) (225g) on a steam bath for 4 hours with stirring. After decomposition of the hot reaction mixture with crushed ice, the keto acid (**V-V**) was isolated by extraction with chloroform. The solvent was distilled off to get the crude keto acid.



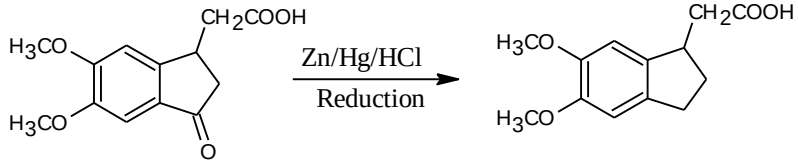
3-(3,4-dimethoxyphenyl) pentanedioic acid
V-IV

(5,6-dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-V

The crude product was finally recrystallized from acetone to get the pure compound. Yield 75-80%; mp 177-178°C; IR (cm^{-1}) (KBr): 3400-2400(br OH stretch), 1680, 1724(C=O stretching), 1045, 1255 (C—O stretch of OCH_3), ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 2.46, 2.57 (dd, CH_2, 2H), 2.79, 2.91 (dd, CH_2, 2H), 3.72 (qn, CH, 1H), 3.91 (s, OCH_3, 3H), 3.98 (s, OCH_3, 3H), 7.00 (s, ArH, 1H), 7.15 (s, ArH, 1H), 10.12 (brs, COOH, 1H).

Note: Polyphosphoric acid was prepared from equivalent weights of phosphorous pentoxide and phosphoric acid.

5.1.1.5. (5,6-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid[152, 153](V-VI).



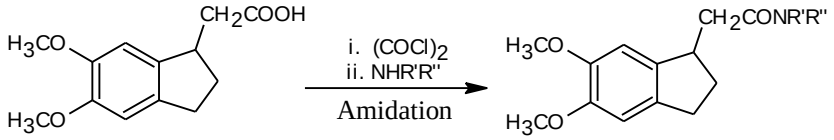
(5,6-dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-V

(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-VI

Compound **V-V** was reduced to **V-VI** using Clemmensen's reduction. Compound **V-V** (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200 ml of benzene was added as a co-solvent. The reaction mixture was refluxed on a steam

bath for about 8 hrs (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was then washed with water to make it free of acidity, dried over anhydrous sodium sulfate and finally distilled off to get the reduced acid **V-VI**. The analytical product was obtained on recrystallization from benzene. Yield 75-80%; mp 152-153^{o}C; IR (cm^{-1}) (KBr): 3400-2800 (br OH stretch), 1712(C=O stretching), 1040, 1256 (C—O stretch of OCH_3); ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS): 1.81 (m, CH_2, 1H), 2.43 (m, CH_2, 1H), 2.49 (dd, CH_2, 1H), 2.80 (dd, CH_2, 1H), 2.89 (m, CH_2, 2H), 3.55 (qn, CH, 1H), 3.86 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 6.76 (s, ArH, 1H), 6.78 (s, ArH, 1H), 10.11 (brs, COOH, 1H), M.S 236(M^{+}).

5.1.1.6. General methods for the synthesis of amide derivatives[151] (DA-1 – DA-34).



(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-VI

Amide Derivatives
DA-1 - DA-34

A solution of compound **V-VI** was made in dry dichloromethane and catalytic amount of dimethylformamide was added. This solution was treated with oxalyl chloride in 1:2.5 molar ratios under ice cold conditions. The reaction mixture was protected with a calcium chloride guard tube to protect it from moisture. The solution was allowed to stand for 24 hours at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation with dry benzene under reduced pressure. The acyl halide thus obtained was not isolated or characterized and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1 mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 hours and was protected with a calcium chloride guard tube. The resulting reaction mixture was then extracted with 0.1N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled to obtain the title compounds (**DA-1 – DA-34**).

All the synthesized amide derivatives were recrystallized from appropriate solvents and their melting points were determined. Their nomenclature and physical data are given in Table 5.1

and Table 5.2 respectively. For few selected compounds the spectral (^{1}H-NMR and Mass) and the elemental analyses were done and are shown in Table 5.3.

5.1.2. NOMENCLATURE AND PHYSICAL DATA OF (5,6-DIMETHOXY-2,3-DIHYDRO -1*H*-INDEN-1- YL)ACETIC ACID AMIDES

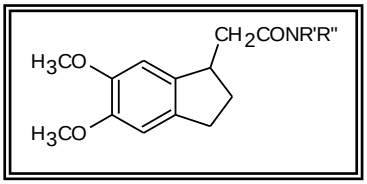


Table 5.1. Nomenclature of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	R'	R''	NOMENCLATURE (IUPAC)
DA-1	H	H	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-2	H	Me	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-methyl acetamide
DA-3	Me	Me	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*,*N*-dimethylacetamide
DA-4	H	Et	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylacetamide
DA-5	Et	Et	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*,*N*-diethylacetamide
DA-6	H	n-Pr	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylacetamide
DA-7	H	i-Pr	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-isopropylacetamide
DA-8	H	n-Bu	*N*-butyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-9	H	tr-Bu	*N*-tert-butyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-10	H	n-Amyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pentylacetamide
DA-11	H	n-Hexyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide
DA-12	H	Cyclopropyl	*N*-cyclopropyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-13	H	Cyclopentyl	*N*-cyclopentyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide

DA-14	H	Cyclohexyl	*N*-cyclohexyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-15	--Pyrrolidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]pyrrolidine
DA-16	--Piperidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]piperidine
DA-17	--Piperazino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]piperazine
DA-18	--Morpholino--		4-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetyl]morpholine
DA-19	H	CH_2CH_2OH	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)acetamide
DA-20	H	Benzyl	*N*-benzyl-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-21	H	Phenyl (C_6H_5)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylacetamide
DA-22	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-23	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-24	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetamide
DA-25	H	C_6H_4(o-CH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-methylphenyl)acetamide
DA-26	H	C_6H_4(m-CH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)acetamide

Contd….

Table 5.1 Contd.

DA-27	H	C_6H_4(p-CH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*n*-(4-methylphenyl)acetamide
DA-28	H	C_6H_4(o-OCH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-methoxyphenyl)acetamide
DA-29	H	C_6H_4(p-OCH_3)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methoxyphenyl)acetamide
DA-30	H	C_6H_4(p-NHAc)	*N*-[4-(acetylamino)phenyl]-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)aa*
DA-31	H	C_6H_4(p-NO_2)	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)acetamide
DA-32	H	2-Pyridyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-2-ylacetamide
DA-33	H	3-Pyridyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylacetamide
DA-34	H	4-Pyridyl	2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylacetamide

*aa- acetamide

Table 5.2. Physical data of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	RECRYSTALLIZATION SOLVENT	YIELD (%)	M.P (°C)	MOLECULAR FORMULA	MOLECULAR WEIGHT	Calc. Log P
DA-1	water	54.0	166-168	$C_{13}H_{17}NO_3$	235	1.45
DA-2	water	59.6	137-138	$C_{14}H_{19}NO_3$	249	1.92
DA-3	dilute alcohol	60.4	117-118	$C_{15}H_{21}NO_3$	263	2.29
DA-4	water	61.8	104-105	$C_{15}H_{21}NO_3$	263	2.44
DA-5	dilute alcohol	64.9	62-63	$C_{17}H_{25}NO_3$	291	3.21
DA-6	dilute alcohol	66.7	107-108	$C_{16}H_{23}NO_3$	277	2.90

DA-7	benzene	66.6	140-141	$C_{16}H_{23}NO_3$	277	2.72
DA-8	cyclohexane	59.2	103-104	$C_{17}H_{25}NO_3$	291	3.36
DA-9	cyclohexane	65.2	156-157	$C_{17}H_{25}NO_3$	291	3.24
DA-10	cyclohexane	65.1	102-103	$C_{18}H_{27}NO_3$	305	3.83
DA-11	cyclohexane	57.9	94-95	$C_{19}H_{29}NO_3$	319	4.31
DA-12	ethyl acetate	62.7	128-129	$C_{16}H_{21}NO_3$	275	2.63
DA-13	cyclohexane	72.5	136-138	$C_{18}H_{25}NO_3$	303	3.34
DA-14	cyclohexane	70.5	152-153	$C_{19}H_{27}NO_3$	317	3.83
DA-15	EtOAc/hexane	64.3	114-115	$C_{17}H_{23}NO_3$	289	2.75
DA-16	chloroform	57.5	101-102	$C_{18}H_{25}NO_3$	303	3.28
DA-17	EtOAc/hexane	63.7	212-213	$C_{17}H_{24}N_2O_3$	304	1.54
DA-18	dilute alcohol	61.6	137-138	$C_{17}H_{23}NO_4$	305	1.90
DA-19	benzene	64.4	72-74	$C_{15}H_{21}NO_4$	279	0.88
DA-20	dilute alcohol	77.4	146-147	$C_{20}H_{23}NO_3$	325	2.95
DA-21	dilute alcohol	72.8	148-149	$C_{19}H_{21}NO_3$	311	2.98

Contd….

Table 5.2 Contd.

DA-22	dilute alcohol	65.6	104-106	$C_{19}H_{20}ClNO_3$	345	3.84
DA-23	dilute alcohol	70.6	118-120	$C_{19}H_{20}ClNO_3$	345	3.79
DA-24	dilute alcohol	65.2	151-152	$C_{19}H_{20}BrNO_3$	390	3.82
DA-25	dilute alcohol	69.5	132-134	$C_{20}H_{23}NO_3$	325	3.26
DA-26	dilute alcohol	72.6	146-148	$C_{20}H_{23}NO_3$	325	3.40
DA-27	dilute alcohol	75.7	128-129	$C_{20}H_{23}NO_3$	325	3.49
DA-28	dilute alcohol	71.5	118-120	$C_{20}H_{23}NO_4$	341	2.97
DA-29	dilute alcohol	60.1	180-181	$C_{20}H_{23}NO_4$	341	3.06
DA-30	EtOAc/hexane	60.7	206-208	$C_{21}H_{24}N_2O_4$	368	2.38
DA-31	EtOAc/hexane	57.5	164-166	$C_{19}H_{20}N_2O_5$	356	3.08
DA-32	dilute alcohol	54.6	112-114	$C_{18}H_{20}N_2O_3$	312	2.19
DA-33	dilute alcohol	55.7	120-122	$C_{18}H_{20}N_2O_3$	312	2.16
DA-34	EtOAc/hexane	58.6	104-106	$C_{18}H_{20}N_2O_3$	312	2.19

Calc. Log P stands for calculated log P (from www. logp.com)

Table 5.3. Spectral and elemental analyses data of (5, 6-dimethoxy-2, 3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMP		**^{1}H-NMR**	**ELEMENTAL ANALYSES**	**MASS**

OUND	IR (cm^{-1}; KBr)	(δ ppm, $CDCl_3$, TMS)	(CALCULATED/FOUND)			(m/z)
			C%	H%	N%	M^+ Peak
DA-1	3391, 3216 (NH str), 1640 (C=O str), 1268, 1087 (OCH_3)	1.77, 2.42 (m, CH_2, 2H), 2.35, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.62 (qn, CH, 1H), 3.83 (s, CH_3, 3H), 3.84 (s, CH_3, 3H), 5.49 (s, NH, 2H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	66.36 66.15	7.28 7.25	5.95 5.93	235
DA-7	3285 (NH), 1644 (C=O), 1265, 1082 (OCH_3)	1.45 (d, CH_3, 6H), 1.81, 2.33 (m, CH_2, 2H), 2.21, 2.47 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.58 (qn, CH, 1H), 3.83 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 3.94 (m, CH, 1H) 5.22 (s, NH, 1H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	69.29 69.35	8.36 8.35	5.05 5.04	277
DA-11	3323 (NH), 1648 (C=O), 1267, 1083 (OCH_3)	0.98 (t, CH_3, 3H), 1.31 (m, CH_2, 6H), 1.53 (qn, CH_2, 2H), 1.78, 2.24 (m, CH_2, 2H), 2.26, 2.52 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.20 (t, CH_2, 2H), 3.60 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 5.39 (s, NH, 1H), 6.75 (s, ArH, 1H), 6.76 (s, ArH, 1H)	71.44 71.60	9.12 9.14	4.38 4.40	319
DA-14	3327 (NH), 1645 (C=O), 1269, 1086 (OCH_3)	1.11 (qn, CH_2, 2H), 1.35 (m, CH_2, 4H), 1.71(m, CH_2, 4H), 1.79, 2.30 (m, CH_2, 2H), 2.19, 2.46 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.58 (qn, CH, 1H), 3.65 (qn, CH, 1H), 3.82 (s, OCH_3, 3H), 3.84 (s, OCH_3,3H), 5.29 (s, NH,1H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	71.89 71.86	8.57 8.58	4.41 4.42	317

Contd….

Table 5.3 Contd.

DA-18	1648 (C=O), 1270, 1081 (OCH_3)	1.74, 2.43 (m, CH_2, 2H), 2.68, 2.45 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.47 (t, CH_2, 4H), 3.60 (qn, CH, 1H), 3.69 (t, CH_2, 4H) 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 6.76 (s, ArH, 1H), 6.77 (s, ArH, 1H)	66.86 66.69	7.59 7.56	4.59 4.60	305
DA-20	3333 (NH), 1643 (C=O), 1272, 1084 (OCH_3)	1.80, 2.48 (m, CH_2, 2H), 2.36, 2.57 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.63 (qn, CH, 1H), 3.81 (s, OCH_3, 3H), 3.83 (s, OCH_3, 3H), 4.46 (s, Bnz CH_2, 2H), 5.71 (s, NH, 1H), 6.74 (s, ArH, 1H), 6.75 (s, ArH, 1H), 7.18-7.33 (m, ArH, 5H**)**	73.82 73.91.	7.12 7.14	4.30 4.31	325
DA-21	3307 (NH), 1645 (C=O), 1265, 1081 (OCH_3)	1.82, 2.41 (m, CH_2, 2H), 2.50, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.64 (qn, CH, 1H), 3.72 (s, OCH_3, 3H), 3.84 (s, OCH_3, 3H), 6.74 (s, ArH, 1H), 6.76 (s, ArH, 1H), 7.09 (s, NH, 1H), 7.26-7.49 (m, ArH, 5H)	73.29 73.08	6.80 6.82	4.50 4.51	311
DA-23	3324 (NH), 1651 (C=O), 1269, 1087	1.82, 2.43 (m, CH_2, 2H), 2.51, 2.66 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.68 (qn,	65.99 65.74	5.83 5.82	4.05 4.06	345

	(OCH_3)	CH, 1H), 3.76 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 7.19 (s, NH, 1H), 7.24 (d, ArH, 2H), 7.53 (d, ArH, 2H)				
DA-26	3294 (NH), 1643 (C=O), 1263, 1078 (OCH_3)	1.82, 2.42 (m, CH_2, 2H), 2.32(s, CH_3, 3H) 2.49, 2.69 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.67 (qn, CH, 1H), 3.74 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 6.91 (d, ArH, 1H), 7.10 (s, NH, 1H), 7.16-7.35 (m, ArH, 3H)	73.82 74.09	7.12 7.14	4.30 4.31	325
DA-29	3317 (NH), 1641 (C=O), 1259, 1077 (OCH_3)	1.81, 2.41 (m, CH_2, 2H), 2.49, 2.61 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.68 (qn, CH, 1H), 3.74 (s, OCH_3, 3H), 3.78 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 6.84 (d, ArH, 2H), 7.14 (s, NH, 1H), 7.38 (d, ArH, 2H)	70.36 70.58	6.79 6.81	4.10 4.11	341
DA-33	3337 (NH), 1649 (C=O), 1268, 1087 (OCH_3)	1.82, 2.42 (m, CH_2, 2H), 2.49, 2.65 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.66 (qn, CH, 1H), 3.75 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 6.75 (s, ArH, 1H), 6.77 (s, ArH, 1H), 7.23 (d, ArH, 1H), 7.29 (s, NH, 1H), 8.11-8.43 (m, ArH, 3H)	69.21 68.99	6.45 6.43	8.97 8.94	312

5.1.3. RESULTS AND DISCUSSION

The starting reagent for the preparation of the key intermediate, 5,6-Dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)acetic acid (**V-VI**) was vanillin (**V-I**). The hydroxyl group of vanillin was methylated by dimethyl sulphate under alkaline conditions to get the methylated product veratraldehyde (**V-II**). This method avoids the use of the more expensive alkyl halides required in the Williamson synthesis. The formation of phenyl alkyl ether is an example of electrophilic substitution reaction where the proton is substituted with the methyl group.

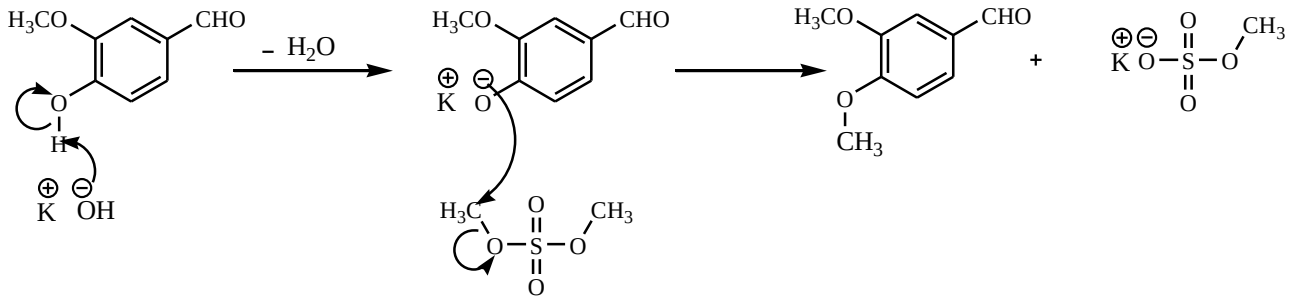


Figure 5.1. Possible reaction mechanism for the synthesis of **V-II**

Veratraldehyde (**V-II**) was reacted with two moles of ethylacetoacetate (EAA) in the presence of catalytic amount of piperidine at room temperature to obtain the benzylic bisacetoacetate **V-VIII.**

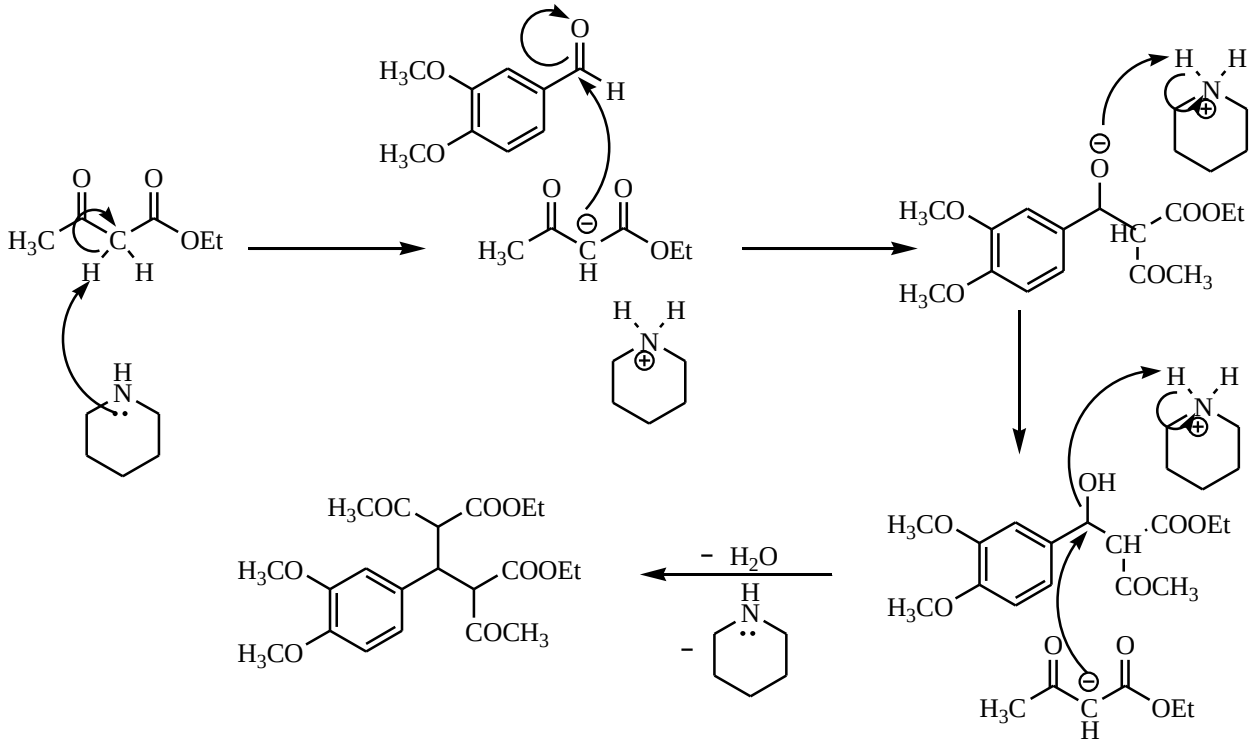


Figure 5.2. Possible reaction mechanism for the synthesis of **V-III**

Piperidine serves as the catalytic base and it abstracts the acidic proton from ethylacetoacetate. The negative charge on the active methylene group attacks the partially positively charged carbonyl carbon of aldehyde group. Similarly another molecule of ethylacetoacetate attacks through its active methylene carbon on the electron deficient carbon to form the benzylic bisacetoacetate (Figure 5.2). The IR spectrum of the compound **V-III** shows the carbonyl C=O stretching at 1720 cm^{-1}

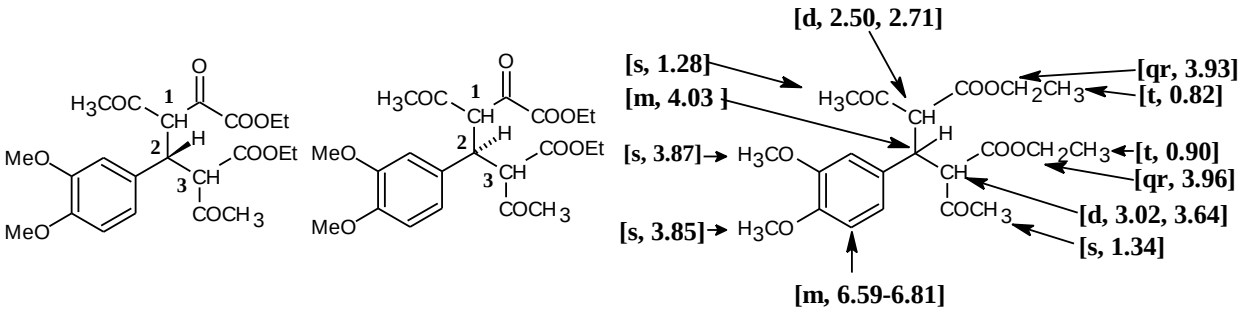


Figure 5.3. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-III**

The ^{1}H-NMR signal from proton present on carbon 1 and 3 are spilt into two doublets at 2.50, 2.71 and 3.02, 3.64 respectively by the neighboring proton of carbon 2 as shown above in Figure 5.3. This is because the proton present on carbon 2 is having two different configurations as shown above in Figure 5.3, causing the same proton to behave as present in two different environments. Similarly the signal from the proton on carbon 2 is split into a multiplet because of different configurational arrangements of proton on carbon 1 and 3.

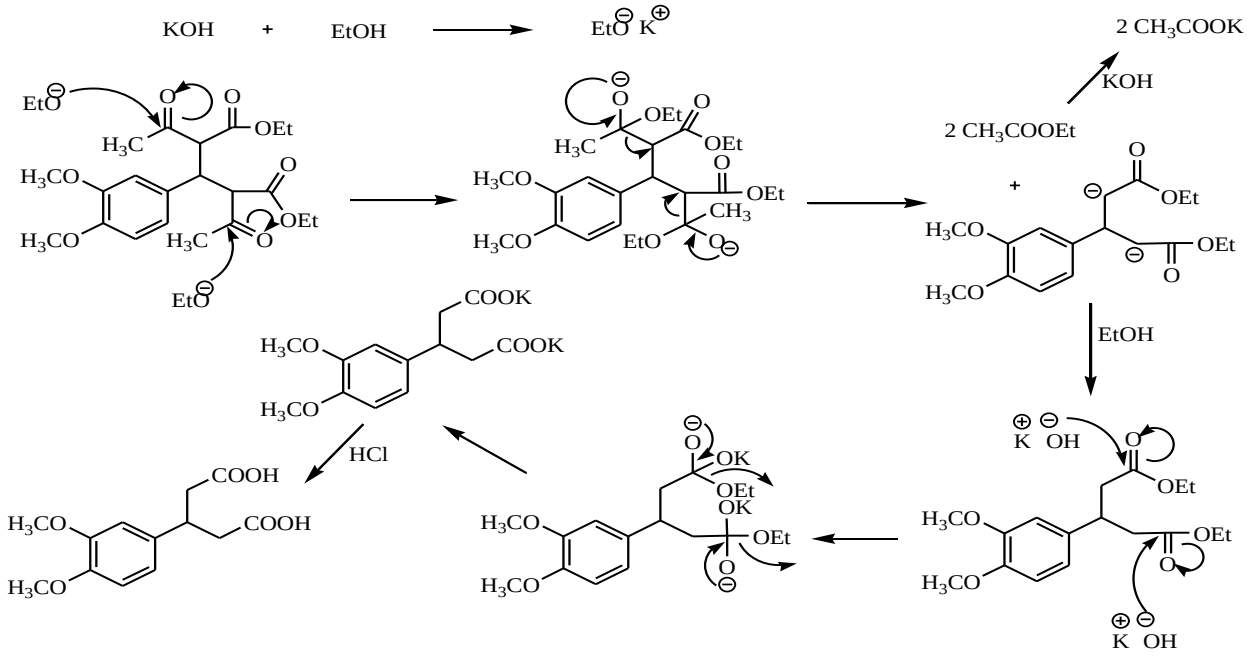


Figure 5.4. Possible reaction mechanism for the synthesis of **V-IV**

Alkaline hydrolysis of ester groups as well as ketonic hydrolysis of acetyl groups of the bisacetoacetate compound **V-III** was carried out with 6N potassium hydroxide (KOH) in 90% ethanol (Figure 5.4). The diacid product obtained was in the form of potassium salt which was liberated in free form by treatment with dilute hydrochloric acid under cold conditions. The IR spectrum of the compound **V-IV** showed broad OH stretch at 3300-2400 cm^{-1} and C=O stretch at 1710 cm^{-1}.

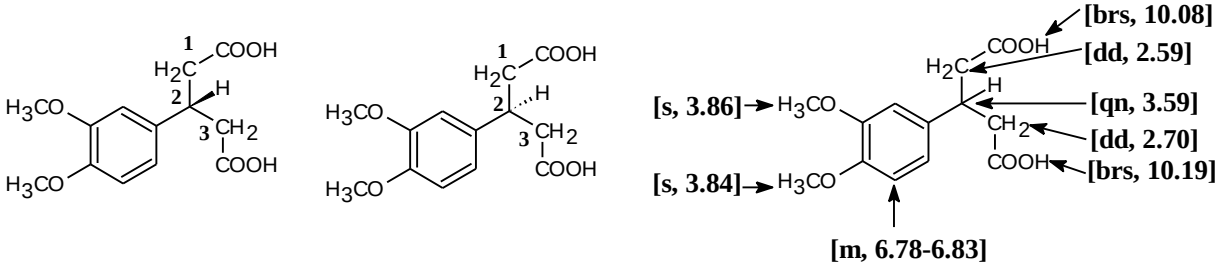


Figure 5.5. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-IV**

^{1}H-NMR of the diacid **(V-IV)** showed singlets at δ 3.84 and 3.86 ppm corresponding to methoxyl protons, doublet of doublet at 2.59 and 2.70 ppm as well as quintet at 3.59 ppm for CH_2 and CH of glutaric acid side chain respectively, multiplet at δ 6.78-6.83 ppm for aromatic protons and small broad singlets at δ 10.08 ppm and δ 10.19 ppm indicating the presence of carboxyl groups. Doublet of doublet was obtained for the protons on carbon 1 and 3 because of the different configurational arrangement of proton on carbon 2 as shown in Figure 5.5. This single proton on carbon 2 present in either equatorial or axial positions behaves in two different environments equivalent to two protons.

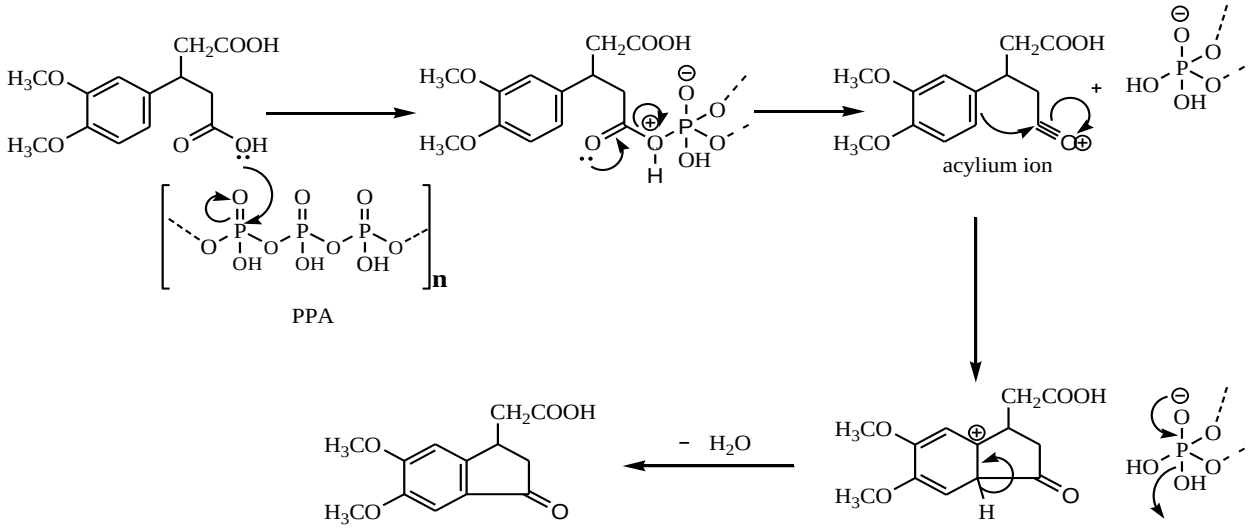


Figure 5.6. Possible reaction mechanism for the synthesis of **V-V**

The diacid compound **V-IV** was treated with polyphosphoric acid (PPA) when intramolecular Friedel-Craft's cyclization and hence ring closure took place to give the cyclic ketonic product. The lone pair of electrons on oxygen of carboxylic group attacks the partially electron deficient polyphosphoric acid and leads to the formation of acylium ion, a highly reactive electrophile. The aromatic electrons attack electron deficient carbon of acylium ion leading to ring closure and formation of the keto compound **V-V** (Figure 5.6).

The IR spectrum of keto acid **V-V** showed a broad OH stretch at 3400-2400 cm^{-1}. Two C=O stretching peaks were obtained at 1680 and 1724 cm^{-1} corresponding to oxo group and to carbonyl of acid group respectively.

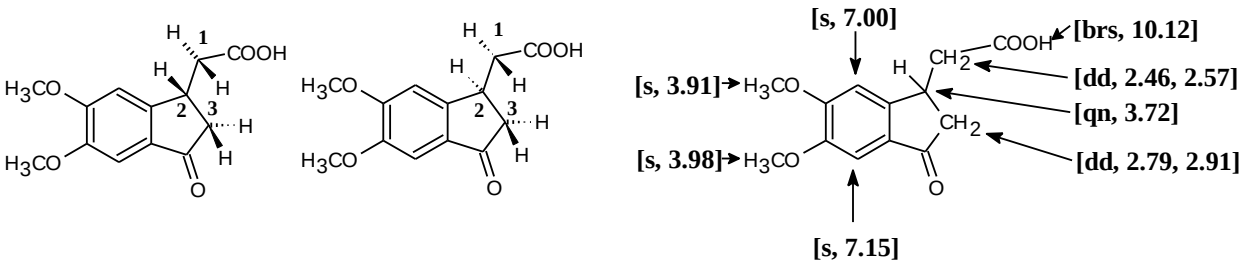


Figure 5.7. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-V**

Presence of doublet of doublet at 2.46 and 2.57 ppm and doublet of doublet at 2.79 and 2.91 ppm corresponding to CH_2 of side chain and CH_2 of the indan ring respectively as well as the presence of quintet at 3.72 ppm in the ^{1}H-NMR spectrum of **V-V** indicates ring closure (Figure 5.7). Doublet of doublets for protons on carbons 1 and 3 were obtained because of different configurational arrangements of the single proton present on carbon 2. The presence of methoxyl protons (singlets at 3.91 and at 3.98 ppm), aromatic protons (singlets at 7.00 and 7.15 ppm) and acid protons (broad singlet at 10.12 ppm) further supports the proposed structure of keto acid (**V-V**).

The ketone group of **V-V** was finally removed using Clemmensen's reduction to give **V-VI**, 5,6-dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)acetic acid. Clemmensen's reduction is a suitable approach for reduction of acid stable ketone compounds. Compound **V-V** is acid stable and hence can be deoxygenated to the corresponding hydrocarbon under the reaction conditions employed for Clemmensen's reduction. The reaction involves heating a carbonyl compound with amalgamated zinc in a hydroxylic solvent (often an aqueous mixture) containing a mineral acid such as HCl. The mercury alloyed with the zinc does not participate in the reaction; it serves only to provide a clean active metal surface. The reaction mechanism involves some sort of zinc ketone complex and is shown in Figure 5.8 [155].

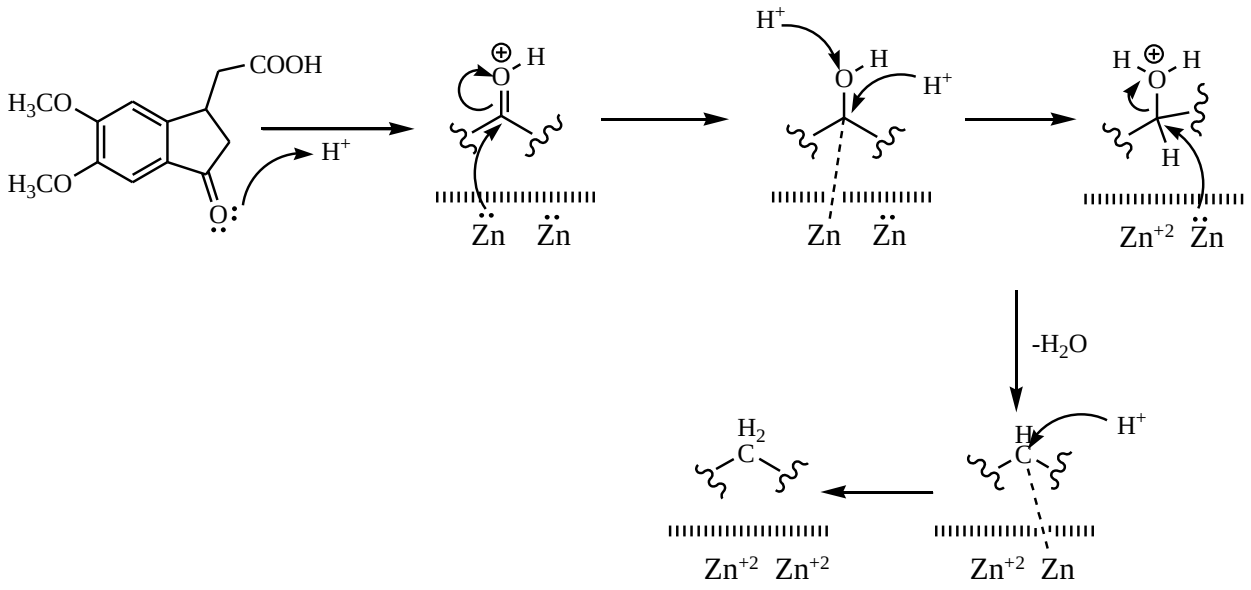


Figure. 5.8. The reaction mechanism of Clemmensen's reduction

Because of the different conformational arrangement of proton present on CH of indan ring both neighbouring CH_2 peaks got split into two peaks (two doublets of doublet at 2.49, 2.80 ppm and two multiplets at 1.81, 2.43 ppm) in the ^{1}H-NMR spectrum of the compound **V-VI** (Figure 5.9). The presence of multiplet at 2.89 ppm equivalent to two protons in addition to peaks obtained for compound **V-V** indicates the reduction of carbonyl group and formation of the indan ring system. The presence of aromatic protons (singlets at 6.76 and 6.78 ppm) and acid protons (broad singlet at 10.11 ppm) further supports the proposed structure of compound **V-VI**.

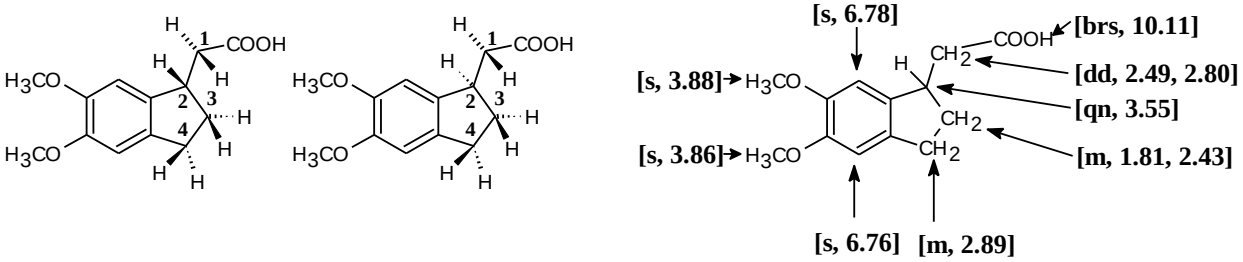


Figure 5.9. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of the compound **V-VI**

Compound **V-VI** was treated with oxalyl chloride in presence of catalytic amount of dimethylformamide to convert the carboxyl group of **V-VI** into acyl halide. The acyl halide thus obtained was not isolated or characterized. It was used directly in the next step. The acyl halide was reacted with various primary and secondary amines under Schotten- Baumann

conditions for the formation of desired amides. The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides. Also disappearance of broad OH stretch at 3400-2400 cm^{-1} indicates the formation of amide bond. The formation of amides was further confirmed by presence of a small singlet at 5-6 ppm for aliphatic amides and 7-8 ppm for the aromatic amides in ^{1}H-NMR. In most of the cases there was an overlap of two peaks at 2.2-2.4 ppm corresponding to doublet of doublet and multiplet; also in case of aromatic amides there was an overlap of peak corresponding to NH of amide group and the peaks corresponding to the aromatic group.

A modification of thionyl chloride method for making acyl halide uses oxalyl chloride plus catalytic amount of DMF. Oxalyl chloride reacts with DMF to produce a highly electrophilic cationic intermediate, plus carbon dioxide and carbon monoxide. As with thionyl chloride these by products are all gases. The first two steps are the nucleophilic substitution of chloride ion at the carbonyl group. The reactive intermediate is highly electrophilic and reacts rapidly with the carboxylic acid producing another intermediate which intercepts chloride ion to give the acyl halide and regenerate DMF (Figure 5.10)[156].

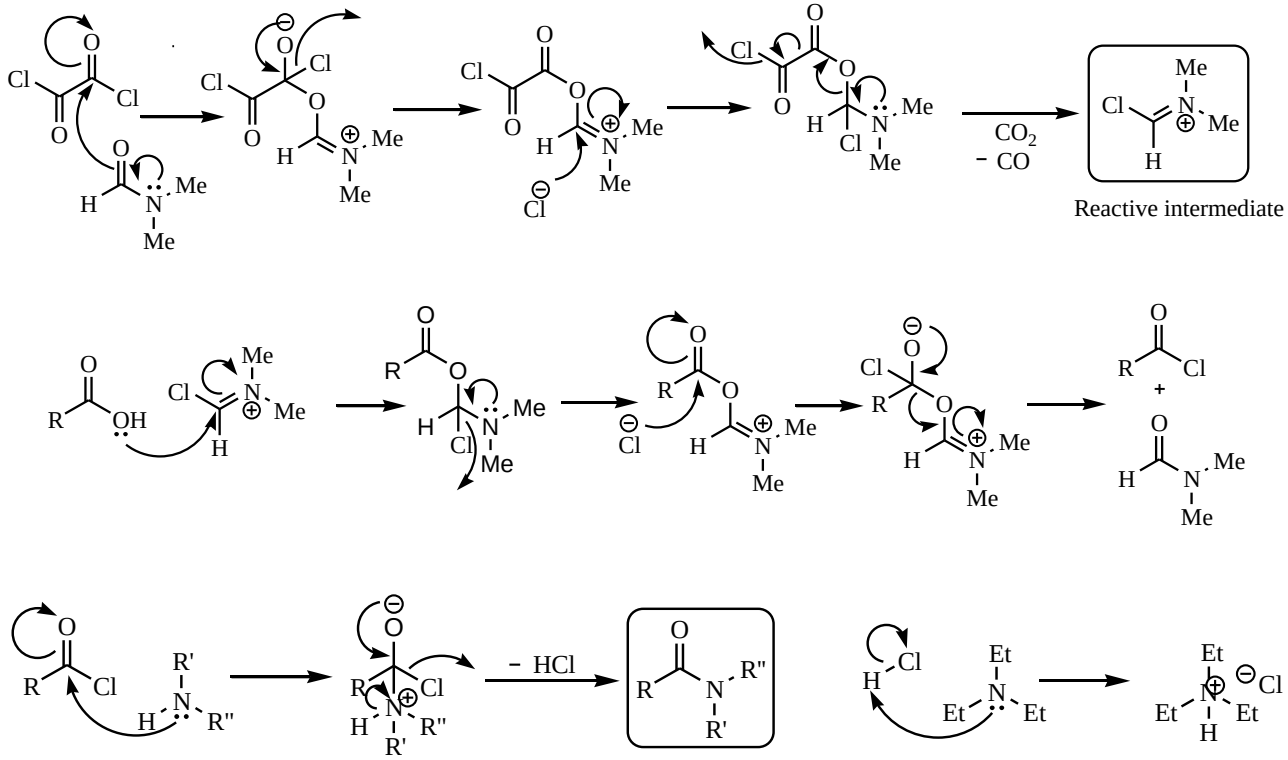


Figure 5.10. The reaction mechanism for formation of amide derivatives from compound **V-VI**

The acyl halide formation was also tried with thionyl chloride, but better yields of amides were obtained when the acyl halide was formed with oxalyl chloride even though the thionyl chloride used was distilled and dried. As heating was involved in the thionyl chloride method, the chances of highly reactive acyl halide's decomposition were more. Also the formation of highly reactive intermediate in oxalyl chloride provides much stronger electrophile to be attacked by the acid. These factors may be the likely reason for the better yields of amides obtained when acyl halide is made by reaction with oxalyl chloride.

In Mass spectroscopy of the compound **V-VI**, 5,6-dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)acetic acid, the M^+ peak was observed as the base peak. The fragmentation pattern has been shown in Figure 5.11. In most of the amide derivatives M^+ peak was observed as the base peak.

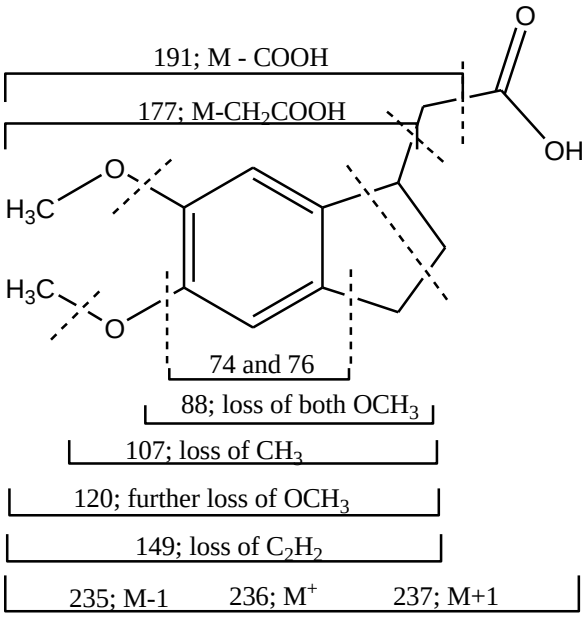
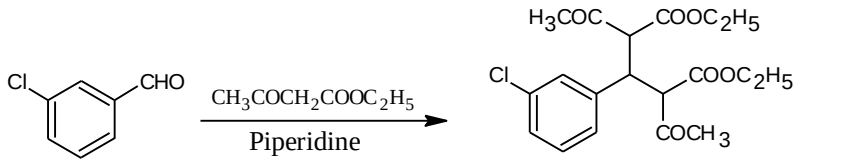


Figure 5.11. Mass spectrum of **V-VI**

5.2. (6-CHLORO-2, 3-DIHYDRO-1*H*-INDEN-1- YL)ACETIC ACID AMIDES

5.2.4. SYNTHESIS

5.2.1.1. Diethyl 2,4-diacetyl-3-(3-chlorophenyl)pentanedionate (V-VIII).



3-chlorobenzaldehyde

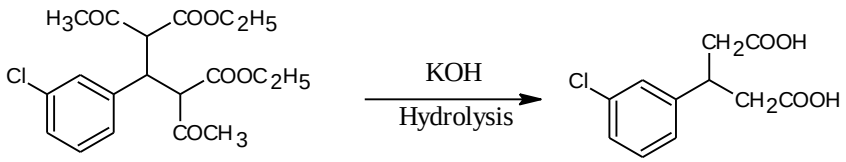
V-VII

diethyl 2,4-diacetyl-3-(3-chloro phenyl)pentanedioate

V-VIII

3-Chlorobenzaldehyde (**V-VII**) (28g, 0.2 mol) was dissolved in ethylacetoacetate (56g, 0.43 mol) in a dry conical flask and piperidine (2.5 ml) was added slowly at ambient temperature and then kept for 3 days or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product **V-VIII** in 70-75% yield. Recrystallization from dil. alcohol gave the analytical product, mp 120-121°C, IR (cm^{-1}) (KBr): 1722, 1682 (C=O stretching), 657 (C-Cl stretch), NMR: δ (ppm) ($CDCl_3$, TMS): 1.23 (t, CH_3, 3H), 1.31 (t, CH_3, 3H), 1.86 (s, CH_3, 3H), 1.93 (s, CH_3, 3H), 2.91, 3.26 (d, CH, H), 3.38, 3.85 (d, CH, H), 4.03 (qr, CH_2, 2H), 4.06 (qr, CH_2, 2H), 4.33 (m, CH, 1H), 6.74-7.17 (m, ArH, 4H).

5.2.1.2. 3-(3-chlorophenyl)pentanedioic acid (V-IX).

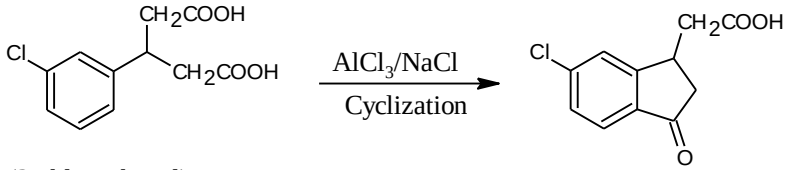


diethyl 2,4-diacetyl-3-(3-chloro phenyl)pentanedioate
V-VIII

3-(3-chlorophenyl) pentanedioic acid
V-IX

Compound **V-VIII** (40g, 0.1 mol) was dissolved in a hot solution of KOH (160g in 120 ml of water) and 160 ml of 50% ethanol was added. The hot reaction mixture was refluxed on a water bath for 1 hour. Alcohol was then removed by distillation, and after dilution with water it was cooled and washed with solvent ether. The aqueous layer on acidification with cold conc. HCl with cooling gave crude **V-IX** which was filtered and recrystallized from hot water. Yield 80-85%; mp 147-148°C; IR (cm^{-1}) (KBr): 3400-2200 (br OH stretch), 1710 (C=O stretching), 663 (C—Cl stretch); ^{1}H NMR:δ (ppm) ($CDCl_3$, TMS): 2.58, 2.70 (dd, CH_2, 4H), 3.60 (qn, CH, 1H), 7.15-7.26 (m, ArH, 4H), 12.01 (brs, COOH, 1H), 12.13 (brs, COOH, 1H)

5.2.1.3. (6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid[157, 158](V-X).

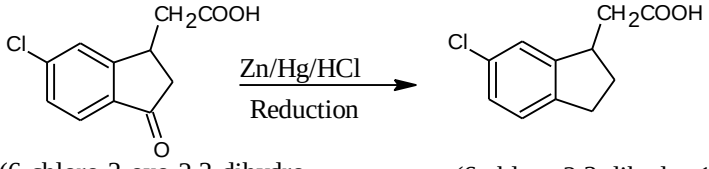


3-(3-chlorophenyl) pentanedioic acid
V-IX

(6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid
V-X

Cyclization of compound **V-IX** was carried by treating this acid (14g, 0.058 mol) with aluminium chloride (49g, 0.37 mol) in presence of sodium chloride (4.47g, 0.08 mol) on an oil bath maintained at 180°C for 30 minutes after cessation of liberation of HCl gas from the reaction mixture, with stirring. After decomposition of the hot reaction mixture with crushed ice, the crude keto acid, **V-X,** thus obtained was filtered. The crude product was finally recrystallized from dilute acetic acid to get the pure compound. Yield 60-65%; mp 188-190°C; IR (cm^{-1}) (KBr): 3400-2400 (br OH stretch), 1680, 1713 (C=O stretching), 657 (C—Cl stretch), ^{1}H NMR: δ (ppm) ($CDCl_3$, TMS): 2.46, 2.58 (dd, CH_2, 2H), 2.84, 2.99 (dd, CH_2, 2H), 3.79 (qn, CH, 1H), 7.38 (d, ArH, 1H), 7.59 (s, ArH, 1H), 7.65 (d, ArH, 1H), 11.18 (brs, COOH, 1H).

5.2.1.4. (6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid (V-XI).



(6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid

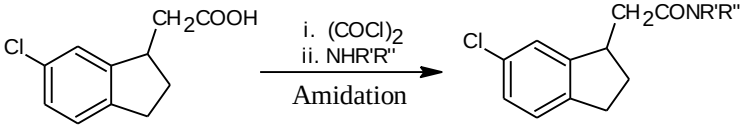
V-X

(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-XI

Compound **V-X** was subjected to Clemmensen's reduction. Compound V-X (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on steam bath for about 24 hrs (until the reaction mixture became keto-negative). After every 8 hrs around 10ml of conc. HCl was added to the reaction mixture. The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free of acidity and then dried over anhydrous sodium sulfate and was finally distilled off to get the reduced acid (**V-XI**). The reduced acid so obtained was a liquid and was subjected to vacuum distillation to get the pure compound. Yield 80-85%; distilled at 148 ºC / 0.3 mm Hg.; IR (cm^{-1}) (thin film): 3400-2400 (br OH stretch), 1700 (C=O stretching), 657 (C—Cl stretch); ^{1}H NMR: δ (ppm) ($CDCl_3$, TMS): 1.78, 2.42 (m, CH_2, 2H), 2.48, 2.81 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.56 (qn, CH, 1H), 7.11-7.16 (m, ArH, 3H), 10.91 (brs, COOH, 1H), M.S 211 (M^+).

5.2.1.5. General methods for the synthesis of amide derivatives (CA-1 - CA-25)



(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid

V-XI

Amide derivatives

CA-1 - CA-25

A solution of compound **V-XI** was made in dry dichloromethane and catalytic amount of dimethylformamide was added. This solution was treated with oxalyl chloride in 1:2.5 molar ratio under ice cold conditions. The reaction flask was attached with a calcium chloride guard tube to protect it from moisture. The solution was allowed to stand for 24 hours at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation

with dry benzene under reduced pressure. The acyl halide thus obtained was not isolated or characterized and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 hours and was kept covered with a calcium chloride guard tube to protect it from moisture. The resulting reaction mixture was then extracted with 0.1N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled to obtain the title compounds (**CA-1 – CA-25**).

All the synthesized amide derivatives were recrystallized from appropriate solvents and their melting points were determined. Their nomenclature and physical data are given in Table 5.4 and Table 5.5 respectively. For few selected compounds the spectral (^{1}H-NMR and Mass) and the elemental analyses were done and are shown in Table 5.6.

5.2.5. NOMENCLATURE AND PHYSICAL DATA OF (6-CHLORO-2,3-DIHYDRO-1*H*-INDEN-1- YL)ACETIC ACID AMIDES

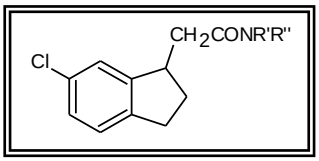


Table 5.4. Nomenclature of (6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	R’	R’’	NOMENCLATURE (IUPAC)
CA-1	H	H	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-2	H	Me	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-methyl acetamide
CA-3	H	Et	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylacetamide
CA-4	H	n-Pr	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylacetamide
CA-5	H	i-Pr	2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)-*N*-isopropylacetamide
CA-6	H	n-Bu	*N*-butyl-2-(6-chloro-2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-7	H	n-Amyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide
CA-8	H	n-Hexyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylacetamide
CA-9	H	Cyclopentyl	*N*-cyclopentyl-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide

CA-10	H	Cyclohexyl	*N*-cyclohexyl-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-11	--Piperidino--		1-[(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetyl]piperidine
CA-12	--Piperazino--		1-[(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetyl]piperazine
CA-13	H	CH_2CH_2OH	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)acetamide
CA-14	H	$CH_2C_6H_5$	*N*-benzyl-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-15	H	C_6H_5	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylacetamide
CA-16	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-17	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-18	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)acetamide
CA-19	H	C_6H_4(m-CH_3)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)acetamide
CA-20	H	C_6H_4(p-CH_3)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methylphenyl)acetamide
CA-21	H	C_6H_4(p-OCH_3)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methoxyphenyl)aa*
CA-22	H	C_6H_4(p-NHAc)	*N*-[4-(acetylamino)phenyl]-2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)aa*
CA-23	H	C_6H_4(p-NO_2)	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)acetamide
CA-24	H	3-Pyridyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylacetamide
CA-25	H	4-Pyridyl	2-(6-chloro -2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylacetamide

*aa- acetamide

Table. 5.5. Physical data of (6-chloro-2, 3-dihydro-1*H*-inden-1-yl)acetic acid amides

COMPOUND	RECRYSTALLIZATION SOLVENT	YIELD (%)	M.P (°C)	MOLECULAR FORMULA	FORMULA WEIGHT	Calc. Log P
CA-1	water	57.8	109-110	$C_{11}H_{12}ClNO$	210	1.92
CA-2	dilute alcohol	58.9	102-103	$C_{12}H_{14}ClNO$	224	2.31
CA-3	dilute alcohol	60.4	98-100	$C_{13}H_{16}ClNO$	238	2.80
CA-4	dilute alcohol	63.6	74-76	$C_{14}H_{18}ClNO$	252	3.27
CA-5	benzene	62.5	104-106	$C_{14}H_{18}ClNO$	252	3.07
CA-6	-	66.8	ss	$C_{15}H_{20}ClNO$	266	3.75
CA-7	-	65.1	ss	$C_{16}H_{22}ClNO$	280	4.24
CA-8	-	67.9	ss	$C_{17}H_{24}ClNO$	294	4.75
CA-9	cyclohexane	66.8	136-138	$C_{16}H_{20}ClNO$	274	3.74
CA-10	cyclohexane	64.3	145-147	$C_{17}H_{22}ClNO$	292	4.24
CA-11	dilute alcohol	69.1	108-110	$C_{16}H_{20}ClNO$	278	3.82
CA-12	dilute alcohol	57.8	215-216	$C_{15}H_{19}ClN_2O$	279	2.00
CA-13	benzene	60.3	60-62	$C_{13}H_{16}ClNO_2$	254	1.57
CA-14	cyclohexane	69.7	106-108	$C_{18}H_{18}ClNO$	300	3.54
CA-15	dilute alcohol	73.7	146-148	$C_{17}H_{16}ClNO$	286	3.59

CA-16	dilute alcohol	72.8	138-140	$C_{17}H_{15}Cl_2NO$	320	4.53
CA-17	dilute alcohol	74.6	145-146	$C_{17}H_{15}Cl_2NO$	320	4.37
CA-18	dilute alcohol	65.8	127-129	$C_{17}H_{15}BrClNO$	365	4.41
CA-19	dilute alcohol	71.3	109-111	$C_{18}H_{18}ClNO$	300	3.90
CA-20	dilute alcohol	70.4	194-195	$C_{18}H_{18}ClNO$	300	4.00
CA-21	dilute alcohol	69.1	176-178	$C_{18}H_{18}ClNO_2$	316	3.76
CA-22	dilute alcohol	67.6	156-158	$C_{19}H_{19}ClN_2O_2$	343	3.14
CA-23	dilute alcohol	65.8	137-139	$C_{17}H_{15}ClN_2O_3$	331	3.83
CA-24	dilute alcohol	60.3	196-198	$C_{16}H_{15}ClN_2O$	287	2.74
CA-25	dilute alcohol	58.6	157-159	$C_{16}H_{15}ClN_2O$	287	2.78

ss: semisolid
Calc. Log P is the calculated log P (from www. logp. com)

Table.5.6. Spectral and elemental analyses data of (6-chloro-2, 3-dihydro-1*H*-inden-1-yl) acetic acid amides

COMPOUND	**IR (cm^{-1}; KBr)**	**^{1}H-NMR (δ ppm, $CDCl_3$, TMS)**	**ELEMENTAL ANALYSES (CALCULATED/FOUND)**			**MASS (m/z) M^+ Peak**
			C%	**H%**	**N%**	
CA-1	3374, 3229 (NH), 1638 (C=O), 657 (C-Cl)	1.77, 2.43 (m, CH_2, 2H), 2.35, 2.64 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.62 (qn, CH, 1H), 5.49 (s, NH, 2H), 7.10-7.18 (m, ArH, 3H)	63.01 62.86	5.77 5.76	6.68 6.70	210
CA-5	3294 (NH), 1631 (C=O), 658 (C-Cl)	1.28 (d, CH_3, 6H), 1.80, 2.40 (m, CH_2, 2H), 2.33, 2.61 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.87 (m, CH, 1H), 5.26 (s, NH, 1H), 7.10-7.18 (m, ArH, 3H)	66.79 66.58	7.21 7.23	5.56 5.57	252
CA-7	3281 (NH), 1640 (C=O), 658 (C-Cl)	0.97 (t, CH_3, 3H), 1.31 (m, CH_2, 4H), 1.52 (qn, CH_2, 2H), 1.79, 2.45(m, CH_2, 2H), 2.37, 2.66 (dd, CH_2, 2H), 2.86 (m, CH_2, 2H), 3.19 (t, CH_2, 2H), 3.64 (qn, CH, 1H), 5.40 (s, NH, 1H), 7.09-7.17 (m, ArH, 3H)	68.68 68.90	7.93 7.91	5.01 4.99	280

CA-9	3310 (NH), 1640 (C=O), 658 (C-Cl) cm^{-1} MS (m/z): 278	1.52 (m, CH_2, 4H) 1.86 (m, CH_2, 4H), 1.79, 2.42 (m, CH_2, 2H), 2.33, 2.64 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.61 (qn, CH, 1H), 3.68 (qn, CH, 1H), 5.32 (s, NH, 1H), 7.11-7.18 (m, ArH, 3H)	69.18 69.41	7.26 7.29	5.04 5.06	278
CA-11	1624 (C=O), 657 (C-Cl) 663 cm^{-1}	1.65 (m, CH_2, 6H),1.75, 2.45 (m, CH_2, 2H), 2.72, 2.46 (dd, CH_2, 2H), 2.85 (m, CH_2, 2H), 3.37 (t, CH_2, 4H), 3.66 (qn, CH, 1H), 7.10-7.17 (m, ArH, 3H)	69.18 69.31	7.26 7.29	5.04 5.06	278
CA-14	3306 (NH), 1642 (C=O), 658 (C-Cl) cm^{-1}	1.77, 2.40 (m, CH_2, 2H), 2.32, 2.57 (dd, CH_2, 2H), 2.84 (m, CH_2, 2H), 3.65 (qn, CH, 1H), 4.46 (s, Bnz CH_2, 2H), 5.81 (s, NH, 1H), 7.09-7.17 (m, ArH, 3H), 7.31 (m, ArH, 5H)	72.11 72.33	6.05 6.06	4.67 4.69	300
CA-15	3282 (NH), 1648 (C=O), 663 (C-Cl) cm^{-1}	(t, 1.81, 2.40 (m, CH_2, 2H), 2.47, 2.75 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.24 (s, NH, 1H), 7.10-7.21 (m, ArH, 4H), 7.31-7.48 (m, ArH, 4H)	71.45 71.67	5.64 5.66	4.90 4.92	286
CA-16	3306 (NH), 1642 (C=O), 662 (C-Cl)	1.80, 2.44 (m, CH_2, 2H), 2.47, 2.76 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.17 (s, NH, 1H), 7.08-7.33 (m, ArH, 6H), 7.64 (s, ArH, 1H)	63.76 63.89	4.72 4.74	4.37 4.39	320

Contd….

Table 5.6 Contd.

CA-20	3320 (NH), 1648 (C=O), 659 (C-Cl) cm^{-1}	1.84, 2.39 (m, CH_2, 2H), 2.30 (s, CH_3, 3H), 2.47, 2.74 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.66 (qn, CH, 1H), 7.10 (s, NH, 1H), 7.05-7.16 (m, ArH, 5H), 7.45 (d, ArH, 2H)	72.11 72.31	6.05 6.03	4.67 4.69	300
CA-24	3321 (NH), 1650 (C=O), 658 (C-Cl) cm^{-1};	1.83, 2.46 (m, CH_2, 2H), 2.53, 2.81 (dd, CH_2, 2H), 2.88 (m, CH_2, 2H), 3.71 (qn, CH, 1H), 7.31 (s, NH, 1H), 7.10-7.30 (m, ArH, 4H), 8.17-8.51 (m, ArH, 3H)	67.02 67.23	5.27 5.25	9.77 9.73	287
CA-25	3330 (NH), 1648 (C=O), 660 (C-Cl) cm^{-1}	1.80, 2.42 (m, CH_2, 2H), 2.49, 2.79 (dd, CH_2, 2H), 2.87 (m, CH_2, 2H), 3.70 (qn, CH, 1H), 7.28 (s, NH, 1H), 7.11-7.19 (m, ArH, 3H), 7.65-8.34 (m, ArH, 4H)	67.02 66.86	5.27 5.29	9.77 9.80	287

5.2.3. RESULTS AND DISCUSSION

The starting reagent for the preparation of the key intermediate, 6-chloro-2,3-dihydro-1*H*-inden(-1-yl)acetic acid **(V-XI)** was 3-chlorobenzaldehyde **(V-VII)**. Compound **V-VII** was reacted with two moles of ethylacetoacetate (EAA) in presence of catalytic amount of piperidine at room temperature to obtain the bisacetoacetate **V-VIII**. The reaction mechanism is same as described in Figure 5.2. The IR spectrum of this bisacetoacetate shows C=O stretches at 1722 and 1682cm^{-1}. In the ^{1}H-NMR spectrum of compound **V-VIII** (Figure 5.12) because of the different configurational arrangement of benzylic proton (as shown for dimethoxy series in Figure 5.3) the signal from the neighboring CH groups are split into two doublets each. The two equivalent side chains of bisacetoacetate showed two different peaks; the chain closer to the chloro group appeared at a slightly higher ppm (more deshielding effect) as compared to the one facing away from the chloro group.

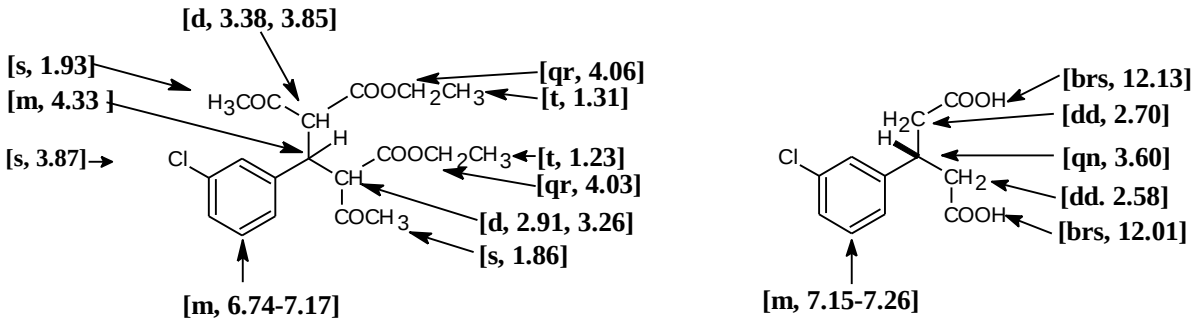


Figure 5.12 ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-VIII** and **V-IX**

Alkaline hydrolysis of **V-VIII** was carried out with 6N potassium hydroxide (KOH) in 50% ethanol to get the diacid **V-IX**. The reaction mechanism is same as shown in Figure 5.4. The IR spectrum of **V-IX** showed broad OH stretch at 3400-2200 cm^{-1} and C=O stretch at 1710 cm^{-1}. ^{1}H-NMR of **V-IX** (Figure 5.12) showed doublet of doublet at δ 2.58 and 2.70 ppm as well as quintet at 3.60 ppm for CH_2 and CH of glutaric acid side chain respectively, multiplet at δ 7.15-7.26 ppm for aromatic protons and small broad singlets at δ 12.13 and 12.01 ppm indicating the presence of carboxyl group. The doublet of doublet for CH2 of glutaric acid was obtained due to different configurational arrangement of the proton present on neighboring CH group. Both CH_2 groups are in different environment and hence behave differently therefore slight difference in δ values of the signals (dd) are observed (as shown in figure 5.12).

Compound **V-IX** was treated with aluminium chloride in presence of catalytic amount of sodium chloride when intramolecular Friedel-Craft's acylation and hence ring closure took

place to give the ketonic product **V-X**. The cyclization was also tried with polyphosphoric acid (PPA) but it gave poorer yield of the keto product with high impurity content. Various reaction conditions were tried to get the better yield of the cyclic oxo compound (Table 5.7 and 5.8). The compound **V-IX** gets deactivated towards the cyclisation by the presence of chloro group at the meta position. Hence aluminium chloride being a stonger lewis acid gave better yields in carrying the cyclization of **V-IX** in comparison to PPA.

Table 5.7. Cyclization of 3-(3-chlorophenylglutaric acid) using $AlCl_3$/ NaCl

S.No.	Temperature(°C)	Time*(min)	Yield (%)
1.	130	0	38.5
2.	160	15	50.54
3.	160	30	56.8
4.	160	60	40.57
5.	180	15	60.85
6.	180	30	63.88
7.	180	60	56.80
8.	190	15	56.80
9.	190	30	50.71
10.	200	15	56.8
11.	200	30	48.74

* Time after cessation of liberation of HCl gas from the reaction mixture
Shaded area shows the most optimum reaction conditions
The oxo compound after single recrystallisation from dilute acetic acid gave melting point of 188-190°C.

Table 5.8. Cyclization of 3-(3-chlorophenylglutaric acid) using Polyphosphoric acid (PPA)

S.No.	3-ClGA : PPA (w /w)	Time (min)	Temperature(°C)	Yield (%)
1.	1 : 25	8hrs	100	26.3
2.	1 : 15	10	120	30.45
3.	1 : 15	10	130	42.6
4.	1 : 15	10	140	25.6

Contd…

Table 5.8. Contd.

5.	1 : 15	10	150	17.05
6.	1 : 15	5	130	56.8
7.	1 : 15	15	130	40.9
8.	1 : 15	30	130	29.8
9.	1 : 15	3	130	44.7
10.	1 : 10	5	130	46.9
11.	1 : 20	5	130	51.5
12.	1 : 25	5	130	40.5

Shaded area shows the most optimum reaction conditions
The oxo compound after single recrystallization from dilute acetic acid gave broad melting point range (176-184°C)

In this reaction sodium chloride is added to aluminium chloride to lower the melting point of the latter and hence to lower the reaction temperature and also the reaction time[159]. Organic reactions have been performed in the binary alkali-metal chloride-aluminium chloride salts, which are lower melting ionic liquids[160]. It has been reported that at higher temperatures the aluminium chloride can cause intramolecular cyclization of the acids to oxo compounds without formation of the acyl halide.

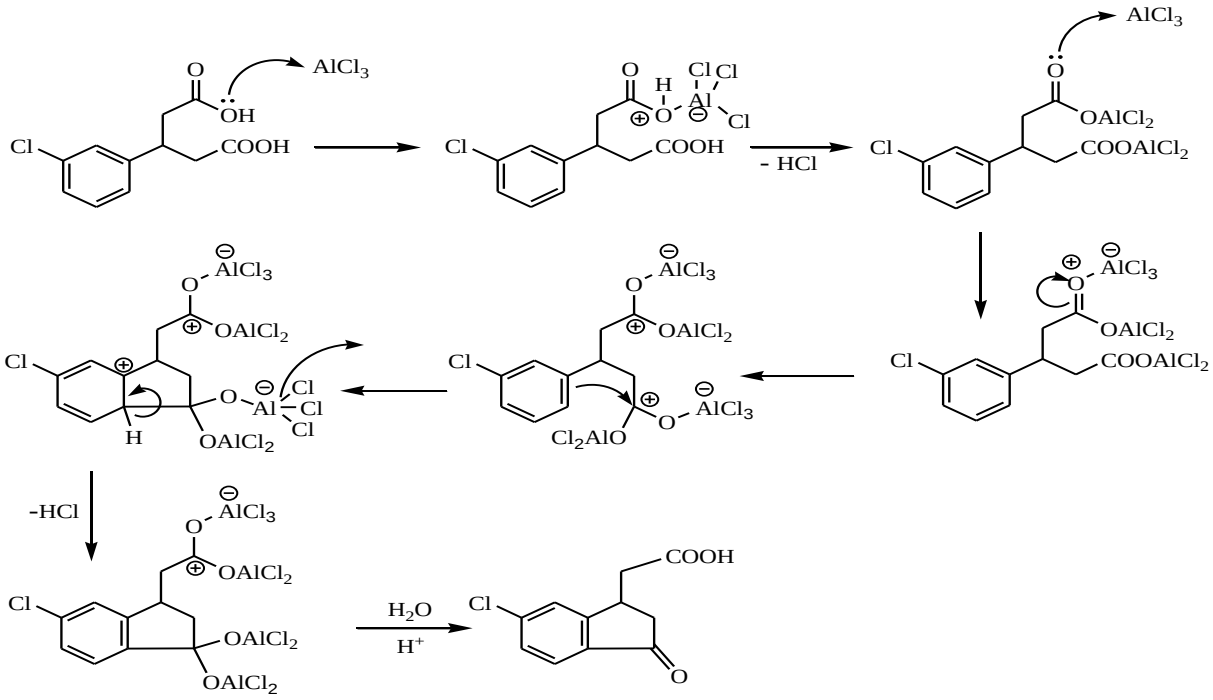


Figure 5.13. Possible reaction mechanism for cyclization of compound **V-IX**[161].

The IR spectrum of compound **V-X** shows broad OH stretch at 3400-2400 cm^{-1}, two carbonyl stretching at 1680, 1713 cm^{-1} and C—Cl stretch at 657 cm^{-1} . Presence of doublet of doublet at 2.46 and 2.58 ppm and doublet of doublet at 2.84 and 2.99 corresponding to CH_2 of side chain and CH_2 of the indan ring respectively as well as the presence of quintet at 3.79 ppm indicates the ring closure and hence formation of compound **V-X** (Figure 5.14). The presence of aromatic protons (doublets at 7.38 and 7.65 ppm and singlet at 7.59) and acid protons (broad singlet at 10.91 ppm) further supports the proposed structure of compound **V-X**.

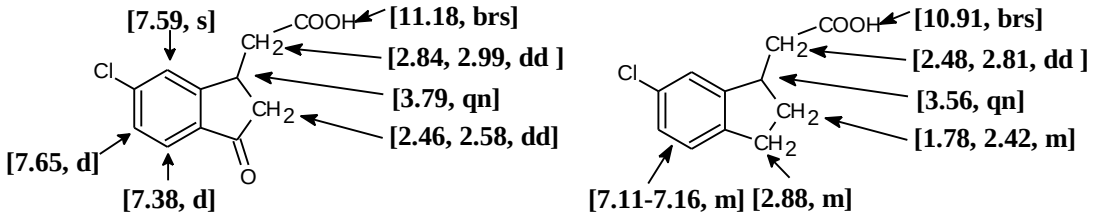


Figure 5.14. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-X** and **V-XI**

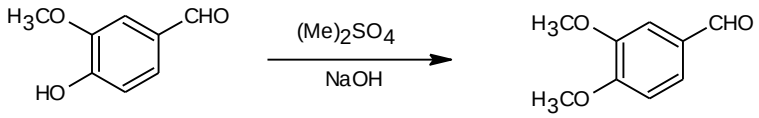
The ketone group of compound **V-X** was removed using Clemmensen's reduction to give 6-chloro-2,3-dihydro-1*H*-inden(-1-yl)acetic acid (**V-XI)**. The mechanism is the same as shown in Figure 5.8. The IR spectrum shows broad OH stretch at 3400-2400, C=O stretch at 1700 and C—Cl stretch 657cm-^{1}. Because of the different conformational arrangement of proton present on CH of indan ring both neighbouring CH_2 peaks got split into two peaks (two doublets of doublet at 2.48 and 2.81 ppm and two multiplets at 1.78 and 2.42 ppm). The presence of multiplet at 2.88 ppm equivalent to two protons in addition to peaks obtained for compound **V-XI** indicates the reduction of carbonyl group and formation of indan ring system. The presence of aromatic protons (multiplet at 7.11-7.16 ppm) and proton of COOH group (small broad singlet at 11.18 ppm) further supports the structure of compound **V-XI**.

Compound **V-XI** was treated with oxalyl chloride in presence of catalytic amount of dimethylformamide to convert carboxyl group of **V-XI** into acyl chloride. The acyl halide thus obtained was not isolated or characterized. It was used directly in the next step. The acyl halide was reacted with various primary and secondary amines under Schotten- Baumann conditions for the formation of desired amides (**CA-1 – CA-25**). The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides respectively. Also disappearance of broad OH stretch at 3400-2400 cm^{-1} indicates the formation of amide bond. The formation of amides was further confirmed by presence of a small singlet at 5-8 ppm in the 1-H NMR spectrum. In most of the cases there was an overlap of two peaks at around 2.3-2.5 corresponding to doublet of doublet and multiplet. The amide peak overlapped with the aromatic peaks around 7.1- 7.3 in case of many aromatic amides. In the mass spectrum of compound **V-XI,** M^+ peak was observed as the base peak at 210 and M+ 2 peak at 212. In most of the amides M^+ peak was observed as the base peak and the corresponding M+2 peaks were also observed.

5.3. (5, 6-DIMETHOXY-2, 3-DIHYDRO-1*H*-INDEN-1- YL)CARBOXLIC ACID AMIDES

5.4.2. SYNTHESIS

5.3.1.1. 3, 4-Dimethoxybenzaldehyde (V-II).

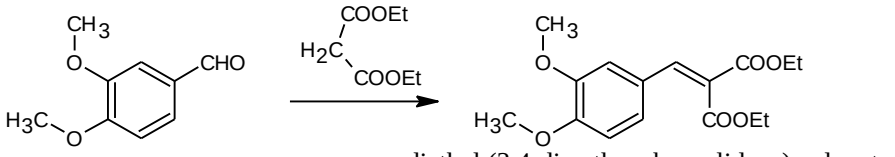


4-hydroxy-3-methoxybenzaldehyde
V-I

3,4-dimethoxybenzaldehyde
V-II

Commercially available vanillin (**V-I**) (15.2g, 0.1 mol) was placed in a 500ml three-necked flask equipped with a magnetic stirrer, two dropping funnels and a reflux condenser. One funnel was charged with potassium hydroxide (8.2g in 12ml) and the other funnel with purified dimethyl sulphate (12 ml, 0.104 mol). Vanillin was melted by warming on water bath, and then KOH was added at the rate of two drops a second. After 20 seconds dimethyl sulphate was also added at the same rate. The heating was stopped after few minutes as the mixture continued to reflux gently from the heat of reaction. The reaction mixture was vigorously stirred throughout. After all reagents have been added the reaction mixture became turbid and separated into two layers. Pale reddish brown color of the reaction mixture indicating the alkaline condition was maintained throughout the reaction. The yellow reaction mixture was poured into a porcelain basin and was left overnight. The hard crystalline mass thus obtained was ground, washed thoroughly with cold water to make it free of basicity, filtered and dried in a vacuum desiccator to get veratraldehyde (**V-II**) in 70-75% yield, mp 43-44°C.

5.3.1.2. Diethyl (3,4-dimethoxybenzylidene)malonate[162] (V-XII).



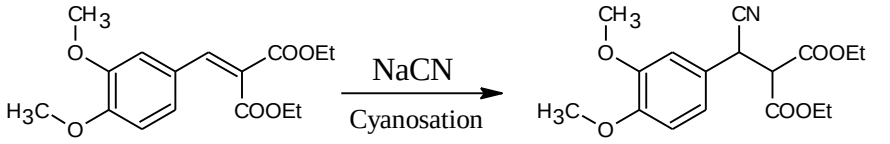
3,4-dimethoxybenzaldehyde
V-II

diethyl (3,4-dimethoxybenzylidene)malonate
V-XII

To the veratraldehyde (**V-II**) (16.6g, 0.1mol) in dry round bottomed flask diethylmalonate (20g, 0.104 mol) was added. To this reaction mixture 1ml of piperidine, 1ml of glacial acetic acid and 100ml of dry benzene was added. The reaction mixture was refluxed in a Dean Stark's apparatus. The reaction was carried until no more water collects in the Dean Stark's apparatus (about 18 hrs). The reaction mixture was washed with water and dried over anhydrous sodium sulphate. Benzene was then removed from the reaction mixture under reduced pressure. The remaining brownish oily liquid was set for vacuum distillation.

Benzylidene malonate, **V-XII,** was distilled at 205-210 °C at 6-7mm Hg as a clear liquid. The overall yield was 85-90%. IR (cm^{-1}) (thin film): 1718 (C=O stretching), 1043, 1260 (C—O stretch of OCH_3); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.26 (t, CH_3, 3H), 1.29 (t, CH_3, 3H), 3.83 (s, OCH_3, 3H), 3.85 (s, OCH_3, 3H), 4.24 (qr, CH_2, 2H), 4.30 (qr, CH_2, 2H), 4.80 (s, CH, 1H), 6.89-7.11 (m, ArH, 3H)

5.3.1.3. Diethyl [cyano(3,4-dimethoxyphenyl)methyl]malonate[162] (V-XIII).

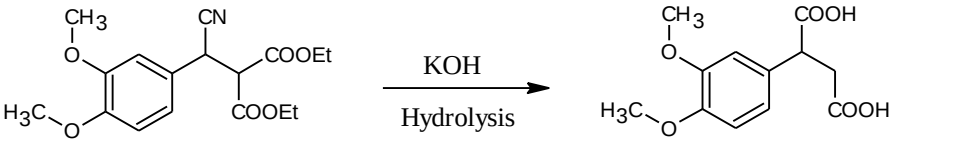


diethyl (3,4-dimethoxy benzylidene)malonate
V-XII

diethyl [cyano(3,4-dimethoxy phenyl)methyl]malonate
V-XIII

To 30.9 g of benzylidene malonate (**V-XII**) (0.1mol) in dry round bottomed flask 5g of sodium cyanide (0.102 mol) dissolved in 90% alcohol was added. The reaction mixture was refluxed for 18 hrs at 100 °C and then alcohol was distilled at reduced pressure. The formed cyanomalonate, **V-XIII,** was not purified and was used directly for the next step.

5.3.1.4. 2-(3, 4-dimethoxyphenyl)succinic acid (V-XIV).

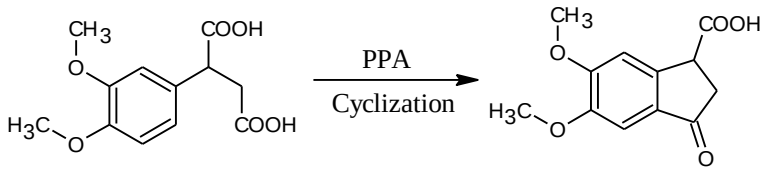


diethyl [cyano(3,4-dimethoxy phenyl)methyl]malonate
V-XIII

2-(3,4-dimethoxyphenyl)succinic acid
V-XIV

To the above reaction mixture 25.2g of KOH dissolved in 75ml of water (6N, 0.45mol) was added. The reaction mixture was refluxed for 16 hrs and then was allowed to cool. The cooled reaction mixture was slowly poured on to ice-sulphuric acid mixture with stirring when the diacid (**V-XIV**) precipitated out. The crude product obtained was filtered, dried and recrystallized from hot water to get the desired succinic acid. Yield 75-80%; mp 171-173°C; IR (cm^{-1}) (KBr): 3400-2400 (OH stretch) 1700 (C=O stretching), 1038, 1256 (C—O stretch of OCH_3); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 2.60 (dd, CH_2, 1H), 3.10 (dd, CH_2, 1H) 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 3.96 (dd, CH, 1H), 6.81-6.88 (m, ArH, 3H), 11.19 (brs, COOH, 1H), 11.26 (brs, COOH, 1H).

5.3.1.5. (5,6-Dimethoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XV)



2-(3,4-dimethoxyphenyl)succinic acid

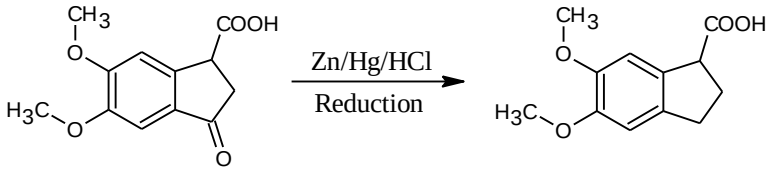
V-XIV

(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XV

Cyclization of compound **V-XIV** was effected by treating the powdered acid (15g, 0.059 mol) with 375 g of polyphosphoric acid (PPA) [1:25 ratio, w/w] on a steam bath for 4 hours with occasional stirring. After decomposition of the hot reaction mixture with crushed ice, the keto acid (**V-XV**) was isolated by extraction with chloroform. The solvent was distilled off to get the crude keto acid. The crude product was finally recrystallized from hot water to get the pure compound. Yield 70-75%; mp 190-191°C; IR (cm^{-1}) (KBr): 3400-2200(br OH stretch), 1726, 1650 (C=O stretching), 1042, 1225 (C—O stretch of OCH_3), ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 2.84 (dd, CH_2, 1H), 3.10 (dd, CH_2, 1H), 4.17 (dd, CH, 1H), 3.91 (s, OCH_3, 3H), 3.98 (s, OCH_3, 3H), 7.21 (s, ArH, 1H), 7.16 (s, ArH, 1H), 10.34 (brs, COOH, 1H).

5.3.1.6. (5,6-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XVI).



(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XV

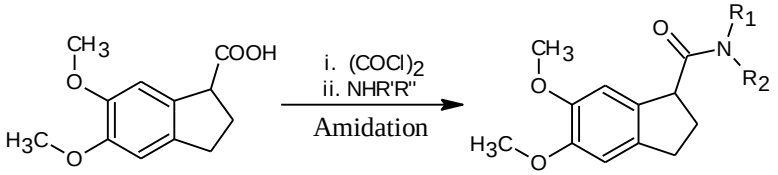
(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XVI

Compound **V-XV** was subjected to Clemmensen's reduction to get the reduced product **V-XVI.** The keto acid, **V-XV,** (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on steam bath until the reaction mixture became keto-negative (about 8 hrs). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free of acidity and then dried over anhydrous sodium sulfate and was finally distilled off to get the reduced acid. The analytical product was obtained on recrystallization from benzene. Yield 70-75%; mp 114-115^{o}C ; IR (cm^{-1}) (KBr): 3400-2400 (br OH stretch), 1700(C=O stretching), 1037, 1220 (C—O stretch of OCH_3); ^{1}H-NMR:δ (ppm) ($CDCl_3$, TMS): 2.36 (m, CH_2, 1H),

2.45 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 3.06 (m, CH_2, 1H), 4.02 (dd, CH, 1H), 3.86 (s, OCH_3, 3H), 3.87 (s, OCH_3, 3H), 6.77 (s, ArH, 1H), 6.94 (s, ArH,1H),10.26 (brs,COOH,1H)

5.3.1.7. General Method for the synthesis of amide derivatives (DC-1 – DC-22).



(5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1yl)carboxylic acid

V-XVI

Amide Derivatives

DC-1 - DC-22

A solution of compound **V-XVI** was made in dry dichloromethane and catalytic amount of dimethylformamide was added. This solution was treated with oxalyl chloride in 1:2.5 molar ratios under ice cold conditions. The reaction flask was fitted with a calcium chloride guard tube to protect the reaction mixture from moisture. The solution was allowed to stand for 24 hours at room temperature with occasional stirring. Excess oxalyl chloride was removed by co-distillation with dry benzene under reduced pressure. The acyl halide thus obtained was not isolated or characterized and was used directly in the next step. To a solution of the acyl halide in dry dichloromethane was added a mixture of triethylamine (1.1mol) and the appropriate amine in dichloromethane with constant stirring under ice-cold conditions. The mixture was kept at ambient temperature for 12 hours and was kept protected from moisture using a calcium chloride guard tube. The resulting reaction mixture was then extracted with 0.1N HCl, water, saturated solution of $NaHCO_3$, brine and water. The organic phase was dried with anhydrous sodium sulfate and then distilled off to obtain the title compounds (**DC-1 – DC-22**).

All the synthesized amide derivatives were recrystallized from appropriate solvents and their melting points were determined. Their nomenclature and physical data are given in Table 5.9 and Table 5.10 respectively. For few selected compounds the spectral (^{1}H-NMR and Mass) and the elemental analyses were done and are shown in Table 5.11.

5.4.3. NOMENCLATURE AND PHYSICAL DATA OF (5,6-DIMETHOXY-2,3-DIHYDRO-1*H*-INDEN-1- YL)CARBOXYLIC ACID AMIDES

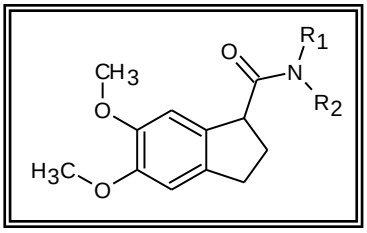


Table 5.9. Nomenclature of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

COMPOUND	R'	R''	NOMENCLATURE (IUPAC)
DC-1	H	H	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -2	H	Me	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-methylcarboxamide
DC -3	H	Et	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylcarboxamide
DC -4	H	n-Pr	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylcarboxamide
DC -5	H	n-Bu	*N*-butyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -6	H	n-amyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pentylcarboxamide
DC -7	H	n-hexyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylcarboxamide
DC -8	H	cyclopentyl	*N*-cyclopentyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -9	H	cyclohexyl	*N*-cyclohexyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -10	--piperidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carbonyl]piperidine
DC -11	--piperazine--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carbonyl]piperazine
DC -12	H	Benzyl	*N*-benzyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -13	H	Phenyl (C_6H_5)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylcarboxamide
DC -14	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -15	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -16	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -17	H	C_6H_4(m-CH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)carboxamide
DC -18	H	C_6H_4(p-CH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methylphenyl)carboxamide
DC -19	H	C_6H_4(p-OCH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)carboxamide
DC -20	H	C_6H_4(p-NO_2)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)carboxamide
DC -21	H	3-pyridyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylcarboxamide
DC -22	H	4-pyridyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylcarboxamide

Table 5.10. Physical data of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

COMPOUND	RECRYSTALLIZATION SOLVENT	YIELD (%)	M.P (°C)	MOLECULAR FORMULA	FORMULA WEIGHT	Calc. Log P
DC-1	dilute alcohol	58.0	150-152	$C_{12}H_{15}NO_3$	221	1.23
DC -2	dilute alcohol	59.1	110-112	$C_{13}H_{17}NO_3$	235	1.76
DC -3	dilute alcohol	61.3	103-105	$C_{14}H_{19}NO_3$	249	2.25
DC -4	EtOAc/hexane	62.5	92-94	$C_{15}H_{21}NO_3$	263	2.71
DC -5	EtOAc/hexane	61.8	104-106	$C_{16}H_{23}NO_3$	277	3.16
DC -6	dilute alcohol	63.4	96-98	$C_{17}H_{25}NO_3$	291	3.62
DC -7	dilute alcohol	65.3	96-97	$C_{18}H_{27}NO_3$	305	4.09
DC -8	alcohol	62.7	169-170	$C_{17}H_{23}NO_3$	289	3.12
DC -9	alcohol	61.6	174-176	$C_{18}H_{25}NO_3$	303	3.60
DC -10	EtOAc/hexane	59.4	132-134	$C_{17}H_{23}NO_3$	289	3.00
DC -11	benzene	60.8	228-230	$C_{16}H_{22}N_2O_3$	290	1.43
DC -12	alcohol	64.8	134-136	$C_{19}H_{21}NO_3$	311	2.80
DC -13	dilute alcohol	71.7	138-139	$C_{18}H_{19}NO_3$	297	2.85
DC -14	dilute alcohol	69.3	131-132	$C_{18}H_{18}NO_3Cl$	332	3.70
DC -15	alcohol	70.9	191-192	$C_{18}H_{18}NO_3Cl$	332	3.65
DC -16	alcohol	68.4	196-198	$C_{18}H_{18}NO_3Br$	376	3.67
DC -17	alcohol	70.6	122-123	$C_{19}H_{21}NO_3$	311	3.25
DC -18	alcohol	71.5	163-164	$C_{19}H_{21}NO_3$	311	3.35
DC -19	alcohol	69.2	162-164	$C_{19}H_{21}NO_4$	327	2.98
DC -20	alcohol	60.4	133-135	$C_{18}H_{18}N_2O_5$	342	2.95
DC -21	dilute alcohol	62.7	174-176	$C_{17}H_{18}N_2O_3$	298	2.06
DC -22	dilute alcohol	64.8	120-122	$C_{17}H_{18}N_2O_3$	298	2.08

Calc. Log P is the calculated log P (from www. logp. com)

Table 5.11. Spectral and elemental analyses data of (5, 6-dimethoxy-2, 3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

			ELEMENTAL ANALYSES (CALCULATED/FOUND)	MASS

COMPOUND	IR (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, $CDCl_3$, TMS)	C%	H%	N%	(m/z) M^+ Peak
DC-6	3314 (NH str), 1648 (C=O str), 1263, 1059 (OCH_3)	0.87 (t, CH_3, 3H), 1.27 (m, CH_2, 4H), 1.44 (qn, CH_2, 2H), 2.28 (m, CH_2, 1H) 2.48 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 2.96 (m, CH_2, 1H), 3.24 (t, CH_2, 2H), 3.86 (s, OCH_3, 3H), 3.88(s, OCH_3, 3H), 3.92 (dd, CH, 1H), 5.42 (s, NH, 1H), 6.79 (s, ArH, 1H), 6.81 (s, ArH, 1H)	70.07 69.85	8.65 8.62	4.81 4.82	291

Contd….

Table 5.11 Contd.

DC-8	3321 (NH), 1643 (C=O), 1269, 1074 (OCH_3)	1.49 (m, CH_2, 4H), 1.62 (m, CH_2, 2H), 1.84 (m, CH_2, 2H), 2.35 (m, CH_2, 1H) 2.47 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 2.98 (m, CH_2, 1H), 3.56 (qn, CH_2, 1H), 3.85 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 4.01 (dd, CH, 1H), 5.34 (s, NH, 1H), 6.78 (s, ArH, 1H), 6.80 (s, ArH, 1H)	70.56 70.79	8.01 8.04	4.84 4.83	289
DC-11	3341 (NH of piperazine), 1638 (C=O), 1256, 1059 (OCH_3)	2.09 (s, NH of piperazine, 1H), 2.30 (m, CH_2, 1H) 2.49 (m, CH_2, 1H), 2.79 (t, CH_2, 4H), 2.89 (m, CH_2, 1H), 3.01 (m, CH_2, 1H), 3.31 (t, CH_2, 4H), 3.85 (s, OCH_3, 3H), 3.87 (s, OCH_3, 3H), 3.99 (dd, CH, 1H), 6.80 (s, ArH, 1H), 6.81 (s, ArH, 1H)	66.18 66.38	7.64 7.66	9.65 9.62	290
DC-12	3327 (NH), 1646 (C=O), 1273, 1085 (OCH_3)	2.34 (m, CH_2, 1H) 2.49 (m, CH_2, 1H), 2.87 (m, CH_2, 1H), 2.96 (m, CH_2, 1H), 3.84 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 4.00 (dd, CH, 1H), 4.42 (s, CH_2, 2H), 5.66 (s, NH, 1H), 6.79 (s, ArH, 1H), 6.81 (s, ArH, 1H), 7.06-7.15 (m, ArH, 5H)	73.29 73.01	6.80 6.78	4.50 4.51	311
DC-14	3311 (NH), 1645 (C=O), 1259, 1061 (OCH_3)	2.44 (m, CH_2, 1H) 2.58 (m, CH_2, 1H), 2.93 (m, CH_2, 1H), 3.07 (m, CH_2, 1H), 3.86 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 4.13 (dd, CH, 1H), 6.85 (s, ArH, 1H), 6.87 (s, ArH, 1H), 7.01 (d, ArH, 1H) 7.10 (s, NH, 1H), 7.21 (t, ArH, 1H), 7.49 (d, ArH, 1H), 7.64 (s, ArH, 1H)	65.16 65.41	5.47 5.49	4.22 4.23	332
DC-18	3318 (NH), 1651 (C=O), 1260, 1058 (OCH_3)	1.57 (s, CH_3, 3H), 2.40 (m, CH_2, 1H) 2.57 (m, CH_2, 1H), 2.91 (m, CH_2, 1H), 3.05 (m, CH_2, 1H), 3.85 (s, OCH_3, 3H), 3.90 (s, OCH_3, 3H), 4.01 (dd, CH, 1H), 6.85 (s, ArH, 1H), 6.86 (s, ArH, 1H), 7.09 (s, NH, 1H), 7.10 (d, ArH, 2H), 7.33 (d, ArH, 2H),	73.29 73.51	6.80 6.82	4.50 4.49	311
DC-20	3324 (NH), 1656 (C=O),	2.42 (m, CH_2, 1H) 2.58 (m, CH_2, 1H), 2.93 (m, CH_2, 1H), 3.07 (m, CH_2, 1H),	63.15 62.94	5.30 5.28	8.18 8.21	342

	1264, 1077 (OCH_3)	3.87 (s, OCH_3, 3H), 3.90 (s, OCH_3, 3H), 4.07 (dd, CH, 1H), 6.78 (s, ArH, 1H), 6.80 (s, ArH, 1H), 7.13 (s, NH, 1H), 7.86 (d, ArH, 2H), 8.09 (d, ArH, 2H),				
DC-22	3321 (NH), 1653 (C=O), 1269, 1072 (OCH_3)	2.41 (m, CH_2, 1H) 2.56 (m, CH_2, 1H), 2.88 (m, CH_2, 1H), 3.05 (m, CH_2, 1H), 3.85 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 4.05 (dd, CH, 1H), 6.77 (s, ArH, 1H), 6.79 (s, ArH, 1H), 7.08 (s, NH, 1H), 7.65 (d, ArH, 2H), 8.38 (d, ArH, 2H)	68.44 68.63	6.08 6.10	9.39 9.43	298

5.2.6. RESULTS AND DISCUSSION:

The starting reagent for the preparation of the key intermediate, 5,6-dimethoxy-2,3-dihydro-1*H*-inden(-1-yl)carboxylic acid was vanillin. The hydroxyl group of vanillin was methylated by dimethyl sulphate under alkaline conditions to get the methylated product veratraldehyde as detailed earlier. The veratraldehyde obtained was reacted with diethylmalonate in presence of catalytic amount of piperidine, an organic base. The base, piperidine, abstracts the acidic methylene proton from the diethylmalonate. The nucleophile thus generated attacks the carbonyl carbon leading to the formation of benzylidene malonate (**V-XII**) after elimination of one molecule of water (Figure 5.15).

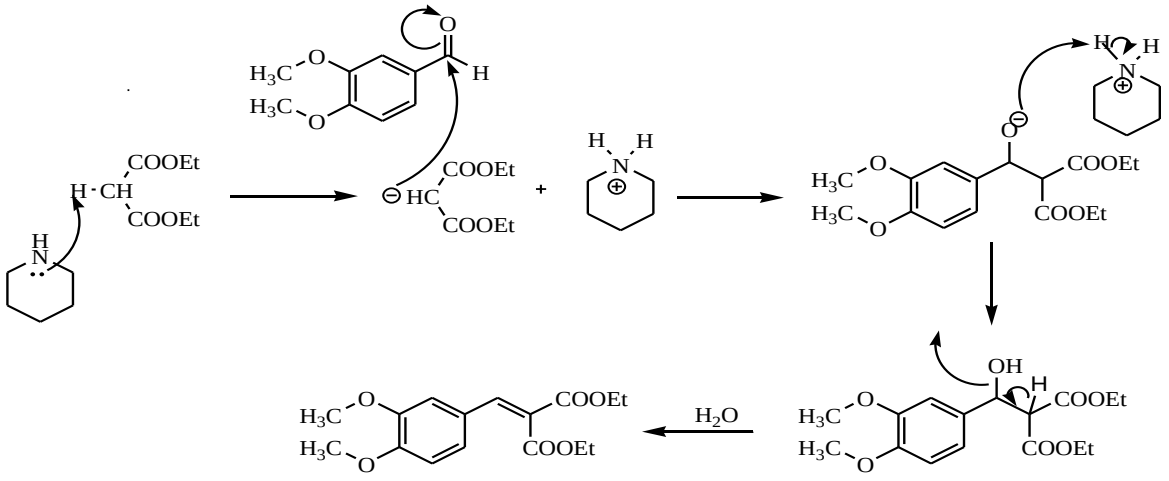


Figure 5.15. Possible reaction mechanism for the synthesis of compound **V-XII**

The IR spectrum of compound V-XII shows the C=O stretching at 1718 cm^{-1} indicating the presence carbonyl group. In ^{1}H-NMR the benzylidene proton is observed far downfield at 4.80 ppm, methoxyl protons at 3.83 and 3.85 ppm and aromatic protons are observed at 6.89-

7.11 ppm. For the side chain of diethyl malonate two triplets (1.26 & 1.29) and two quartet (4.24 & 4.30) are observed at slightly different δ value due to the presence of same protons in slightly different environment as shown in Figure 5.16

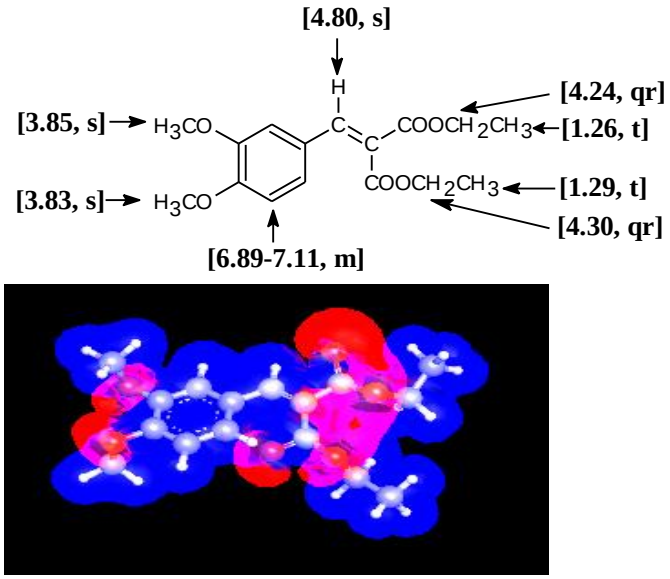


Figure 5.16. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) and electrostatic potential map of compound **V-XII**

The benzylidene malonate (**V-XII**) obtained was reacted with sodium cyanide when cyanosation took place at the double bond to give compound **V-XIII**. The mechanism of reaction is shown in Figure 5.17.

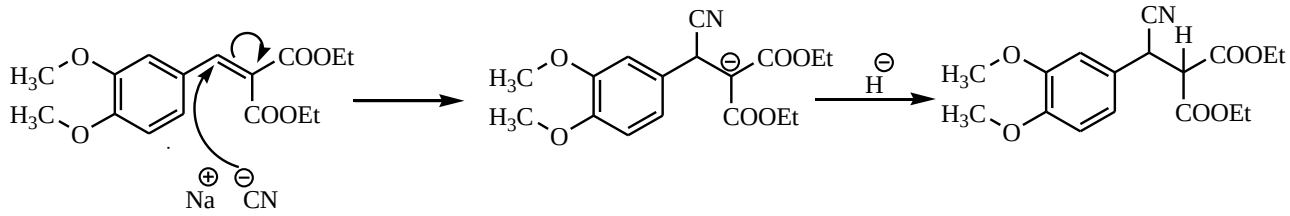


Figure 5.17. Possible reaction mechanism for the synthesis of compound **V-XIII**

The cyano group and the two ester groups were further hydrolyzed with 6N potassium hydroxide solution. The diacid thus obtained got converted into potassium salt due to excess potassium hydroxide in the reaction mixture. This potassium salt was neutralized with dilute hydrochloric acid under ice cold conditions to liberate the free diacid **V-XIV** (Figure 5.18).

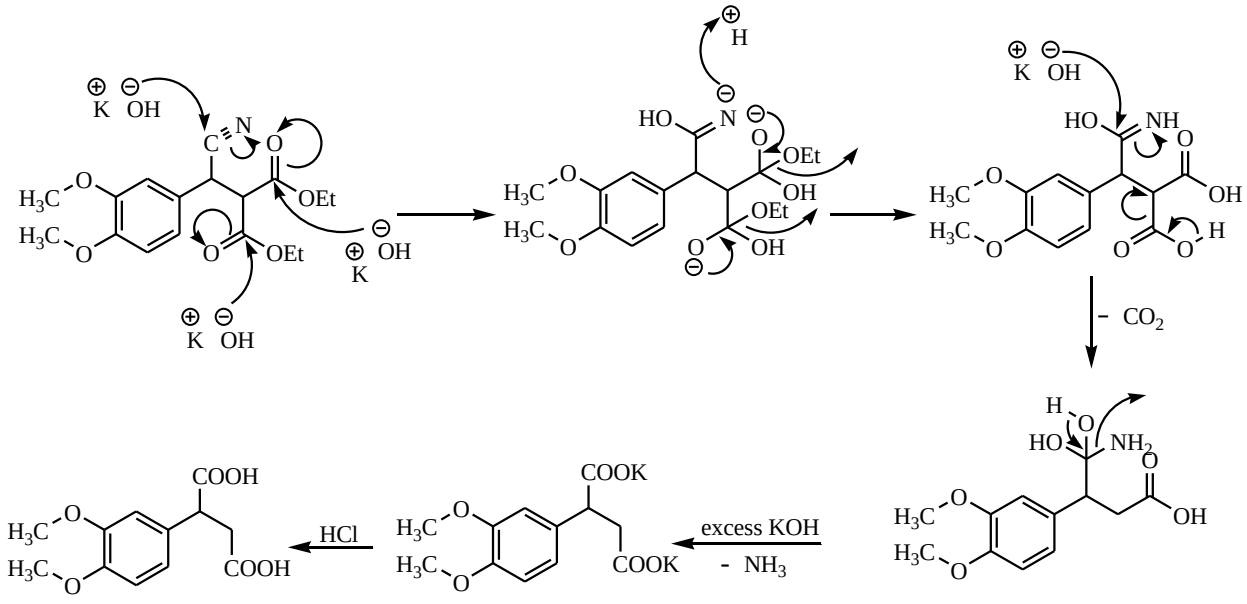


Figure 5.18. Possible reaction mechanism for the synthesis of compound **V-XIV**

The broad OH stretch at 3400-2400 cm^{-1} and C=O stretch at 1700 cm^{-1} indicated the presence of carboxylic group. The proton NMR showed broad singlet at 11.19 and 11.26 ppm indicating the presence of two carboxylic groups. Due to different configurational arrangements of protons on carbon 1 and 2 (as shown in figure) two doublets of doublets and single doublets of doublets were obtained for CH_2 and CH of succinic acid side chain respectively (Figure 5.19).

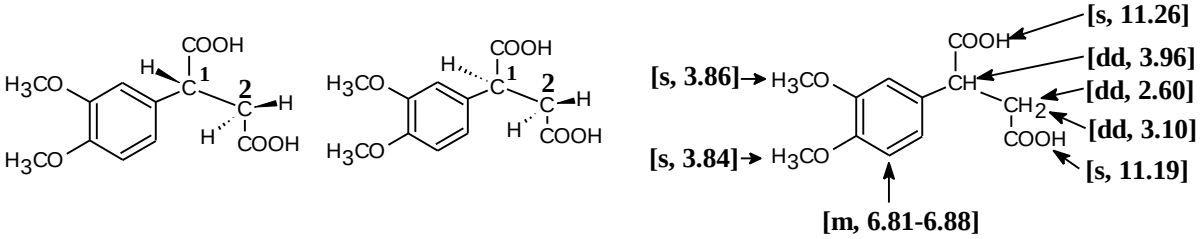


Figure 5.19. ^{1}H- NMR:δ (ppm) ($CDCl_3$, TMS) of compound **V-XIV**

The compound V-XIV was also synthesized through acrylate route[163] where **V-II** was reacted with ethylcyanoacetate in the presence of an organic base. The cyanoacrylate thus obtained was cyanosated to form dicyanopropanoate, which was further hydrolyzed to the required succinic acid **V-XIV.** However**,** the yields obtained by the malonate route were much better than the acrylate route.

The diacid (**V-XIV**) was treated with polyphosphoric acid when intramolecular Friedel Craft's cyclization took place to form a keto acid. The reaction mixture is poured onto ice water to decompose the excess of polyphosphoric acid. The reaction mechanism is similar to as shown in Figure 5.6. In case of synthesis of keto acid (**V-XV**) more PPA was required as compared to keto acid (**V-V**) because of lesser reactivity of succinic acid (**V-XIV**) towards cyclization. The compound **V-XIV** has only one COOH group available whereas in case of **V-V** both COOH groups are separated from the central ring by two carbons and hence are available for cyclization.

The IR spectrum of compound **V-XV** shows broad OH stretch at 3400-2200 and C=O stretching at 1726 and 1650cm^{-1} indicating the presence of carboxylic group, carbonyl of acid and carbonyl of keto functionalities respectively. The proton NMR showed two doublets of doublets for CH_2 group and a doublet of doublet for CH group. As shown in Figure 5.20 this appearance of signals at slightly different δ values was due to different arrangements of protons on carbon 1 and 2. The broad singlet at 10.34 indicated the presence of acidic proton of carboxylic acid group. The two aromatic protons showed singlets at δ values of 7.16 ppm and 7.21 ppm

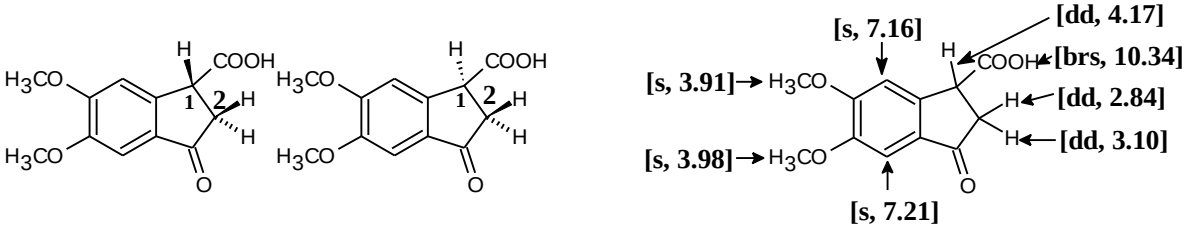


Figure 5.20. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XV**

The keto group was finally removed by Clemmenson's reduction to obtain the final 5,6-dimethoxy indan(-1-yl)carboxylic acid. The IR spectrum of this compound showed broad OH stretch at 3400-2200 and C=O stretching at 1700 indicating the presence of carboxylic group. The proton NMR showed two singlets at 3.86 and 3.87 equivalent to six protons indicating the presence of two methoxyl groups. The broad singlet equivalent to one proton indicated the presence of carboxylic group. Two multipets were obtained for each of the two CH_2 groups (carbon 2 and 3) and a doublet of doublet for CH (carbon 1) of the indan ring system (Figure 5.21). Such splitting pattern was observed due to different configurational arrangement of protons making the protons present on the same carbon to behave differently (in different environments)

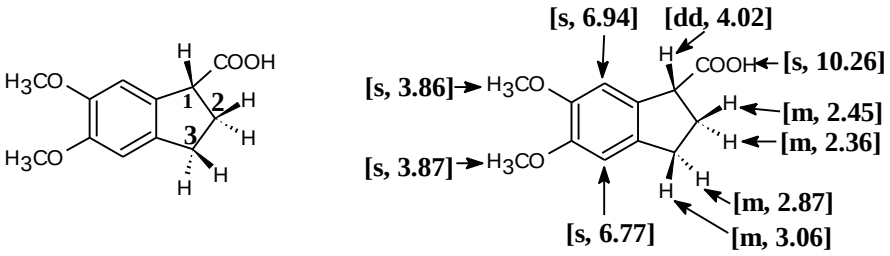


Figure 5.21. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XVI**

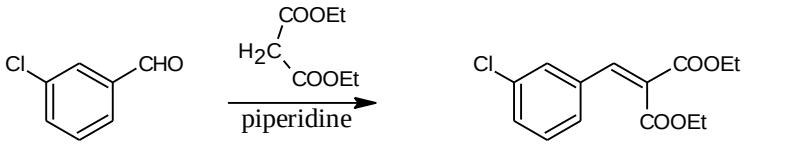
The acid obtained was converted to amides via acyl halide under Schotten- Baumann conditions.

The structure of amide derivatives were confirmed by the presence of two, one or no peak around 3300 cm^{-1} (NH stretch) corresponding to primary, secondary and tertiary amides respectively. Also disappearance of broad OH stretch at 3400-2400 cm^{-1} indicates the formation of amide bond. The formation of amides was further confirmed by presence of a small singlet at 5-6 ppm (aliphatic amide derivatives) and at 7-8 ppm (aromatic amides) in ^{1}H-NMR spectrum. In case of many aromatic amides the amide peak was overlapped with the aromatic peaks around 7.1- 7.3 ppm. In the mass spectrum of compound **V-XVI**, base peak was observed at 177 and M^+ peak at 222. However in amides M^+ peak was observed as the base peak.

5.5. (6-CHLORO-2, 3-DIHYDRO-1H-INDEN-1- YL)CARBOXYLIC ACID

5.5.1. SYNTHESIS

5.4.1.1. Diethyl (3-chlorobenzylidene)malonate (V-XVII).

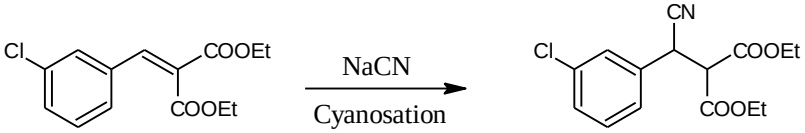


3-chlorobenzaldehyde
V-VII

diethyl (3-chlorobenzylidene)malonate
V-XVII

To 14.05g of m-chlorobenzaldehyde (0.1mol) in a dry round bottomed flask 20g of diethylmalonate (0.104 mol) was added. To this reaction mixture 1ml of piperidine, 1ml of glacial acetic acid and 100ml of dry benzene was added. The reaction mixture was refluxed in a Dean Stark's apparatus (for nearly 18hrs) until no more water collects. The reaction mixture was washed with water to remove the water soluble reactants and then it was dried over anhydrous sodium sulphate. The solvent (benzene) was removed from the reaction mixture under reduced pressure. The remaining brownish oily liquid was set for vacuum distillation. The product benzylidene malonate was distilled at 172-175 °C at 2mm Hg as a clear liquid. The overall yield was 80-85%. The IR spectrum (thin film) showed C-Cl stretch at 661cm^{-1} and C=O stretch at 1713cm^{-1}.

5.4.1.2. Diethyl [cyano(3-chlorophenyl)methyl]malonate (V-XVIII).

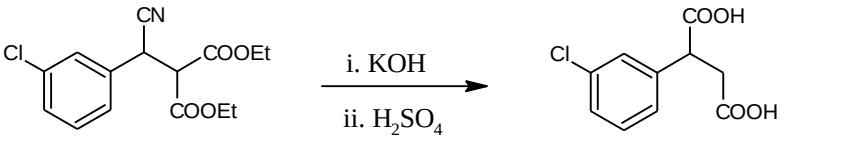


diethyl (3-chlorobenzyl idene)malonate
V-XVII

diethyl [cyano(3-chloro phenyl)methyl]malonate
V-XVIII

To 22.86 g of benzylidene malonate (0.1mol) in a dry round bottomed flask 5g of sodium cyanide (0.102 mol) dissolved in 90% alcohol was added. The reaction mixture was refluxed for 18 hrs at 100 °C on water bath and then alcohol was distilled out at reduced pressure. The formed cyanomalonate was not purified and was used directly for the next step.

5.4.1.3. 2-(3-chlorophenyl)succinic acid (V-XIX).



diethyl [cyano(3-chloro phenyl)methyl]malonate

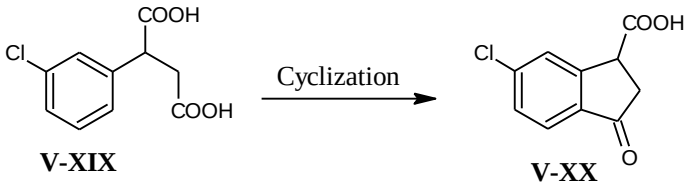
V-XVIII

2-(3-chlorophenyl)succinic acid

V-XIX

To the above reaction mixture 25.2g of KOH dissolved in 75ml of water (6N, 0.45mol) was added. The reaction mixture was refluxed for 16 hrs and then was allowed to cool. The cooled reaction mixture was slowly poured onto ice-sulphuric acid mixture with stirring when the diacid precipitated out. The crude product obtained was filtered, dried and recrystallized from hot water to get the desired succinic acid derivative. Yield 70-75%; mp 163-164°C; IR (cm^{-1}): 3400-2400(br OH stretch), 1703(C=O stretching), 641 (C-Cl stretch)

5.4.1.5. (6-chloro-3-oxo-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XX)



V-XIX **V-XX**

This reaction was tried using various reagents under varying reaction conditions. Following are the different cyclization reagents used for carrying out this intramolecular cyclization.

11. Phosphorous pentoxide
12. Methanesulphonic acid
13. Mixture of phosphorous pentoxide and methanesulphonic acid
14. Polyphosphoric acid(1:1)
15. Polyphosphoric acid(1:1.8)
16. Anhydrous aluminium chloride / sodium chloride
17. Acyl halide / anhydrous aluminium chloride in nitrobenzene as a solvent
18. Acyl halide / anhydrous aluminium chloride in dichloromethane as a solvent
19. Sulphuric acid
20. Heteropolyacid (Phosphotungstic acid)

These reactions were tried at various temperatures for varying durations of time. In most of the cases the starting acid (**V-XIX**) was recovered back and if drastic reaction conditions

(higher temperatures) were applied it resulted in charring of the compound. Taking into consideration the reaction mechanism for cyclization it can be seen that the electron withdrawing chloro group at meta position deactivates the ring system towards the intramolecular cyclisation (electrophilic substitution) as shown in Figure 5.22. Also in comparison to glutaric acid side chain the chances of cyclization are halved in case of succinic acid side chain.

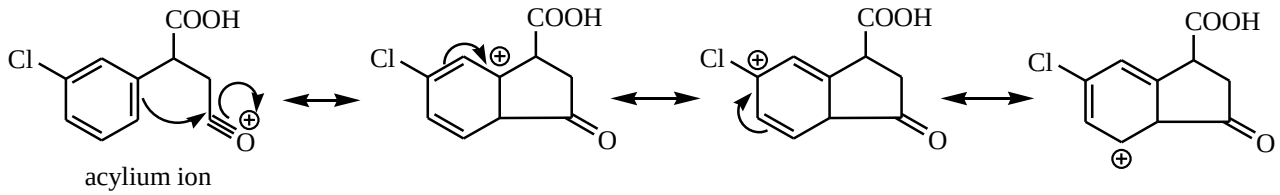


Figure 5.22. Mechanism for hindered cyclization of compound **V-IX**

It has been reported that ring closure of β-m- and - β-p-nitrophenylglutaric acid failed to undergo ring closure with (a) aluminium chloride in carbon disulphide or (b) aluminium chloride in nitrobenzene at 150°C. This free acid also failed to undergo ring closure with anhydrous hydrogen fluoride[149]. It has been reported that for cyclization of phenylsuccinic acid, the corresponding acyl halide was treated with aluminium chloride in nitrobenzene to yield (60%) indan-3-one-1-carboxylic acid. However the cyclization of phenylsuccinic acid was achieved in 22% yield by use of concentrated sulfuric acid[164].

5.5. 5-(CYCLOPENTYLOXY)-6-METHOXY-INDAN-1-ALKANOIC ACIDS.

Literature survey reveals that (17) 6-chloro-5-(cyclopentylmethyl)-indan-1-carboxylic acid, an isomer of 6-chloro-5-cyclohexylindan-1-carboxylic acid (clidanac), was found to be non-ulcerogenic after acute and chronic treatment in rats and monkeys. This compound did not show any mucosal damage in the rat stomach after single oral application up to highest tested dose of 400mg/kg p.o. whereas clidanac has been shown to be ulcerogenic in anti-inflammatory active doses (≤ 3mg/kg). Also this cyclopentyl methyl compound did not exhibit any gastrointestinal toxicity in rats and monkeys during chronic daily drug treatment upto 400 mg / kg p.o. for 4 weeks. This prompted us to synthesize and evaluate the activity of 5-(cyclopentyloxy)-6-methoxy-indan-1-alkanoic acid (an isostere of 6-chloro-5-

(cyclopentylmethyl)-indan-1-carboxylic acid) and its amide derivatives as potential nonsteroidal anti-inflammatory compounds with lesser ulcerogenecity.

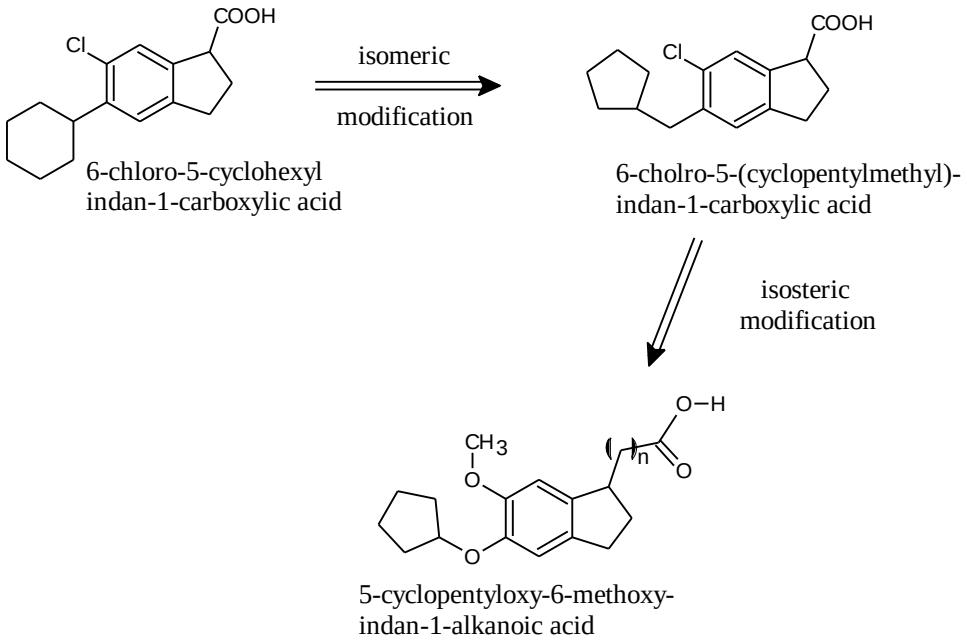
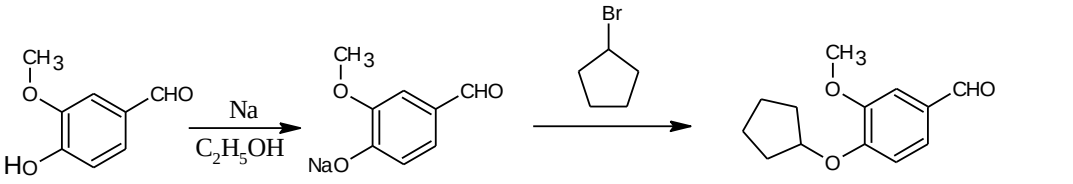


Figure 5.23. Design of 5-cyclopentyloxy-6-methoxy-indan-1-alkanoic acid

5.5.1 SYNTHESIS OF (5-CYCLOPENTYLOXY -6-METHOXY-2, 3-DIHYDRO-1*H*-INDEN-1- YL) ACETIC ACID

5.5.1.3. 5-cyclopentyloxy-6-methoxybezaldehyde[165] (V-XXII).



4-hydroxy-3-methoxybenzaldehyde **I**

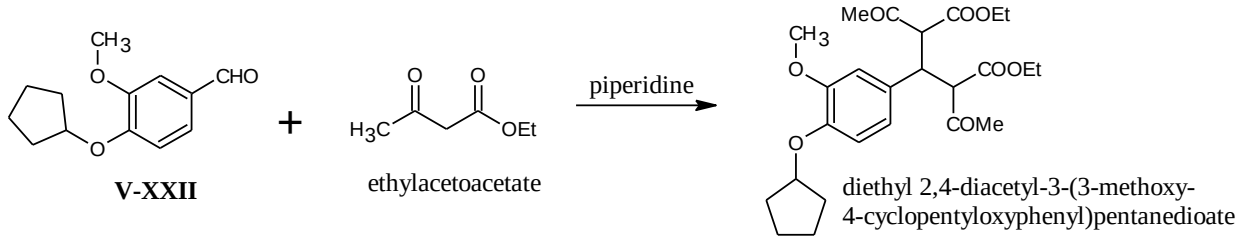
Sodium vanillate **V-XXI**

4-(cyclopentyloxy)-3-methoxybenzaldehyde **V-XXII**

Clean sodium (1.55g, 0.067mol) was placed in a 250ml of round bottomed flask equipped with a reflux condenser. To this 40ml of absolute alcohol was added. Once the vigorous reaction has subsided, the flask was warmed on water bath until the whole amount of sodium went into solution. The flask was cooled and to this 10g (0.066mol) of vanillin (**I**) dissolved

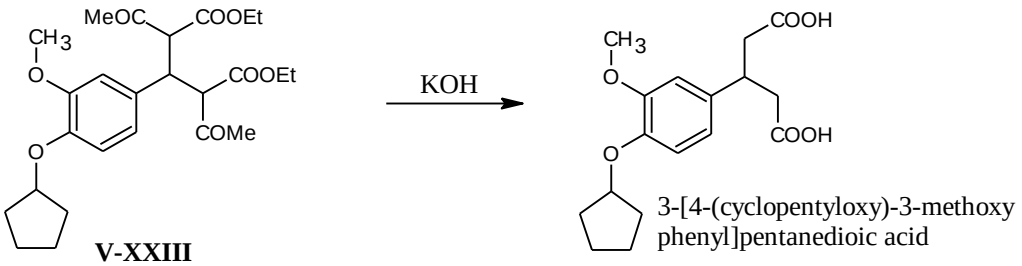
in alcohol was added. After refluxing the reaction mixture on water bath for 30min sodium vanillate (**V-XXI**) separated out. Then alcohol was distilled out and a sufficient quantity of dimethylformamide was added to sodium vanillate until a clear solution was obtained. To this solution cyclopentyl bromide (0.1mol) was slowly added under ice cold conditions. The reaction mixture was heated on water bath for six hours and finally poured onto 150ml of water. The system was kept protected with a calcium chloride guard tube through out the reaction. It was extracted with two 100ml portions of benzene. The combined benzene extracts was washed with three portions of 10% sodium hydroxide solution. Benzene and the unreacted cyclopentyl bromide was removed under reduced pressure and the residue was further distilled at 138-140 °C at 0.4mm Hg to get 5-cyclopentyloxy-6-methoxy benzaldehyde (**V-XXI**) in 45-50% yield, IR (cm^{-1}) (thin film): 1680 (C=O stretching) and 1037, 1264 (C-O stretch of ether linkage).

5.5.1.4. Diethyl 2,4-diacetyl-3-(5-cyclopentyloxy-6-methoxy)pentanedionate (V-XXIII)



5-cyclopentyloxy-6-methoxy benzaldehyde (11g, 0.05 mol) was dissolved in ethylacetoacetate (15.6g, 0.12mol) in a dry conical flask and piperidine (0.85 ml) was added slowly at ambient temperature and then kept for 3 days or more (up to seven days, depending on room temperature) with the mouth stoppered. The solid product thus obtained was crushed and then washed with solvent ether to get the desired product in 50-55% yield[14]. Recrystallization from dil. alcohol gave the analytical product, mp 142-144 °C IR (cm^{-1}) (KBr): 1720 (C=O stretching), 1036, 1260 (C-O stretch of ether linkage).

5.5.1.3. 3-(5-cyclopentyloxy-6-methoxy)pentanedioic acid (V-XXIV).



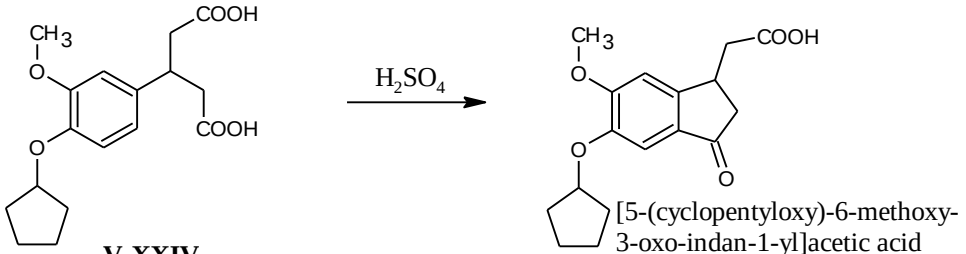
V-XXIII

3-[4-(cyclopentyloxy)-3-methoxy phenyl]pentanedioic acid

V-XXIV

Compound **V-XXIII** (9.24g, 0.02mol) was dissolved in a hot solution of KOH (37g in 37 ml of water) and 74ml of absolute alcohol was added. The hot reaction mixture was refluxed on a water bath for 1 hour. Alcohol was then removed by distillation, and after dilution with water the reaction mixture was cooled and washed with solvent ether. The aqueous layer on acidification with cold conc. HCl with cooling gave crude diacid which was filtered and recrystallized from hot water [14]. Yield 70-75 %, mp 131-132°C, IR (cm^{-1}) (KBr): 3400-2600 (O-H stretch), 1730 (C=O stretching), 1261, 1022 (C-O stretch of ether linkage). ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.62 (m, CH_2, 2H), 1.85 (m, CH_2, 6H), 2.69 (dd, CH_2, 2H), 2.73 (dd, CH_2, 2H), 3.67 (qn, CH, 1H), 3.84 (s, OCH_3, 3H), 4.73 (m, CH, 1H), 6.71-6.83 (m, ArH, 3H), 10.11 (brs, COOH, 1H), 10.25 (brs, COOH, 1H)

5.5.1.4. (5-cyclopentyloxy-6-methoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetic acid[166](V-XXV).



V-XXIV

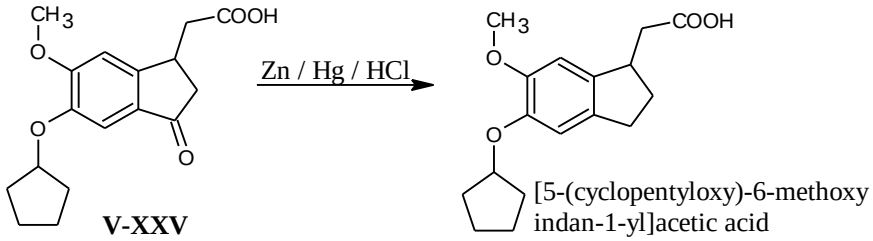
[5-(cyclopentyloxy)-6-methoxy-3-oxo-indan-1-yl]acetic acid

V-XXV

The substituted glutaric acid **V-XXIV** (5g, 0.0155 mol) was taken in a round bottomed flask. To this 12.4 ml of concentrated sulphuric acid (15times) was added. The flask was fitted with a calcium chloride guard tube and was left overnight at room temperature. The solution was poured onto ice and extracted with ether for 2-3 times. The ethereal extract was washed with water to remove any sulphonated products and finally dried and distilled to yield the crude keto product (V-XXV). Yield 55-60%, mp 232-234 °C, IR (cm^{-1}) (KBr): 3400-2400 (O-H stretch), 1721, 1640 (C=O stretching), 1245, 1033 (C-O stretch of ether linkage); ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS): 1.63 (m, CH_2, 2H), 1.87 (m, CH_2, 6H), 2.43, 2.67 (dd, CH_2, 2H), 2.73,

2.99 (dd, CH_2, 2H), 3.71(qn, CH, 1H), 3.84 (s, OCH_3, 3H), 4.78 (m, CH, 1H), 6.89 (s, ArH, 1H), 7.31 (s, ArH, 1H), 10.28 (brs, COOH, 1H).

5.5.1.6. (5-cyclopentyloxy-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)acetic acid (V-XXVI).



V-XXV

[5-(cyclopentyloxy)-6-methoxy indan-1-yl]acetic acid

V-XXVI

The keto compound, **V-XXV,** was subjected to Clemmensen's reduction to obtain the title compound. The keto acid (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on a steam bath for about 8 hrs (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free from acidity. After drying over anhydrous sodium sulfate it was finally distilled to get the reduced acid **V-XXVI**. The analytical product was obtained upon recrystallization from benzene. Yield 65-70 %; mp 114-115^oC; IR (cm^{-1}): 3400-2400 (O-H stretch), 1724 (C=O stretching), 1251, 1036 (C-O stretch of ether linkage). ^1H- NMR: δ (ppm) ($CDCl_3$, TMS): 1.59 (m, CH_2, 2H), 1.81 (m, CH_2, 6H), 1.75, 2.33 (m, CH_2, 2H), 2.39, 2.63 (dd, CH_2, 2H), 2.81 (m, CH_2, 2H), 3.67 (qn, CH, 1H), 3.85 (s, OCH_3, 3H), 4.67 (m, CH, H), 6.81 (s, ArH, 1H), 6.85 (s, ArH, 1H), 10.21 (brs, COOH, 1H).

5.5.2. RESULTS AND DISCUSSION

The compound **I** was converted to compound **V-XXII** by Williamson's synthesis. The reaction is a nucleophilic substitution reaction where the phenolic oxygen attacks the electron deficient carbon of cyclopentyl bromide, leading to formation of **V-XXIII** by elimination of bromine as sodium bromide (Figure 5.24). As the alkyl halide involved in this reaction was secondary in nature the yield obtained for the compound **V-XXIII** was comparatively less. The reaction was tried for longer durations (maximum of 36 hrs), under varying reaction

conditions including microwave and phase transfer catalysis, but the yields could not be improved further.

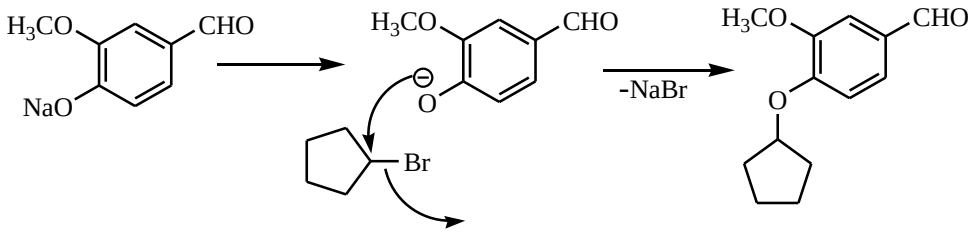


Figure 5.24. The reaction mechanism for the synthesis of compound **V-XXII**

The IR spectrum showed C=O stretching 1680 cm^{-1} indicating the presence of carbonyl group and also C-O stretch of ether linkage 1037 and 1264 cm^{-1}. Absence of broad O-H stretch also supported the structure of **V-XXIII.**

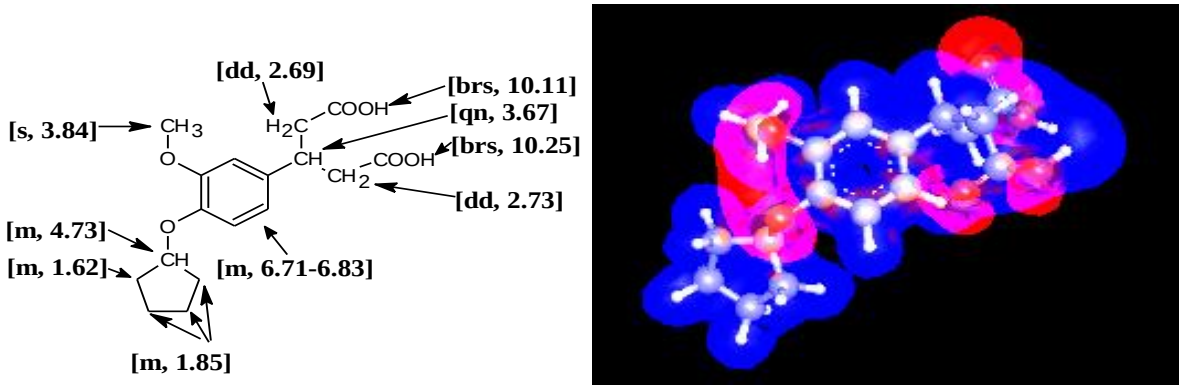


Figure 5.25. ^{1}H- NMR:δ (ppm) ($CDCl_3$, TMS) and electrostatic potential map of compound **V-XXV**

The compound **V-XXIII** was converted into the benzylic bisacetoacetate **V-XXIV** under the reaction conditions as explained earlier. The IR spectra showed C=O stretching at 1720 cm^{-1}corresponding to carbonyl of an ester group and C-O stretch of ether linkage at 1036 and 1260 cm^{-1}. The hydrolysis of compound **V-XXIV** gave the glutaric acid derivative **V-XXV.** The IR spectrum shows a broad O-H stretch at 3400-2600cm^{-1} indicating the formation of carboxylic group. The ^{1}H-NMR spectrum of compound shows small broad singlets at 10.11 and 10.25 δ values indicating the presence two carboxylic acid protons. The two CH_2 groups in the glutaric acid side chain of the compound **V-XXV** give two doublet of doublet at slightly different δ values. This splitting pattern was observed due to equatorial and axial arrangement of proton on CH of glutaric acid side chain of the compound **V-XXV.** The CH of

cyclopentyloxy ring gives multiplet at higher δ value. Because of the existence of axial and equatorial arrangements of protons in a rigid cyclopentane ring, the multiplets are observed for all the protons as shown in Figure 5.25.

The compound **V-XXV** was cyclized to from the keto compound **V-XXVI** by treating it with sulfuric acid. Sulfuric acid being a dehydrating agent has been reported as cyclizing agent for phenylglutaric acid. The yields were comparatively low because of the probability of formation of sulfonated by products. This reaction was also tried with polyphosphoric acid and aluminium chloride at different temperatures and varying duration of time. However the cyclization could not be achieved by these methods.

The mechanism of the reaction (Figure 5.26) may be as follows:

(d) Sulfuric acid leads to the formation of anhydride.

(e) The anhydride adds on sulfuric acid.

(f) Ring formation occurs owing to the production of sulfuric acid from appropriate nuclear hydrogen atom

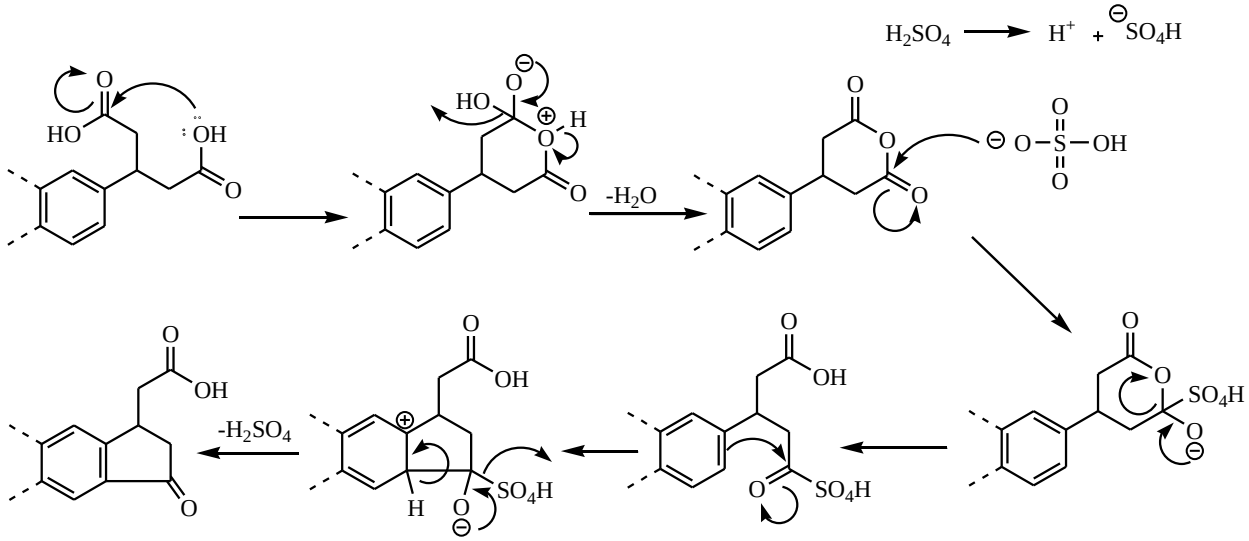


Figure 5.26. Possible reaction mechanism for synthesis of **V-XXVI**

The IR spectra showed broad O-H stretch at 3400-2400 cm^{-1} indicating the presence of free carboxylic group. The C=O stretch was obtained at 1721 and 1640 corresponding to carbonyl of acid group and the keto goup introduced after cyclization respectively. Presence of quintet for CH group and doublet of doublet for the neighbouring CH_2 groups indicated the ring closure as shown in the Figure 5.27.

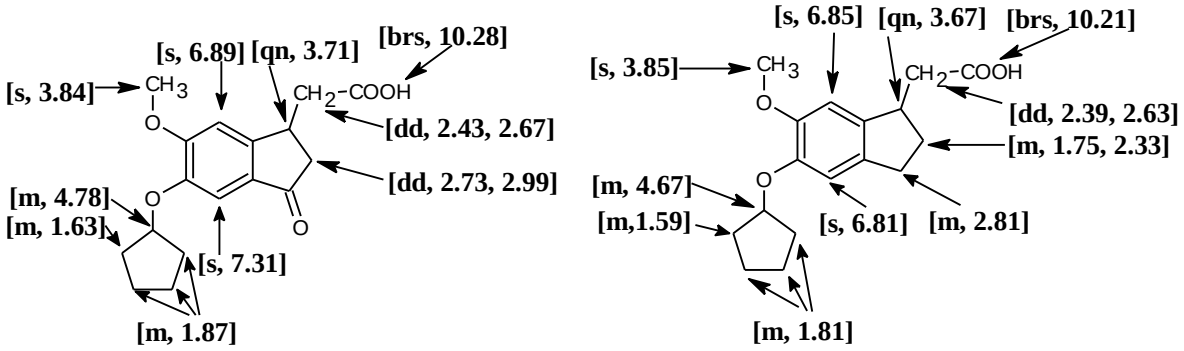
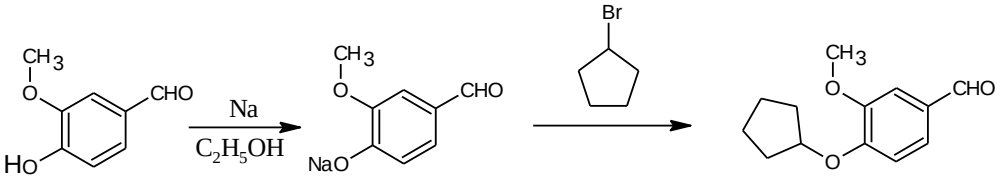


Figure 5.27. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XXVI and V-XXVII**

The keto group of compound **V-XXVI** was reduced to methylene by Clemmensen's reduction; converting indanone to indan **V-XXVII**. The IR spectra showed broad O-H stretch and C=O stretch in confirmation of the structure. The NMR spectra as shown in Figure 5.27 further support the proposed structure of V-**XXVII.**

5.5.3. SYNTHESIS OF (5-CYCLOPENTYLOXY -6-METHOXY-2, 3-DIHYDRO-1*H*-INDEN-1- YL) CARBOXYLIC ACID

5.5.3.2. 5-cyclopentyloxy-6-methoxybezaldehyde (V-XXII).



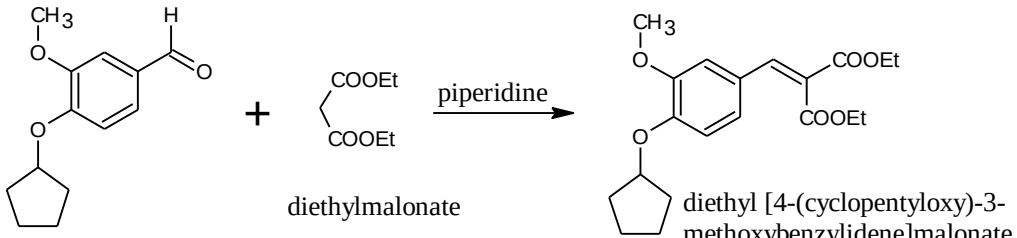
4-hydroxy-3-methoxybenzaldehyde **I**

Sodium vanillate **V-XXI**

4-(cyclopentyloxy)-3-methoxybenzaldehyde **V-XXII**

The methodology adopted for synthesis of this compound has been described in section 5.5.1.1.

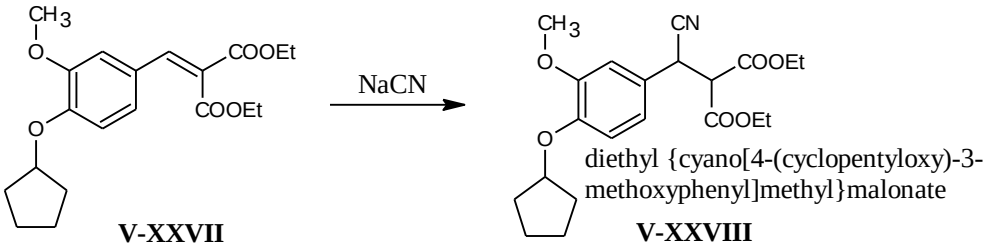
5.5.3.2. Diethyl (4-cyclopentyloxy-3-methoxy benzylidene)malonate (V-XXVII).



V-XXII diethylmalonate diethyl [4-(cyclopentyloxy)-3-methoxybenzylidene]malonate **V-XXVII**

To 11 g (0.05mol) of 5-cyclopentyloxy-6-methoxy benzaldehyde, **V-XXII,** in a dry round bottomed flask 8.5 g (0.053 mol) of diethylmalonate was added. To this reaction mixture 0.5ml of piperidine, 0.5ml of glacial acetic acid and 50ml of dry benzene was added. The reaction mixture was refluxed for about 18 hrs in Dean Stark's apparatus. The reaction was carried until no more water collects in the Dean Stark's apparatus. The reaction mixture was washed with water to remove the added piperidine and acetic acid. After drying over anhydrous sodium sulfate benzene was removed from the reaction mixture under reduced pressure. The remaining brownish oily liquid was set for vacuum distillation. The benzylidene malonate, **V-XXVII,** was distilled at 215-220 °C at 0.5mm Hg as a clear liquid. The overall yield was 85-90%, IR (cm^{-1}) (thin film): 1710 (C=O stretching), 1038, 1252 (C-O stretch of ether linkage), 1620 (C=C stretch); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.31 (t, CH_3, 3H), 1.34 (t, CH_3, 3H), 1.62 (m, CH_2, 2H), 1.89 (m, CH_2, 6H), 3.83 (s, OCH_3, 3H), 4.29 (qr, CH_2, 2H), 4.35 (qr, CH_2, 2H), 4.80 (m, CH, 1H), 6.85 (d, ArH, 1H), 7.02(s, ArH, 1H), 7.05(d, ArH, 1H), 7.64 (s, CH, 1H).

5.5.3.4. Diethyl [cyano(4-cyclopentyloxy-3-methoxyphenyl) methyl] malonate (V-XXVIII).

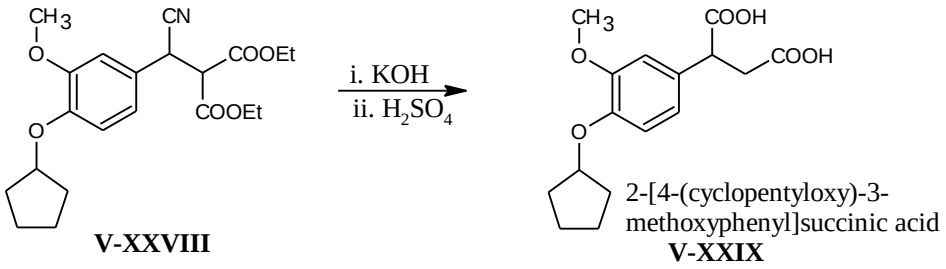


V-XXVII diethyl {cyano[4-(cyclopentyloxy)-3-methoxyphenyl]methyl}malonate **V-XXVIII**

To benzylidene malonate, **V-XXVII,** (9 g, 0.025mol) in a dry round bottomed flask 1.3g (0.026 mol) of sodium cyanide dissolved in 90% alcohol was added. The reaction mixture

was refluxed for 18 hrs at 100 °C and then alcohol was distilled out at reduced pressure. The formed cyanomalonate **V-XXVIII** was not purified and was used directly in the next step.

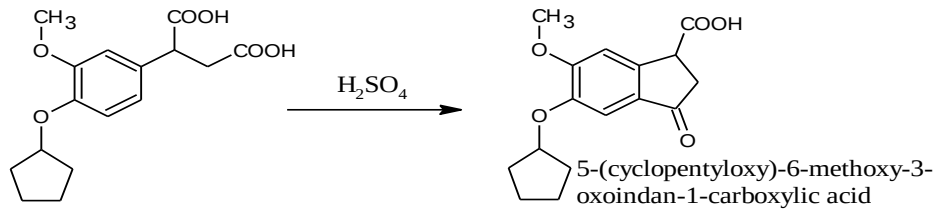
5.5.3.4. 2-(4-cyclopentyloxy-3-methoxy phenyl)succinic acid (V-XXIX).



V-XXVIII **V-XXIX**

To the above reaction mixture (vide 5.5.2.3) 7.4g of KOH dissolved in 22ml of water (6N, 0.45mol) was added. The reaction mixture was refluxed for 16 hrs and then allowed to cool. The cooled reaction mixture was slowly poured on to calculated amount of ice-sulphuric acid mixture with stirring when the diacid precipitated out. The crude product obtained was filtered, dried and recrystallised from hot water to get the desired succinic acid. Yield 75-80%; mp 171-173°C; IR (cm^{-1}) (KBr): 3400-2400 (br OH stretch), 1700(C=O stretching), 1040, 1248 (C—O stretch ether linkage); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.60 (m, CH_2, 2H), 1.79 (m, CH_2, 4H), 1.88 (m, CH_2, 2H), 2.54 (dd, CH_2, 1H), 3.01 (dd, CH_2, 1H), 3.79 (s, OCH_3, 3H), 3.86 (dd, CH, 1H), 4.73 (m, CH, 1H), 6.79-6.83 (m, ArH, 3H), 10.81 (brs, COOH, 1H), 10.92 (brs, COOH, 1H).

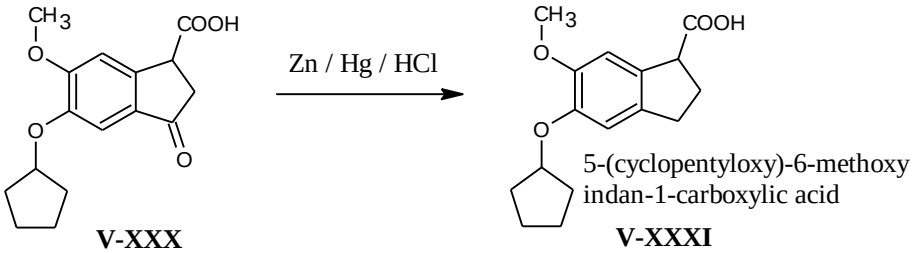
5.5.4.5. (5-cyclopentyloxy-6-methoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XXX).



V-XXIX **V-XXX**

The appropriate succinic acid **V-XXIV** (5g, 0.0162 mol) was taken in a round bottomed flask. To this 21.6 ml of concentrated sulphuric acid (25times) was added. The flask was fitted with a calcium chloride guard tube and was left overnight at room temperature. The solution was poured onto ice and extracted with ether for 2-3 times. The ethereal extract was washed with water to remove any sulphonated product and inorganic acid and finally dried and distilled to yield the crude keto product **V-XXX** which was recrystallized from dilute alcohol. Yield 65-70% mp 125-127 ^{o}C, IR (cm^{-1}) (KBr): 3500-2400 (br OH stretch), 1703, 1668 (C=O stretching), 1042, 1263 (C—O stretch ether linkage); ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.61 (m, CH_2, 2H), 1.84 (m, CH_2, 6H), 2.91, 3.23 (dd, CH_2, 2H), 4.18 (dd, CH, 1H), 3.83 (s, OCH_3, 3H), 4.77 (m, CH, 1H), 7.10 (s, ArH, 1H), 7.19 (s, ArH, 1H), 10.31 (brs, COOH, 1H).

5.5.4.6. (5-cyclopentyloxy-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid (V-XXXI).



V-XXX **V-XXXI**

Clemmensen's reduction was employed to obtain the title compound. The keto acid **V-XXX** (0.1 mol) was treated with 50 g of zinc amalgam, 50 ml of conc. HCl and 75ml of water. About 200ml of benzene was added as a co-solvent. The reaction mixture was refluxed on a steam bath for about 8 hrs (until the reaction mixture became keto-negative). The organic layer was separated and the aqueous layer and zinc granules were further extracted with benzene. The pooled organic phase was washed with water to make it free of acidity. After drying over anhydrous sodium sulfate it was distilled to get the reduced acid V-XXXI. The analytical product was obtained on recrystallization from benzene. Yield 70-75%; mp 94-96^{o}C (benzene); IR (cm^{-1}) (KBr): 3600-2400 (br OH stretch), 1720 (C=O stretching), 1047, 1262 (C—O stretch ether linkage) ^{1}H-NMR: δ (ppm) ($CDCl_3$, TMS): 1.62 (m, CH_2, 2H), 1.85 (m, CH_2, 6H), 2.33, 2.51 (m, CH_2, 2H), 2.91 (m, CH_2, 2H), 3.84 (s, OCH_3, 3H), 3.94 (dd, CH, 1H), 4.65 (m, CH, 1H), 6.79 (s, ArH, 1H), 6.91 (s, ArH, 1H), 10.28 (brs, COOH, 1H).

5.5.5. RESULTS AND DISCUSSION

The hydroxyl group of vanillin was changed to cyclopentyloxy group by Williamson's synthesis. The aldehyde group of compound **V-XXII** was reacted with diethyl malonate in the presence of base to form benzylidene malonate **V-XXVII**. The reaction mechanism is the same as described in the Figure 5.23. The IR and the NMR spectra also confirm the structure of compound **V-XXVII**. The protons on CH of benzylidene part of **V-XXVII** being most desheilded give signal far downfield at δ value of 7.64 ppm as shown in the Figure 5.28.

The malonate **V-XXVII** was cynosated at the double bond and then hydrolyzed to succinic acid derivative **V-XXIX**. The broad O-H signal in IR and two small broad singlets far downfield (Figure 5.28) indicated the presence of two carboxylic groups in **V-XXIX**.

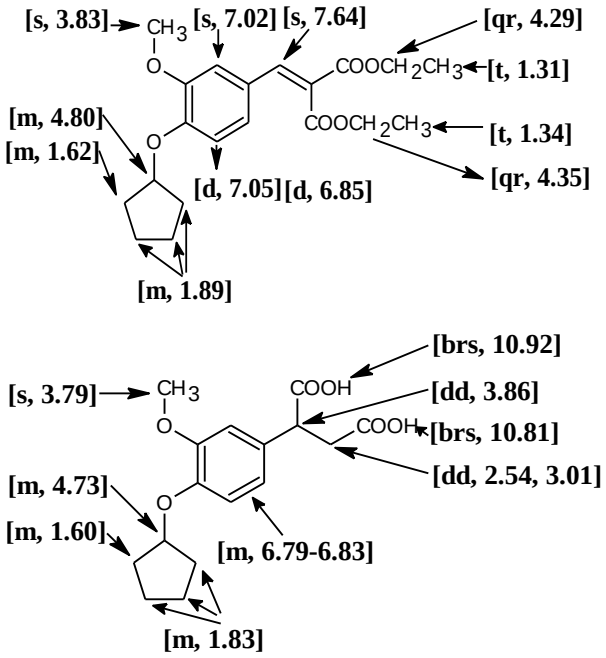


Figure 5.28. ^{1}H- NMR:δ (ppm) ($CDCl_3$, TMS) of compounds **V-XXVII** and **V-XXIX**

Compound V-XXIX was cyclized to indanone derivative V-XXX by treatment with sulfuric acid. This reaction was also tried with polyphosphoric acid and aluminium chloride at different temperatures and durations of time. However the cyclization could not be achieved by these methods. The doublet of doublet for CH and two doublet of doublet for CH_2 on indanone ring confirmed the ring closure and formation of compound **V-XXX** (Figure 5.29).

The keto group of compound **V-XXX** was reduced to CH_2 to form the indan derivative **V-XXXI**. The IR spectra showed broad O-H stretch at 3600-2400 $cm^{-1}$and C=O stretch at 1720 cm^{-1}. The doublet of doublet at 3.94 ppm corresponding to CH, two multiplets at δ 2.33 ppm

and 2.51 ppm corresponding to CH_2 and another multiplet at 2.91 ppm corresponding to CH_2 indicates the presence of indan ring system in **V-XXXI** (Figure 5.29). Other signals obtained in ^{1}H-NMR are also in consonance with the proposed structure of the compound **V-XXXI**.

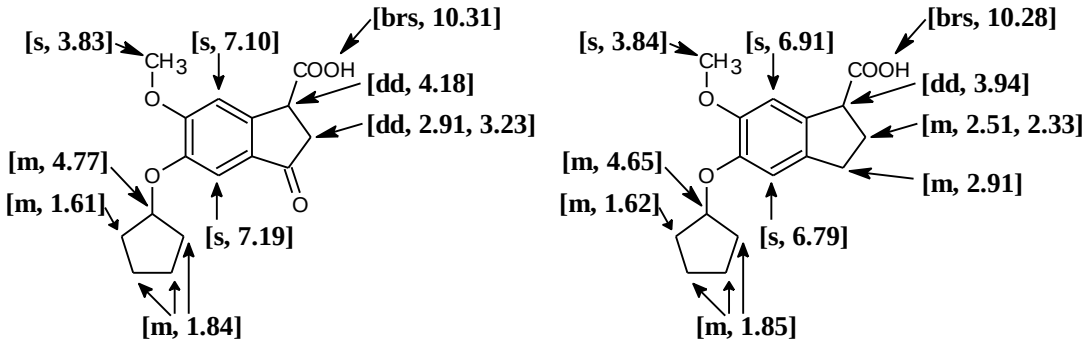


Figure 5.29. ^{1}H- NMR: δ (ppm) ($CDCl_3$, TMS) of compound **V-XXX** and **V-XXXI**

CHAPTER 6
PHARMACOLOGY

6.1. GENERAL

6.1.1. Anti-inflammatory Activity: Methods based on the inhibition of an induced swelling of the rat's paw have been among the most popular with the pharmacologists for search of new anti-inflammatory substances. The general procedure is to inject a small amount of a suspension or solution of an edemogen into the subplantar tissue of a hind paw of the rat. Assessment of the response is usually made at the time of maximum swelling. Methods for measuring the amount of swelling of the paw include determining its thickness, its weight, and the volume of water or mercury it displaces. Of many edemogen that can be used, carrageenan-induced rat paw edema is the most widely used method for evaluation of anti-inflammatory activity[76]. The rat carrageenan-induced paw edema model has been used as a popular assay for such anti-inflammatory agents which are primarily cyclooxygenase (COX) inhibitors[167]. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss (*Chondrus crispus*). Only λ-type galactan is effective in eliciting the inflammatory response. A certain structural integrity of the polysaccharide is essential for edemogenic activity, and heat denaturation of a sufficient degree to cause the release of esterified sulfuric acid from the substance results in the loss of part of its activity[76]. The edema of the rat paw induced by carrageenan injection develops slowly and reaches its peak in 3-4 h[168]. It is uninfluenced by pretreatment with antihistamine agents but is inhibited by salicylate congeners. On the other hand, the edema produced by dextran, yeast or egg albumin develops rapidly, reaching its maximum in ½ to 1 h and then slowly subsides. It is blocked by pretreatment with antihistamine and anti-5-hydroxytryptamine agents[161]. Carrageenan-induced edema is a nonspecific inflammation resulting from a complex of diverse mediators. Thus edema following carrageenan is attributable to capillary permeability from the release of kinins while edema from dextran, yeast, egg albumin mainly follows the release of histamine and 5-hydroxytryptamine[169]. Swelling of the rat's paw after the carrageenan injection is not normally distributed, the curve is leptokurtic, i.e., it has more items near the mean and at the tails than does the normal distribution. The development of edema after carrageenan injection has been described as a biphasic event. The existence of

two phases of swelling probably explains the failure of standard anti-inflammatory drugs to effect a complete inhibition of edema. Different investigators have reported the maximal inhibition of the edema induced by carrageenan of 40-80% with standard anti-inflammatory drugs. The initial phase of edema has been attributed to the release of histamine and serotonin; the edema maintained during the plateau phase, to kinin like substances; and the second accelerating phase of swelling to the release of prostaglandin like substances. The evaluation of anti-inflammatory drugs is, therefore, generally done against the second phase of the edema. Because edemas of this type are highly sensitive to NSAIDs, carrageenan has been accepted as a useful agent for studying new anti-inflammatory drugs[71]. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs, and during the second phase, it detects compounds that are anti-inflammatory agents as a result of inhibition of prostaglandin amplification. Moreover it has been shown that when carrageenan is injected into the paws of the rats, a substantial induction of COX-2 is observed at 3 hours, coinciding with enhanced TxB_2 levels and local edema[170]. The direct role of prostaglandins in the rat carrageenan-induced paw edema model was demonstrated by using an anti-PGE_2 antibody to inhibit paw edema as effectively as indomethacin[171].

6.1.2. Analgesic Activity[76, 172]: The most successful methods used to evaluate the analgesic activity of non-narcotic analgesics involve the assessment of drug's ability to modify the inflammatory pain. The assessment of the ability of a drug to modify inflammatory pain appears a *priori* to be the most relevant test for the anti-inflammatory analgesics because this is presumably the type of pain that is present in most of the conditions for which such drugs are prescribed. The most widely used methods for evaluating the analgesic activity are phenylquinone (2-phenyl-1,4-benzoquinone) induced writhing assay and the acetic acid-induced writhing assay. These methods are sensitive, simple, and reproducible for screening weak analgesics. Pain is induced by an injection of irritant (acetic acid or phenylquinone) into the peritoneal cavity of mice. The animals react with a characteristic stretching behaviour called writhing. The response occurring after intraperitoneal administration of a nociceptive agent to mice is " a wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs". For the present study acetic acid-induced writhing assay has been used. Acetic acid as an irritant is injected intraperitoneally to mice and the stretching reaction is evaluated.

6.1.3. Antipyretic Activity: Fever is the most outstanding component of a complex host response to invading agents called "acute-phase response", which also includes neutrophilia, activation of the hypothalamic-pituitary-adrenal axis, and changes in serum metal levels. By definition, fever is a regulated increase in body core temperature (T_b) characterized by a raised thermoregulatory set point. A number of studies have reported that, besides endothermic species, a variety of ectothermic vertebrates (as well as invertebrates) develop fever in response to injections of exogenous pyrogens, such as lipopolysaccharide (LPS; endotoxin), viruses, gram positive bacteria, and yeast. Lipopolysaccharide, which is the most purified form of a compound from the cell wall of gram-negative bacteria, usually *Escherichia coli*, has been extensively used to induce fever in experimental animals. In mammals, it is generally believed that LPS-induced fever is mediated by stimulation of immune cells to produce and release a complex variety of soluble mediators, called endogenous pyrogens. Among these, the cytokines interleukin (IL)-1β, IL-6, interferons, and tumor necrosis factor (TNF) are thought to convey the pyrogenic message to the brain region where fever is regulated, namely the preoptic area of the anterior hypothalamus (POAH). The ability of endogenous pyrogens to cause fever depends importantly on the generation, within the brain, of at least two mediators: corticotropin releasing hormone (CRH), and prostaglandins[173, 174].

Classically, PGE_2 is considered to be the most proximal mediator of fever, acting on the POAH. Evidence in favor of this hypothesis is that the levels of PGE_2 rise in the POAH in conjunction with the generation of fever, and administration of PGE_2 into the POAH causes a rise in T_b. Moreover, pretreatment of humans and experimental animals with inhibitors of cyclooxygenase (COX), the key enzyme in PG biosynthesis, attenuates fever, and COX inhibitors do not affect fever induced by the PGE series. However, not all fevers are mediated via PGs, because, in rats, some endogenous pyrogens, such as macrophage inflammatory protein-1, IL-8, and endothelin-1, have been reported to evoke PG-independent fever, i.e., a fever that is not blocked by a COX inhibitor. The antipyretic actions of NSAIDs have generally been ascribed to their ability to inhibit COX-1/-2 in central nervous system. However some NSAIDs seem also to display antipyretic properties unrelated to COX-inhibition[175, 176].

Although fever is the most common thermoregulatory change accompanying inflammation, hypothermia can also be seen in response to LPS treatment, especially in rodents[177, 178]. It has been proposed that hypothermia is triggered by TNF-α, which is possibly released from

peripheral macrophages. A significant increase in serum TNF-α level occurs just before the development of hypothermia. In experimental animals the inhibition of TNF-α bioactivity by several methods also attenuates LPS induced hypothermia[179, 180]. Thus LPS causes a dual thermoregularity response in rats in which hypothermia precedes fever.

It is generally accepted that the NSAIDs exhibit their anti-inflammatory effect primarily through inhibition of prostaglandin synthesis. However, relatively recent *in vitro* studies have indicated that NSAIDs also interfere with peripheral proinflammatory cytokine production [181].

It is well known that LPS produces a biphasic response in the rat. It shows an initial hypothermia up to 3rd h of its administration. It has been shown that this hypothermia is triggered by TNF-α.

6.1.4. Anti-arthritic Activity [141]: Inadequate knowledge of the etiology and pathogenesis of rheumatoid arthritis in man has seriously impaired the development of laboratory tests for compounds with specific anti-arthritic activity. Adjuvant-induced arthritis seems more akin to human rheumatoid arthritis than any other laboratory model investigated so far[76]. A single subplantar injection of Freund's adjuvant into the hind paw induces arthritic symptoms in rats. Freund's adjuvant consists of dead cells of *Mycobacterium butyricum* suspended in heavy paraffin oil. Injection of complete Freund's adjuvant into the rat paw induces inflammation as primary lesion with maximum after 3-5 days. Thereafter the swelling slowly subsides until the 8th day when the foot begins to swell again. Secondary lesions occur after a delay of approximately 10-12 days which are characterized by inflammation of non-injected sites (hind leg, forepaws, ears, nose and tail), a decrease of weight and immune responses. The primary swelling is basically an indication of acute inflammatory response whereas secondary polyarthritis signs indicate a delayed cellular type hypersensitivity reaction. The procedure has been modified by several authors in order to differentiate between the anti-inflammatory activity and the immunosuppressive activity. Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented by immunosuppressive agents[144]. Two protocols termed preventive or prophylactive and therapeutic or established adjuvant arthritis have gained wide usage for assessing a drug's potential anti-arthritic activity[77]. It has also been shown that in adjuvant arthritis model, high levels of COX-2 protein develop rapidly throughout the hind limb joints, both contra and ipsilateral to the site of Freund's adjuvant administration. Induction of COX-2 precedes or parallels the local production of PGE_2 and symptoms of arthritis[182].

In our hands adjuvant-induced arthritis test in rats produced a biphasic response. The initial response started immediately after adjuvant administration and reached maximum on 3rd day and then it started subsiding. The 2nd phase started from the 9th day and continued till the end of the experiment (14th day). Secondary lesions (appearance of nodules, and erythema in tail, nose and ears) started developing from the 10th day.

6.2. PHARMACOLOGICAL SCREENING OF 5,6-DIMETHOXYINDAN-1-ACETIC ACID AMIDES

6.2.1. Screening of 5,6-dimethoxyindan-1-acetic acid amides for anti-inflammatory activity

The anti-inflammatory activity of the test compounds was evaluated by carrageenan-induced rat paw edema model. The results (Table 6.1) show that the test compounds exhibit variable anti-inflammatory activity, and a few among them have significant acute as well as residual anti-inflammatory activity at 24h after a single oral dose.

Table 6.1. The anti-inflammatory activity profile of 5,6-dimethoxyindan-1-acetic acid amides against carrageenan-induced rat paw edema

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]					
	1H	2H	3H	4H	6H	24H
DA-1	0.26±0.0158 [18.75]ns	0.345±0.016 [31.0]*	0.48±0.0126 [30.43]*	0.44±0.0127 [31.25]*	0.39±0.0145 [27.78]*	0.22±0.0126 [26.67]*
DA-2	0.27±0.006 [15.62]ns	0.35±0.0072 [30.0]*	0.49±0.0152 [28.99]*	0.45±0.0179 [29.69]*	0.41±0.0179 [24.07]*	0.20±0.0153 [33.33]*
DA-3	0.24±0.0367 [25.0]ns	0.32±0.0148 [36.0]*	0.40±0.0273 [34.78]*	0.41±0.0273 [35.94]*	0.36±0.0165 [33.33]*	0.23±0.0125 [23.33]ns
DA-4	0.27±0.0058 [15.62]ns	0.37±0.0216 [26.0]*	0.51±0.027 [26.09]*	0.47±0.0225 [26.56]*	0.43±0.212 [20.37]*	0.20±0.0159 [33.33]*
DA-5	0.22±0.0138 [31.25]*	0.30±0.0086 [40.0]*	0.43±0.0154 [37.68]*	0.40±0.085 [37.5]*	0.37±0.0195 [31.48]*	0.24±0.0308 [16.67]ns
DA-6	0.26±0.0203 [18.75]ns	0.36±0.0157 [28.0]*	0.49±0.0148 [28.98]*	0.47±0.0212 [26.56]*	0.42±0.0212 [22.22]*	0.22±0.0083 [26.67]*
DA-7	0.22±0.0115 [31.25]*	0.315±0.019 [37.0]*	0.48±0.0143 [30.43]*	0.39±0.0125 39.06]*	0.34±0.0191 [37.03]*	0.21±0.0065 [30.00]*
DA-8	0.22±0.016 [31.25]*	0.35±0.0240 [24.0]*	0.47±0.013 [27.54]*	0.45±0.0178 [25.0]*	0.39±0.0105 [24.07]*	0.19±0.0141 [36.67]*
DA-9	0.22±0.0158 [31.25]*	0.34±0.0203 [32.0]*	0.46±0.0173 [33.33]*	0.43±0.0167 [32.81]*	0.37±0.0155 [31.48]*	0.18±0.0151 [40.00]*
DA-10	0.21±0.0134 [34.38]*	0.33±0.018 [34.0]*	0.43±0.013 [37.68]*	0.38±0.0105 [40.62]*	0.35±0.0156 [35.19]*	0.14±0.0073 [46.67]*
DA-11	0.17±0.0186 [46.88]*	0.345±0.017 [31.0]*	0.41±0.014 [40.58]*	0.39±0.0183 [39.06]*	0.325±0.015 [39.81]*	0.17±0.0173 [43.33]*
DA-12	0.25±0.006	0.43±0.0108	0.56±0.0142	0.53±0.0142	0.48±0.0084	0.33±0.0154

	[21.88]ns	14.0]ns	[18.84]ns	[17.19]*	[11.11]ns	[0]ns
DA-13	0.18±0.0169 [40.62]*	0.19±0.0189 [52.0]*	0.39±0.0204 [43.48]*	0.39±0.0196 [45.31]*	0.32±0.018 [40.74]*	0.16±0.019 [46.67]*
DA-14	0.10±0.0073 [50.0]*	0.20±0.0183 [56.0]*	0.32±0.0171 [49.27]*	0.30±0.0142 [50.0]*	0.26±0.0185 [48.15]*	0.14±0.0131 [53.33]*
DA-15	0.305±0.016 [4.69]ns	0.40±0.017 [20.0]ns	0.51±0.0182 [26.09]*	0.485±0.013 [24.22]*	0.42±0.0147 22.22]*	0.20±0.00963 [33.33]*
DA-16	0.20±0.0152 [18.75]ns	0.39±0.0167 [22.0]ns	0.47±0.017 [31.88]*	0.435±0.012 [32.03]*	0.37±0.158 31.48]*	0.18±0.013 [40.00]*
DA-17	0.12±0.0131 [62.5]*	0.28±0.0125 [44.0]*	0.40±0.022 [42.03]*	0.36±0.0197 [40.62]*	0.36±0.0197 [33.33]*	0.105±0.0099 [65.00]*

Contd……

Table 6.1. Contd.

DA-18	0.27±0.0068 [15.62]ns	0.43±0.0154 [14.0]ns	0.47±0.0247 [19.57]*	0.555±0.034 [13.28]ns	0.46±0.0265 [14.8]ns	0.23±0.0154 [23.33]*
DA-19	0.35±0.012 [0]ns	0.52±0.0208 [0]ns	0.605±0.021 [12.31]ns	0.58±0.0114 [9.37]ns	0.47±0.0208 12.96]*	0.26±0.0193 13.33]ns
DA-20	0.20±0.01 [18.75]ns	0.38±0.0206 [24.0]*	0.59±0.0128 14.45]ns	0.52±0.0105 18.75]*	0.375±0.015 [30.55]*	0.17±0.0133 43.33]*
DA-21	0.22±0.0113 [31.25]*	0.34±0.0058 [32.0]*	0.44±0.0071 [36.23]*	0.40±0.008 [37.5]*	0.33±0.0174 [38.88]*	0.21±0.0087 [30.0]*
DA-22	0.22±0.0141 [31.25]*	0.36±0.0198 [28.0]*	0.52±0.0250 [24.64]*	0.475±0.016 [25.78]*	0.33±0.0213 [38.88]*	0.11±0.006 [63.33]*
DA-23	0.09±0.0125 [71.88]*	0.185±0.014 [63.0]*	0.27±0.0123 [60.88]*	0.25±0.0108 [60.94]*	0.21±0.0088 [61.11]*	0.09±0.008 [70.00]*
DA-24	0.16±0.0130 [50.00]*	0.30±0.0117 [40.0]*	0.39±0.0138 43.48]*	0.38±0.0125 [40.62]*	0.29±0.0131 [46.30]*	0.10±0.0019 [66.67]*
DA-25	0.25±0.0136 [21.88]ns	0.41±0.0186 [18.00]ns	0.51±0.0164 [26.09]*	0.48±0.0182 [25.0]*	0.40±0.0135 [25.93]*	0.23±0.0158 [23.33]*
DA-26	0.20±0.0163 [37.5]*	0.355±0.0143 29.0]*	0.43±0.0151 [37.68]*	0.38±0.0137 [40.62]*	0.335±0.008 [37.96]*	0.175±0.0112 [41.67]*
DA-27	0.21±0.0129 [34.38]*	0.37±0.0256 [26.0]*	0.45±0.0154 [34.78]*	0.43±0.0254 [32.81]*	0.34±0.0102 [37.04]*	0.21±0.0065 [30.00]*
DA-28	0.23±0.0142 [28.12]*	0.42±0.0204 [16.0]ns	0.54±0.0161 [21.74]ns	0.49±0.0168 [23.44]*	0.41±0.0135 [24.07]*	0.21±0.0143 [30.0]*
DA-29	0.21±0.0117 [34.38]*	0.41±0.0093 [18.0]ns	0.51±0.0073 [26.09]*	0.465±0.0171 [27.34]*	0.38±0.0137 [29.63]*	0.18±0.0106 [40.00]*
DA-30	0.24±0.014 [25.0]*	0.41±0.0138 [18.0]ns	0.53±0.0169 [23.19]*	0.495±0.0149 [22.66]*	0.41±0.0184 [24.07]*	0.20±0.0119 [33.33]*
DA-31	0.20±0.0181 [37.50]*	0.32±0.0364 [36.0]*	0.43±0.0275 [37.68]*	0.40±0.0131 [37.5]*	0.34±0.0215 [37.04]*	0.17±0.0056 [43.33]*
DA-32	0.24±0.0161 [25.0]*	0.34±0.0138 [32.0]*	0.48±0.0142 [30.43]*	0.45±0.0152 [29.69]*	0.38±0.0114 [29.63]*	0.20±0.0154 [30.0]*
DA-33	0.24±0.0147 [25.0]*	0.35±0.0058 [30.0]*	0.46±0.0176 [33.33]*	0.42±0.0148 [34.38]*	0.35±0.0208 [35.18]*	0.19±0.0114 [36.67]*
DA-34	0.185±0.013 [42.19]*	0.28±0.013 [44.0]*	0.40±0.0068 [42.03]*	0.35±0.0143 [45.31]*	0.29±0.0183 [46.29]*	0.16±0.0143 [46.67]*
DA**	0.26±0.034 [18.75]ns	0.38±0.034 [24.0]*	0.50±0.0335 [27.53]*	0.49±0.0256 [23.94]*	0.43±0.0226 [20.37]*	0.22±0.0125 [26.67]*
Indomethacin	0.16±0.0145 [50]*	0.21±0.0125 [58]*	0.24±0.0131 [65.22]*	0.27±0.0113 [57.8]*	0.28±0.0154 [48.15]*	0.26±0.0141 [13.33]ns
Control	0.32±0.0221	0.50±0.0187	0.69±0.0263	0.64±0.0169	0.54±0.0196	0.30±0.0154

Species used: Female Wistar rats (160±20g); Dose: 100mg/kg of the test compounds and 10mg/kg for the standard indomethacin was administered orally, 1 h before the carrageenan injection (0.1ml of 1% solution in saline).
Each value represents the mean ± SEM (n=6)
Data was analyzed by one way ANOVA followed by Post-hoc test
* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$
DA refers to the 5,6-dimethoxyindan-1-acetic acid (compound **V-VI)

The 1st h data indicate that the test compounds are slow acting and the activity of these compounds reaches peak after 3-4 h of oral administration, however, residual activity is very high and the duration of action often exceeded 24 hours. The higher residual activity may be associated with the high lipophilicity of the amide derivatives (Table 5.2) in comparison to the free acid **DA** having logP value of 2.11. Due to high lipophilicity these amide derivatives will not only have better absorption profile but are also likely to form lipid depots in the body which may be responsible for longer activity profile of these compounds. The peak activity as well as residual activity is maximal in case of long chain amides (**DA-10** and **DA-11**) having comparatively high calculated log P values (Table 5.2). However, the peak activity of the test compounds was found to be lower than that of indomethacin (10mg/kg, p.o.) but their residual activity at 24th h. exceeded that of the latter (Figure 6.1).

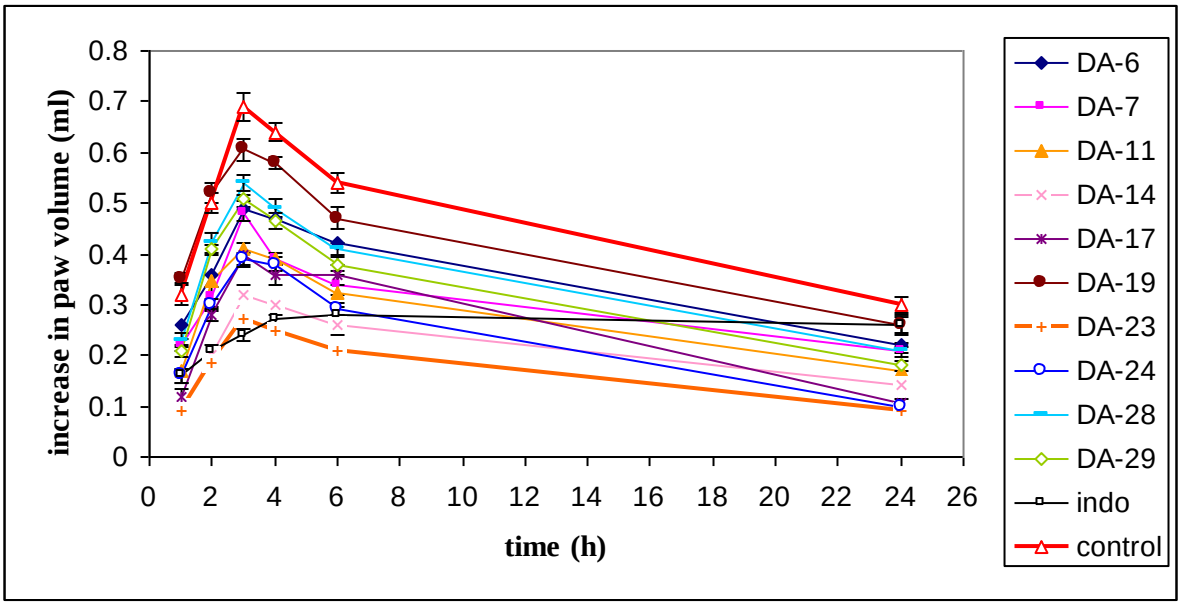


Figure 6.1. Anti-inflammatory screening of 5,6-dimethoxyindan-1-acetic acid amides

In the aliphatic amides it was found that the activity increased with increasing bulk of the amide function. The dimethyl (**DA-3**), diethyl (**DA-5**), isopropyl (**DA-7**) and tertiary butyl (**DA-9**) derivatives were comparatively more active than methyl (**DA-2**), ethyl (**DA-4**), propyl (**DA-6**) and butyl (**DA-8**) derivatives. The cyclic analogs (**DA-13, DA-14**) were also found to

be more active as compared to their linear counterparts (**DA-10, DA-11**). The ethanolamine derivative (**DA-19**) showed least activity which may have resulted from its low log P value and hence poor absorption. Among the cyclic amine derivatives, the piperazino derivative (**DA-17**) showed good activity in spite of its low log P value, which may be correlated to some possible hydrogen bonding interactions of –NH of piperazine ring system with the receptor.

In the case of the aromatic amides the activity seemed to be dependent on the type and position of substituents on the phenyl ring of these aromatic amides. 4-chlorophenyl derivative (**DA-23**) was found to be most active and showed longer activity profile with 70.0 % inhibition at 24th hour. Replacement of chloro group at para position with the bulkier bromo group (**DA-24**) decreased activity. There was substantial decrease in activity with electron donating substituents (**DA-27**, **DA-29** and **DA-30**) at para position. The ortho substituted derivatives (**DA-25**, **DA-28** and **DA-32**) showed poor activity possibly because of steric hindrance to interaction with the receptor.

Based on the anti-inflammatory activity profile of the test compounds (Table 6.1) we selected compound **DA-23** for dose response studies. Compound **DA-23** was found to have an ED_{30} value of 17.94 mg/kg at 3rd h.

Table 6.2. Anti-inflammatory screening for DA-23 for calculation of ED_{30}

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]					
	1H	2H	3H	4H	6H	24H
DA-23 [200mg/kg]	0.07±0.0154 [76.67]*	0.13±0.0141 [70.45]*	0.18±0.0162 [71.43]*	0.18±0.0148 [70.0]*	0.15±0.0136 [70.59]*	0.07±0.0133 [74.07]*
DA-23 [100mg/kg]	0.09±0.0147 [70.0]*	0.16±0.0138 [63.64]*	0.24±0.0167 [61.90]*	0.23±0.0153 [61.67]*	0.20±0.0141 [60.78]*	0.09±0.0136 [66.67]*
DA-23 [50mg/kg]	0.14±0.0161 [53.33]*	0.22±0.0177 [50.0]*	0.34±0.0171 [46.03]*	0.31±0.0167 [48.33]*	0.26±0.0163 [49.02]*	0.13±0.0127 [51.85]*
DA-23 [25mg/kg]	0.18±0.0151 [40.0]*	0.27±0.0168 [38.64]*	0.40±0.0153 [36.51]*	0.37±0.0175 [38.33]*	0.31±0.0165 [39.22]*	0.16±0.0131 [40.74]*
Control	0.30±0.0172	0.44±0.0184	0.63±0.0164	0.60±0.0159	0.51±0.0177	0.27±0.0142

Species used: Female Wistar rats (160±20g); Dose: 100mg/kg was administered orally, 1 h before the carrageenan injection (0.1ml of 1%solution in saline).
Each value represents the mean ± SEM (n=6)
Data was analyzed by one way ANOVA followed by Post-hoc test
* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$

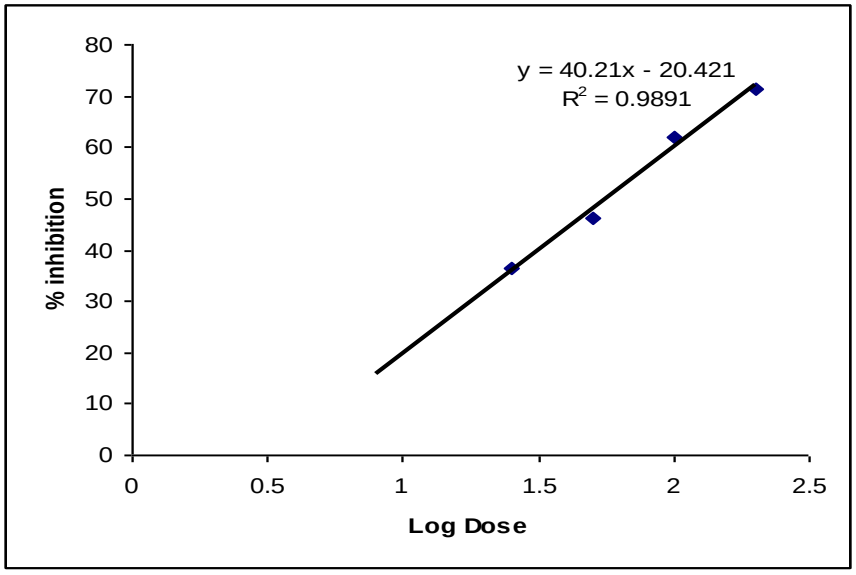


Figure 6.2. Calculation of ED_{30} for the compound DA-23

6.2.2. Screening of 5,6-dimethoxyindan-1-acetic acid amides for analgesic activity

The analgesic activity was determined by acetic acid-induced writhing assay. The results (Table 6.3) reveal that the beneficial effect of increasing chain length was also apparent for analgesic activity of these compounds. The activity increased with increasing chain length and reaches the maximum with the hexyl derivative, compound **DA-11**. The cyclic analogs (**DA-13, DA-14**) were comparatively less active than their linear counterparts (**DA-10, DA-11**). The cyclic amine derivatives showed less activity while the piperazino derivative (**DA-17**) exhibited good analgesic properties. From the results described in Table 6.3 it was seen that for aromatic amides the activity was dependent on the type and position of substituents on the phenyl ring of the aromatic amides.

Table 6.3. Analgesic activity profile of 5,6-dimethoxyindan-1-acetic acid amides against acetic acid-induced writhing

COMPOUND	WRITHING ± S.E.M	% INHIBITION	COMPOUND	WRITHING ± S.E.M	% INHIBITION
DA-1	26.13±1.41*	24.62	**DA-18**	26.5±1.91*	23.56
DA-2	27.33±1.36*	21.17	**DA-19**	24.33±2.08*	29.82
DA-3	26.83±1.19*	22.61	**DA-20**	18.0±2.65*	48.05
DA-4	26.5±1.78*	23.57	**DA-21**	19.83±2.48*	42.80
DA-5	25.17±1.94*	27.40	**DA-22**	16.83±0.79*	51.46
DA-6	25.33±1.76*	26.94	**DA-23**	9.17±1.85*	73.55
DA-7	24.5±1.23*	29.33	**DA-24**	21.67±0.49*	37.50

DA-8	21.0±0.97*	39.43	**DA-25**	24.26±1.85*	30.03
DA-9	20.19±1.37*	41.76	**DA-26**	15.83±1.33*	54.34
DA-10	9.83±1.47*	71.65	**DA-27**	20.33±1.15*	41.36
DA-11	6.17±1.17*	82.20	**DA-28**	26.17±1.12*	24.52
DA-12	23.33±1.28*	32.71	**DA-29**	14.33±1.17*	58.67
DA-13	21.83±2.07*	37.03	**DA-30**	24.00±1.37*	30.78
DA-14	19.17±1.78*	44.70	**DA-31**	15.33±1.54*	55.78
DA-15	28.0±0.73*	19.24	**DA-32**	18.00±2.25*	48.05
DA-16	19.17±2.80*	44.70	**DA-33**	10.17±1.56*	70.67
DA-17	11.0±1.06*	68.83	**DA-34**	13.83±2.09*	60.11
DA	19.41±1.68*	44.01	**Aspirin**	13.33±1.09*	61.55
Indomethacin	16.83±1.30*	51.46	**Control**	34.67±1.67	

Species used: Female Swiss albino mice (20±5g); Dose: 100mg/kg of the test compounds and aspirin and 10mg/kg of the standard indomethacin were administered orally 1 h before the intraperitoneal injection of acetic acid injection (0.1ml/10g of 1% v/v acetic acid.)
Data was analyzed using student's t test. * Represents data significantly different from control at $p < 0.05$.
Each value represents the mean ± SEM (n=6)

The 4-chlorophenyl (**DA-23**) derivative showed good activity while 3-chlorophenyl (**DA-22**) and 4-bromophenyl (**DA-24**) derivatives were comparatively less active (Figure 6.3). The 3-pyridyl (**DA-33**) and 4-pyridyl (**DA-34**) derivatives were found to have good analgesic activity compared to the standard drug aspirin. The m-toluidine derivative (**DA-26**) was more active than the ortho (**DA-25**) and para (**DA-27**) substituted toluidine derivatives. The 4-methoxyphenyl (**DA-29**) derivative which showed poor anti-inflammatory activity was found to have relatively good analgesic activity.

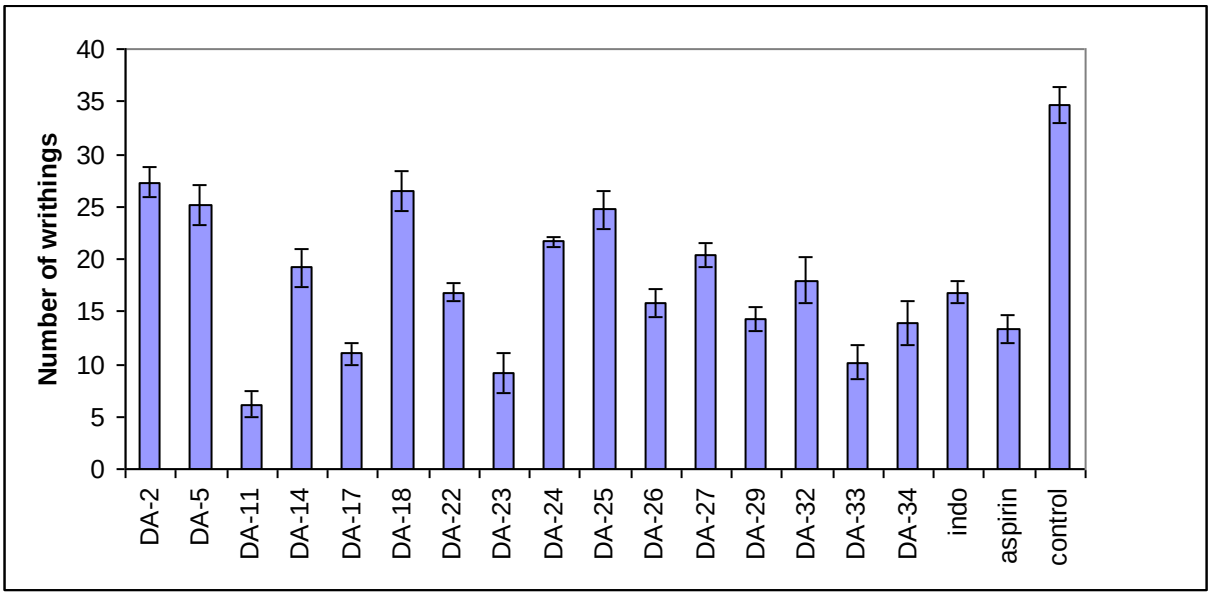


Figure 6.3. Analgesic screening of 5,6-dimethoxyindan-1-acetic acid amides

6.2.3. Screening of 5,6-dimethoxyindan-1-acetic acid amides for antipyretic activity

Few selected compounds were subjected to lipopolysaccharide (LPS)-induced hyperthermia assay. The test compounds (Table 6.4) exhibited significant antipyretic property, but none of these compounds exhibited antagonism of the initial LPS-induced hypothermia as indicated by first temperature index at the end of 3rd h in Table 6.4 thus indicating that the antagonism of TNF-α is not possibly involved in the mechanism of action of these compounds[11]. However, the test compounds significantly reduce the hyperthermia induced by LPS as shown by the second temperature index at end of 7th h in Table 6.4. Compounds **DA-11** and **DA-17** were found to be the most potent in this regard. Compounds **DA-23** and **DA-29** showed antipyretic activity comparable to that of the standard drug aspirin (Figure 6.4). It can be observed from Table 6.4 and Figure 6.4 that the standard drug indomethacin lowers the initial LPS-induced hypothermia. This lowering of initial hypothermia can be correlated to the inhibition of TNF-α by indomethacin.

Table 6.4. Antipyretic activity profile of 5,6-dimethoxyindan-1-acetic acid amides against LPS-induced-pyresis

COMPOUND	CHANGE IN RECTAL TEMPERATURE (ºC) AND TEMPERATURE INDEX								
	1H	2H	3H	TI	4H	5H	6H	7H	TI
DA-7	-0.41±0.013	-1.51±0.014	-0.39±0.016	**-2.31**	0.19±0.017	0.43±0.018	0.55±0.011	0.59±0.015	**1.76**
DA-11	-0.56±0.019	-1.38±0.018	-0.33±0.011	**-2.27**	0.11±0.015	0.38±0.016	0.53±0.013	0.54±0.017	**1.56**
DA-14	-0.33±0.011	-1.26±0.012	-0.67±0.017	**-2.26**	0.05±0.022	0.51±0.016	0.45±0.013	0.43±0.019	**1.44**
DA-17	-0.45±0.018	-1.23±0.015	-0.85±0.016	**-2.53**	-0.18±0.019	0.11±0.021	0.19±0.017	0.16±0.020	**0.28**
DA-19	-0.65±0.017	-1.93±0.018	0.17±0.011	**-2.72**	0.34±0.016	0.57±0.018	0.65±0.012	0.66±0.015	**2.22**
DA-22	-0.35±0.016	-1.62±0.014	-0.24±0.018	**-2.21**	0.05±0.019	0.23±0.013	0.44±0.019	0.42±0.019	**1.14**
DA-23	-0.47±0.013	-1.69±0.015	-0.54±0.019	**-2.70**	-0.11±0.011	0.17±0.014	0.41±0.011	0.40±0.014	**0.87**
DA-26	-0.19±0.015	-1.34±0.016	-0.51±0.013	**-2.04**	0.22±0.018	0.46±0.017	0.71±0.015	0.82±0.018	**2.21**
DA-29	-0.12±0.019	-1.84±0.012	-0.66±0.017	**-2.62**	-0.15±0.018	0.14±0.014	0.26±0.017	0.43±0.017	**0.68**
DA-34	-0.11±0.019	-2.23±0.013	-0.87±0.016	**-3.21**	0.13±0.013	0.35±0.015	0.74±0.016	0.42±0.013	**1.64**
Aspirin	-0.43±0.016	-1.45±0.015	-0.51±0.013	**-2.39**	0.05±0.012	0.11±0.019	0.17±0.013	0.23±0.019	**0.56**
Indomethacin	-0.39±0.011	-	-	**-**	0.22±0.019	0.30±0.022	0.35±0.014	0.55±0.018	**1.42**

		1.03±0.017	0.11±0.015	**1.53**					
Control	-0.61±0.014	-1.28±0.018	-0.19±0.014	**-2.08**	0.34±0.016	0.76±0.011	1.03±0.019	1.30±0.021	**3.43**

Species used: Female Wistar rats (170±20g); Dose: 100mg/kg for the test compounds and aspirin and 10mg/kg for the standard indomethacin was administered orally, half an hour before the intraperitoneal administration of LPS 100µg/kg; each value represents the mean ± SEM (n=6)

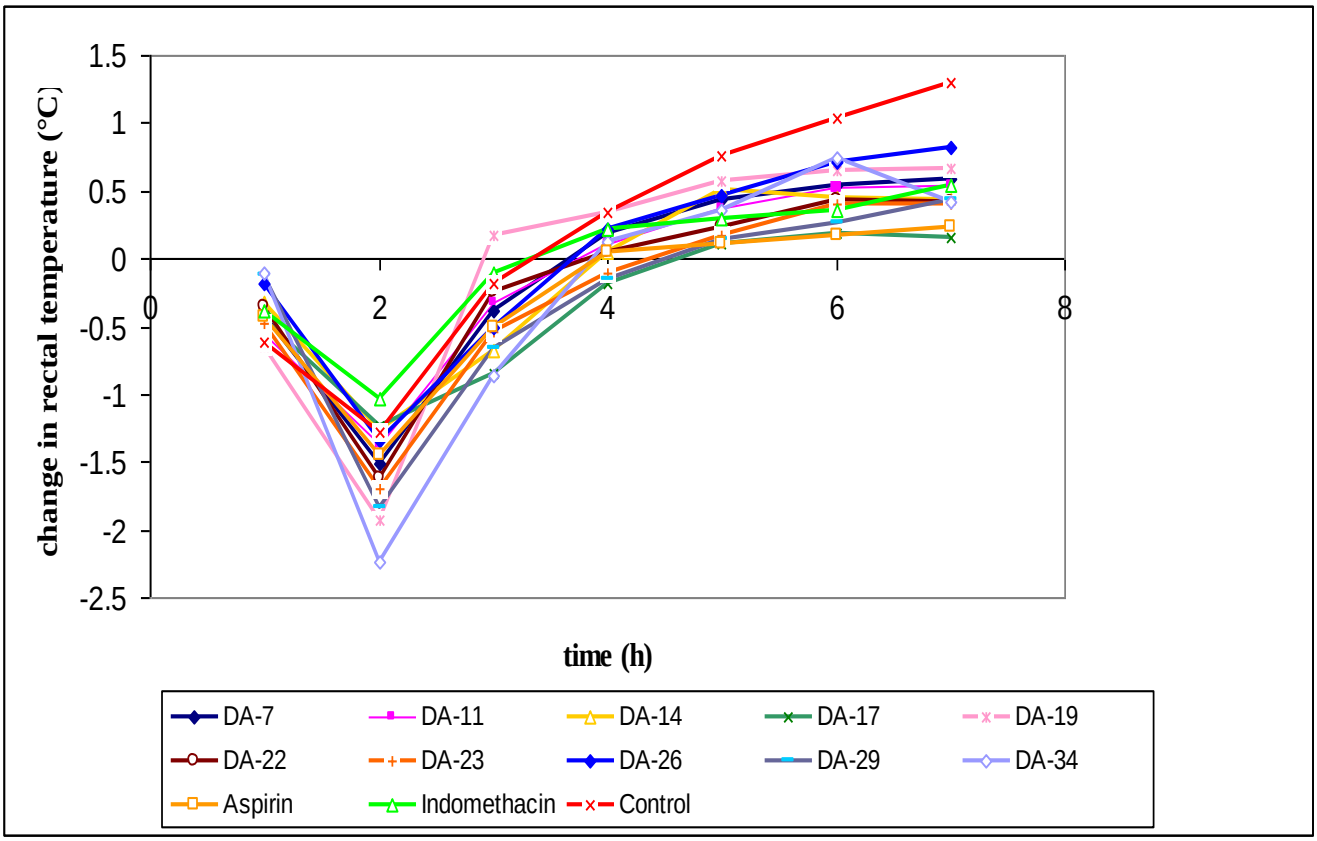


Figure 6.4. Antipyretic activity profile of 5,6-dimethoxyindan-1-acetic acid amides

6.2.4 Screening of 5,6-dimethoxyindan-1-acetic acid amides for anti-arthritic activity

Compounds were selected for adjuvant–induced arthritis assay based on the inhibition of carrageenan-induced edema, analgesic and antipyretic activity profile. Adjuvant-induced arthritis test in rats produced a biphasic response. Results of this test summarized in Table 6.5 showed that these compounds possess comparatively poor anti-arthritic activity. The 3rd day data revealed that there was mild to moderate inhibition of inflammation in all the tested compounds but only few compounds (**DA-11**, **DA-14**, and **DA-22**) showed inhibition of secondary inflammation (30-35%) in the left uninjected paw on the 13th day. Also there was better weight change for these test compounds as compared to indomethacin treated animals

indicating their better toxicity profile. Compound **DA-23** which showed best activity profile in anti-inflammatory screening was inactive in this assay. There was severe development of nodules in most of the cases. 3-chloro substituted (**DA-22**) derivative which showed intermediate activity profile in anti-inflammatory screening exhibited best activity in the adjuvant induced arthritis.

Table 6.5. Anti-arthritic activity profile of 5,6-dimethoxyindan-1-acetic acid amides against adjuvant-induced arthritis

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]				SECONDARY LESIONS	WEIGHT CHANGE
	3rd DAY	8th DAY	13th DAY	13th DAY#		(g)
DA-11	1.54±0.0164 [31.25]*	1.41±0.0176 [28.79]*	1.29±0.0185 [39.44]*	1.02±0.0163 [35.44]*	Moderate	7.64±0.167
DA-14	1.59±0.0175 [29.02]*	1.35±0.0179 [31.82]*	1.42±0.0168 [33.33]*	1.10±0.0203 [30.38]*	Moderate	6.84±0.154
DA-17	1.45±0.0156 [35.27]*	1.41±0.0169 [28.79]*	2.08±0.0159 [2.35]	1.55±0.181 [1.90]	Severe	3.11±0.132
DA-22	1.40±0.0213 [37.5]*	1.47±0.0191 [25.76]*	1.37±0.0206 [35.68]*	1.08±0.0196 [31.65]*	Moderate	7.21±0.140
DA-23	1.60±0.0182 [28.57]*	1.82±0.0225 [8.08]	2.03±0.0219 [4.69]	1.53±0.0179 [3.16]	Severe	3.51±0.131
DA-26	1.87±0.0221 [16.52]*	1.60±0.0235 [19.19]*	1.69±0.0197 [20.66]*	1.32±0.0213 [16.46]*	Severe	4.56±0.129
DA-31	1.63±0.0203 [16.52]*	1.63±0.0217 [17.68]*	1.90±0.0239 [10.80]	1.46±0.0175 [7.60]	Severe	4.01±0.133
DA-34	1.86±0.0188 [16.96]*	1.76±0.0196 [11.11]	1.71±0.0261 [19.72]	1.35±0.0179 [14.56]	Severe	4.25±0.154
Indomethacin	1.40±0.0178 [37.50]*	1.11±0.0185 [43.94]*	1.14±0.0213 [46.48]*	1.02±0.0181 [35.44]*	Moderate	5.68±0.124
Control	2.24±0.0175	1.98±0.0189	2.13±0.0201	1.58±0.0165	Severe	2.88±0.117

Species used: Female Wistar rats (160±10g); Dose: 100mg/kg for the test compounds and 1mg/kg for the standard indomethacin was administered orally for 14 days; 0.1ml of adjuvant administered was injected in subplantar region of the right hind paw on the second day. Each value represents the mean ± SEM (n=6)
Data was analyzed by one way ANOVA followed by Post-hoc test
* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$
# uninjected left paw

6.2.4. Evaluation of ulcerogenic potential of 5,6-dimethoxyindan-1-acetic acid amides

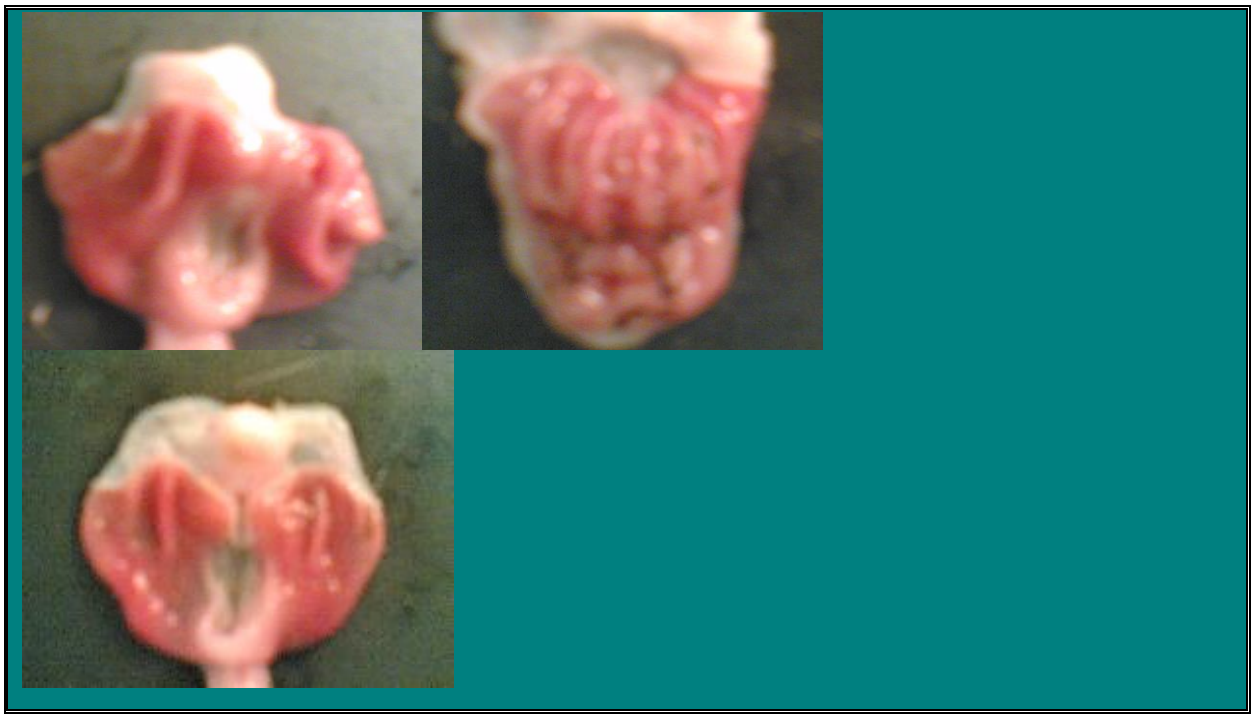
Some selected compounds (based on anti-inflammatory and analgesic activity profile) were tested for their ulcerogenecity potential. No animal treated with test compounds at the level of 100mg/kg p.o. developed ulcer (Table 6.6 and Figure 6.5). Compounds DA-14 and DA-23 did not show any ulceration at the highest tested dose level of 500mg/kg. It may, however, be noted here that the administration of the test compounds at the dose level of 100mg/kg p.o.

even for 14 days did not cause any ulceration of the gastric mucosa as revealed in the post mortem studies of sacrificed animals at the end of the adjuvant-induced arthritis study

Table 6.6. Evaluation of ulcer index for 5,6-dimethoxy indan-1-acetic acid amides

COMPOUND	DOSE (mg/kg)	TIME	ULCER INDEX	COMPOUND	DOSE (mg/kg)	TIME	ULCER INDEX
DA-7	100	6h	0	**DA-22**	100	6h	0
DA-11	100	6h	0	**DA-23**	100	6h	0
DA-14	100	6h	0	**DA-23**	500	6h	0
DA-14	500	6h	0	**DA-27**	100	6h	0
DA-17	100	6h	0	**DA-31**	100	6h	0
Indomethacin	30	6h	30 ± 2.74	**Control**	-	6h	0
DA-11	100	14days	0	**DA-22**	100	14days	0
DA-14	100	14days	0	**DA-26**	100	14days	0
Indomethacin	1	14days	21.50 ± 2.60	**Control**	-	14days	0

The compounds were given orally to 24 h fasted male Wistar rats (200±20g);
Each value represents the mean ± SEM (n=4)



A **B** **C**

Figure 6.5. Photographs of rat stomachs showing ulcer development by Indomethacin and compound DA-23 in comparison to control; A-control, B-Indomethacin, C-**DA-23**

6.2.6. Acute toxicity study

The rats employed in anti-inflammatory screening were observed during 24 h. No mortality was found with any of these compounds at the end of observation period. The compounds **DA-14** and **DA-23** were also studied for their toxicity profile at higher dose levels. No toxicity was observed with these compounds up to the highest tested dose of 1000 mg/kg p.o.

6.3. PHARMACOLOGICAL SCREENING OF 6-CHLOROINDAN-1-ACETIC ACID AMIDES

6.3.1. Screening of 6-chloroindan-1-acetic acid amides for anti-inflammatory activity

The anti-inflammatory activity of the test compounds was evaluated by carrageenan-induced rat paw edema model. The results (Table 6.7) show that the test compounds exhibit variable anti-inflammatory activity, and a few among them have significant acute as well as residual anti-inflammatory activity at 24 h after a single oral dose. Most of these amide derivatives were found to have more activity than the parent acid **CA** but in general the activity profile was slightly lower in comparison to 5,6-dimethoxyindan-1-acetic acid amides.

Table 6.7. The anti-inflammatory activity profile of 6-chloroindan-1-acetic acid amides against carrageenan-induced rat paw edema

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]					
	1H	2H	3H	4H	6H	24H
CA-1	0.25±0.0145 [19.35]*	0.26±0.0136 [46.94]*	0.32±0.0161 [48.39]*	0.38±0.0125 [41.15]*	0..32±0.0141 [37.25]*	0.13±0.0116 [55.17]*
CA-2	0.29±0.0149 [6.45]ns	0.45±0.0117 [8.16]ns	0.57±0.0132 [8.06]ns	0.58±0.0132 [10.77]ns	0.45±0.0128 [11.76]ns	0.25±0.0133 [13.79]ns
CA-3	0.28±0.0173 [9.68]ns	0.43±0.0148 [12.24]ns	0.56±0.0273 [9.68]ns	0.59±0.0273 [9.23]ns	0.45±0.0165 [11.76]ns	0.27±0.0125 [6.90]ns
CA-4	0.29±0.0149 [6.45]ns	0.44±0.0117 [10.20]ns	0.55±0.0132 [11.29]ns	0.58±0.0132 [10.77]ns	0.46±0.0128 [9.80]ns	0.26±0.0133 [10.34]ns
CA-5	0.28±0.0118 [9.68]ns	0.37±0.0166 [24.48]*	0.48±0.0134 [22.58]*	0.50±0.0125 [23.08]*	0.44±0.0145 [13.72]*	0.26±0.0108 [10.34]ns
CA-6	0.27±0.0124 [12.90]ns	0.42±0.0153 [14.28]ns	0.54±0.0148 [12.90]ns	0.58±0.0132 [10.77]ns	0.45±0.0173 [11.76]ns	0.26±0.0128 [10.34]ns
CA-7	0.26±0.0136 [16.13]ns	0.38±0.0149 [22.45]*	0.48±0.0147 [22.58]*	0.51±0.0129 [21.54]*	0.37±0.0166 [27.45]*	0.21±0.0091 [27.59]*
CA-8	0.24±0.0154 [22.58]*	0.34±0.0192 [30.61]*	0.42±0.0144 [32.26]*	0.46±0.0165 [29.23]*	0.35±0.0127 [31.37]*	0.20±0.0162 [31.03]*
CA-9	0.25±0.0116	0.38±0.0137	0.44±0.0147	0.46±0.0132	0.35±0.0151	0.19±0.0131

	[19.35]*	[22.45]*	[29.03]*	[29.23]*	[31.37]*	[34.48]*
CA-10	0.27±0.0143 [12.90]ns	0.40±0.0161 [18.37]*	0.48±0.0153 [22.58]*	0.50±0.0136 [23.08]*	0.41±0.0163 [19.61]*	0.20±0.0085 [31.03]*

Contd….

Table 6.7. Contd.

CA-11	0.28±0.0112 [9.62]ns	0.39±0.0121 [20.41]*	0.55±0.0134 [11.29]ns	0.56±0.0150 [13.85]ns	0.46±0.0088 [9.80]ns	0.23±0.0125 [20.69]*
CA-12	0.21±0.0138 [32.26]*	0.36±0.0118 [26.53]*	0.46±0.0136 [25.81]*	0.48±0.0132 [26.15]*	0.42±0.0158 [17.65]*	0.21±0.0138 [27.59]*
CA-13	0.29±0.0178 [6.45]ns	0.43±0.0163 [12.24]ns	0.55±0.0152 [11.29]ns	0.59±0.0186 [9.23]ns	0.47±0.0159 [7.84]ns	0.27±0.0153 [6.90]ns
CA-14	0.23±0.0155 [25.81]*	0.36±0.0154 [26.53]*	0.44±0.0148 [29.03]*	0.44±0.0164 [32.31]*	0.38±0.0135 [25.49]*	0.21±0.0113 [27.59]*
CA-15	0.20±0.0114 [35.48]*	0.31±0.0159 [36.73]*	0.42±0.0121 [32.25]*	0.48±0.0137 [26.15]*	0.42±0.0162 [17.65]*	0.25±0.0142 [13.79]ns
CA-16	0.15±0.0138 [51.61]*	0.25±0.0146 [48.98]*	0.31±0.0165 [50.0]*	0.36±0.0159 [44.62]*	0.31±0.0132 [39.22]*	0.13±0.0083 [55.17]*
CA-17	0.26±0.0133 [16.13]ns	0.32±0.0126 [34.69]*	0.40±0.0138 [35.48]*	0.48±0.0142 [26.15]*	0.40±0.173 [26.15]*	0.21±0.0121 [27.59]*
CA-18	0.23±0.0125 [25.81]*	0.34±0.0161 [30.61]*	0.44±0.0178 [29.03]*	0.47±0.0136 [27.69]*	0.41±0.0179 [19.61]*	0.20±0.0130 [31.03]*
CA-19	0.26±0.0169 [16.13]ns	0.36±0.0141 [26.53]*	0.52±0.0177 [16.13]ns	0.55±0.0134 [15.38]*	0.43±0.0156 [15.69]*	0.26±0.0144 [10.34]ns
CA-20	0.19±0.0143 [38.71]*	0.26±0.0187 [46.94]*	0.36±0.0151 [41.94]*	0.39±0.0149 [40.0]*	0.34±0.0164 [33.33]*	0.21±0.0126 [27.59]*
CA-21	0.28±0.0186 [9.68]ns	0.42±0.0162 [14.29]ns	0.53±0.0167 [14.52]ns	0.58±0.0179 [10.77]ns	0.44±0.0206 [13.72]*	0.21±0.0165 [27.59]*
CA-22	0.30±0.0145 [3.22]ns	0.46±0.0134 [6.12]ns	0.56±0.0129 [9.67]ns	0.59±0.0180 [9.23]ns	0.47±0.0161 [7.80]ns	0.26±0.0114 [10.34]ns
CA-23	0.17±0.0157 [45.16]*	0.24±0.0149 [51.02]*	0.34±0.0176 [45.16]*	0.40±0.0136 [38.46]*	0.33±0.0182 [35.29]*	0.15±0.0142 [48.28]*
CA-24	0.26±0.0139 [16.34]ns	0.35±0.0156 [28.57]*	0.42±0.0162 [32.26]*	0.52±0.0148 [20.0]*	0.45±0.0151 [11.76]ns	0.23±0.0112 20.69]*
CA-25	Toxic at 100mg/kg, p.o.					
CA-25 (25 mg)	0.24±0.0142 [22.58]	0.33±0.0136 [32.65]	0.29±0.0154 [53.22]	0.12±0.0147 [81.54]	0.14±0.0135 [72.54]	0.08±0.0123 [72.41]
CA-25 (12.5 mg)	0.25±0.0133 [19.35]	0.35±0.0148 [28.57]	0.37±0.0156 [40.32]	0.37±0.0141 [43.08]	0.33±0.0146 [35.29]	0.17±0.0135 [41.38]
CA-25 (6.25 mg)	0.26±0.0154 [16.31]	0.41±0.0144 [16.33]	0.48±0.00138 [22.58]	0.51±0.0157 [21.54]	0.43±0.0151 [15.68]	0.22±0.0143 [24.17]
CA**	0.27±0.0165 [12.90]ns	0.40±0.0129 [18.37]*	0.50±0.0146 [19.35]*	0.52±0.0158 [20.0]*	0.43±0.0143 [15.69]*	0.22±0.0126 [24.14]*
indomethacin	0.16±0.0145 [48.39]*	0.22±0.0125 [55.10]*	0.23±0.0131 [62.90]*	0.26±0.0113 [60.0]*	0.28±0.0154 [45.10]*	0.25±0.0141 [13.79]ns
control	0.31±0.0163	0.49±0.0159	0.62±0.0185	0.65±0.0174	0.51±0.0166	0.29±0.0156

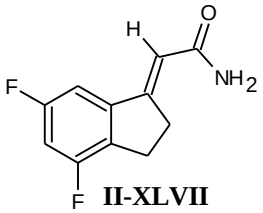
Species used: Female Wistar rats (160±20g); Dose: 100mg/kg for the test compounds and 10mg/kg for the standard indomethacin was administered orally, 1 h before the carrageenan injection (0.1ml of 1%solution in saline).

Each value represents the mean ± SEM (n=6)

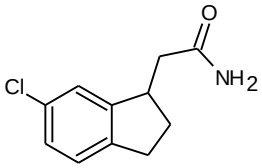
Data was analyzed by one way ANOVA followed by Post-hoc test

* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$
CA refers to the 6-chloroindan-1-acetic acid (compound **V-XI)

The compounds were slow acting and showed longer duration of action. In this series also the peak activity of the test compounds was also found to be lower than that of indomethacin (10mg/kg, p.o.) but their residual activity at 24th h exceeded that of the latter. The compounds **CA-1**, **CA-16**, **CA-23** showed nearly 50% inhibition as the residual activity. The activity can be partially correlated to the lipophilicity of these compounds. The lower members in the aliphatic amides showed almost negligible activity, however compound **CA-1** was found to have good anti-inflammatory activity. This may be due to close resemblance of this compound to recently discovered centrally acting muscle relaxant, (E)-2-(4,6-difluoro-1-indanylidene)acetamide, **II-XLVII**, possessing potent anti-inflammatory and analgesic activity[110]. This compound has been taken in phase I clinical trials.



II-XLVII



CA-1

Amongst the aliphatic amides the hexyl derivative (**CA-8**) showed intermediate activity profile. The tertiary amides (**CA-11** and **CA-12**) and the cyclic amides (**CA-9** and **CA-10**) showed intermediate activity. Among the aromatic series no generalized pattern of activity was found.

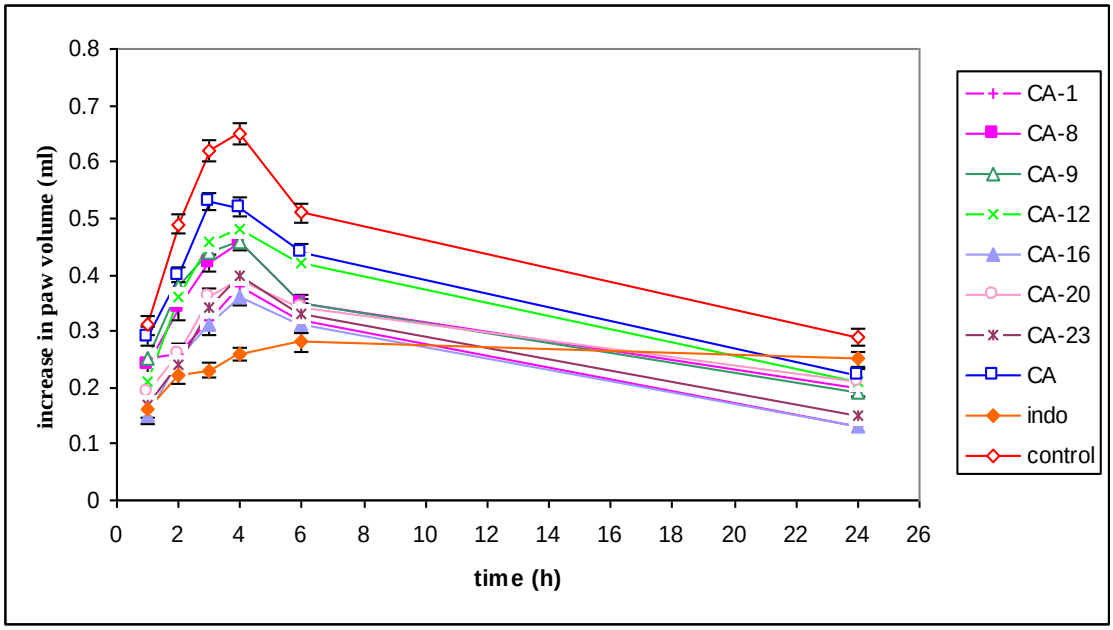
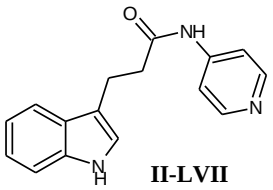


Figure 6.6. Anti-inflammatory activity profile of 6-chloroindan-1-acetic acid amides

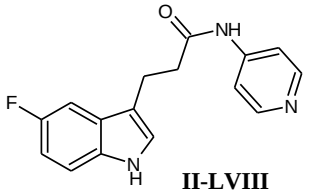
The m-chloroaniline derivative (**CA-16**) was found to be the most active. The p-chloro derivative (**CA-17**) and p-bromo derivative (**CA-18**) were comparatively less active and showed intermediate activity (Figure 6.6). The p-nitroaniline (**CA-23**) and p-toluidine (**CA-20**) derivatives also showed good anti-inflammatory activity profile while p-acetamidoaniline (**CA-22**) derivative showed least activity. The 4-aminopyridine derivative (**CA-25**) was found to be toxic at 100mg / kg p.o. whereas its positional isomer 3-aminopyridine derivative (**CA-24**) didn't show any toxicity at the same dose level. The animals died within half an hour to one hour of oral administration of this compound. The animals showed very unusual behaviour (jumping, piloerection, convulsions). Also after death the rigor mortis developed very soon (within five minutes). One of the 4-aminopyridine derivative of (indol-3-yl)alkylcarboxamide has also been reported to be toxic at higher dose levels of 0.4mM/kg however this compound showed excellent anti-inflammatory activity of 95±3 % at 0.1mM/kg. Pharmacomodulation showed that its toxicity could be markedly attenuated by introduction of a fluorine atom at C5 or a methoxycarbonylborane grouping in the pyridinyl nucleus[117].



II-LVII

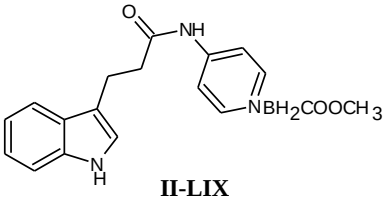
0.4mM/kg - toxic

0.1mM/kg - 95 % inhibition of edema



II-LVIII

0.1mM/kg - 86 % inhibition of edema



II-LIX

0.1mM/kg - 84 % inhibition of edema

This compound CA-25 was also not found to be toxic at lower dose level of 25 mg/kg. At this dose level it showed best anti-inflammatory activity among the chloro series with ED_{30} of 6.45mg/kg at end of 4^{th} h (Table 6.7 and Figure 6.7).

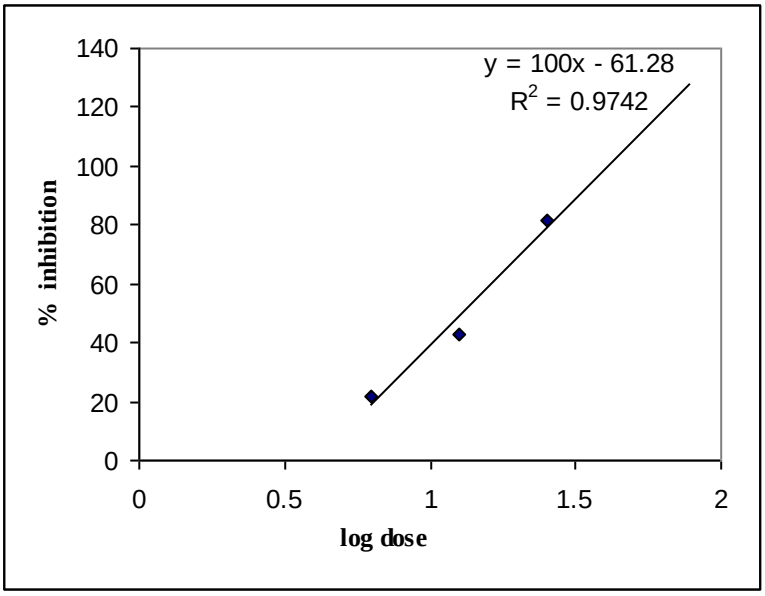


Figure 6.7. Calculation of ED_{30} of compound CA-25

6.3.2. Screening of 6-chloroindan-1-acetic acid amides for analgesic activity

The analgesic activity was determined by acetic acid-induced writhing assay. The results (Table 6.8) reveal that the beneficial effect of increasing amide chain length was also apparent for analgesic activity to some extent with few exceptions. The amide derivative (**CA-1**) showed good analgesic properties with 66.82 % of inhibition in writhing assay. Among the cyclic analogs the cyclopentyl derivative (**CA-9**) was equipotent to its linear counterpart, (**CA-7**) whereas the cyclohexyl derivative (**CA-10**) was less active than its linear counterpart (**CA-8**) (Figure 6.8). The cyclic piperidino derivative (**CA-11**) showed intermediate activity while the piperazino derivative (**CA-12**) exhibited good analgesic properties. Among aromatic amides, halogen substitution (**CA-16, CA-17, CA-18**) resulted in intermediate activity. The aniline derivative (**CA-15**) was more active than the benzyl derivative (**CA-14**). Though m-toluidine derivative (**CA-19**) showed poor anti-inflammatory activity than para derivative (**CA-20**) the results were opposite for analgesic activity. The 3-aminopyridinyl derivative (**CA-24**) showed maximum inhibition of 94 % at the dose level of 100mg/kg p.o. and was more potent than standard drugs.

Table 6.8. Analgesic activity profile of 6-chloroindan-1-acetic acid amides against acetic acid- induced writhing

COMPOUND	WRITHING	% INHIBITION	COMPOUND	WRITHING	% INHIBITION

	± S.E.M			± S.E.M	
CA-1	11.22±0.65*	66.82	**CA-14**	22.33±1.05*	33.68
CA-2	27.74±0.87*	17.34	**CA-15**	14.17±0.95*	57.92
CA-3	26.5±0.85*	21.29	**CA-16**	15.17±0.60*	54.94
CA-4	20.5±0.76*	39.11	**CA-17**	17.33±1.05*	48.53
CA-5	19.5±0.80*	42.08	**CA-18**	16.5±0.99*	50.99
CA-6	22.17±0.95*	34.16	**CA-19**	11.33±0.71*	66.35
CA-7	20.5±1.18*	39.11	**CA-20**	23.33±0.88*	30.71
CA-8	19.33±0.99*	42.59	**CA-21**	23.83±0.91*	29.22
CA-9	20.17±0.87*	40.10	**CA-22**	19.5±0.89*	42.08
CA-10	22.33±0.88*	33.68	**CA-23**	15.67±0.76*	53.46
CA-11	24.5±0.78*	27.23	**CA-24**	2.0±0.37*	94.06
CA-12	13.33±0.80*	60.41	**CA-25**	toxic	
CA-13	25.17±0.70*	25.24	**CA**	20.17±0.96*	40.10
Indomethacin	15.26±0.95*	54.68	**Aspirin**	12.18±1.13*	63.82
Control	33.67±1.12				

Species used: Female Swiss albino mice (20±5g); Dose: 100mg/kg for the test compounds and aspirin and 10mg/kg for the standard indomethacin were administered orally, 1 h before the intraperitoneal injection of acetic acid injection (0.1ml/10g of 1% v/v acetic acid.)
Data was analyzed using student's t test. * Represents data significantly different from control at $p < 0.05$
Each value represents the mean ± SEM (n=6)

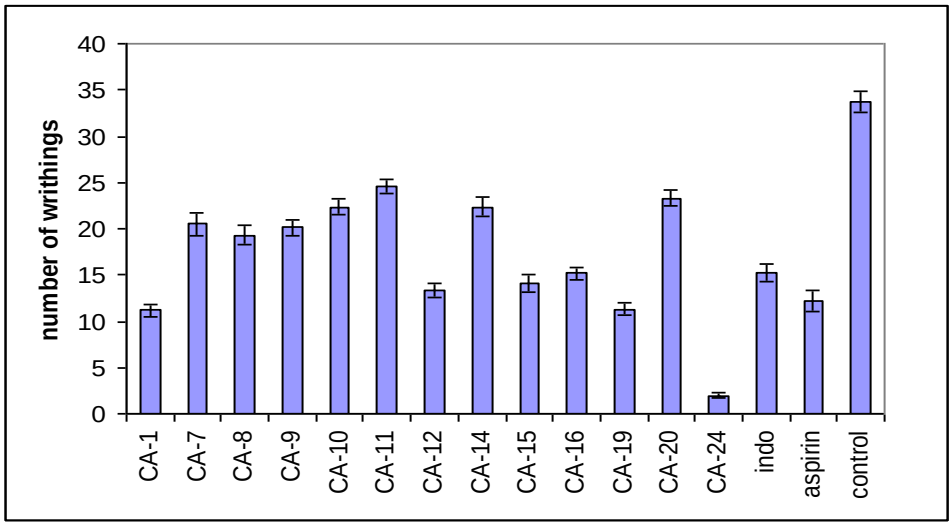


Figure 6.8. Analgesic screening of 6-chloroindan-1-acetic acid amides

6.3.3. Screening of 6-chloroindan-1-acetic acid amides for antipyretic activity

Few selected compounds were subjected to lipopolysaccharide (LPS)-induced hyperthermia assay. As compared to earlier reported protocol for testing the antipyretic activity some changes were made in testing the compounds of chloro series. Here the LPS was given first and after three hours of its administration the drug was given orally, the rectal temperature was recorded hourly for next 4 h. The change in rectal temperature was determined in comparison to the intial temperature of the rectum before the administration of LPS. This

change was adopted to prevent the initial hypothermia induced by LPS which was not assessed because these compounds were similar to the DA series. The test compounds (Table 6.9 and Figure 6.9) exhibited significant antipyretic property, as indicated by the temperature index. The compound **CA-1** showed antipyretic activity comparable to that of aspirin. The isopropyl (**CA-5**), halogen substituted amine (**CA-16, CA-18**), and 3-aminopyridino (**CA-24**) amide derivatives showed appreciable antipyretic activity. The piperazine derivative CA-12 showed poor antipyretic activity.

Table 6.9. Antipyretic activity profile of 6-chloroindan-1-acetic acid amides against LPS induced pyresis

COMPOUND	CHANGE IN RECTAL TEMPERATURE (ºC) AND TEMPERATURE INDEX				
	4H	5H	6H	7H	TI
CA-1	-0.06± 0.0125	0.05± 0.0142	0.08±0.0133	0.13±0.0126	**0.17**
CA-5	0.28± 0.0161	0.18±0.0130	0.13±0.0152	0.13±0.0152	**0.70**
CA-9	0.12± 0.0153	0.30±0.0148	0.62±0.0128	0.71±0.0137	**1.75**
CA-12	0.28±0.0173	0.70±0.0162	0.76±0.0141	0.84±0.0148	**2.58**
CA-16	0.20± 0.0141	0.11±0.0115	0.18±0.0112	0.30±0.0120	**0.79**
CA-18	0.24±0.0144	0.15± 0.0136	0.11±0.0138	0.11±0.0125	**0.61**

Contd….

Table 6.9. Contd.

CA-19	0.18±0.0126	0.38± 0.0152	0.44±0.0134	0.56±0.0155	**1.56**
CA-20	0.37±0.0133	0.55±0.0128	0.55± 0.0 147	0.64±0.0134	**2.11**
CA-23	0.23±0.0136	0.42±0.0164	0.51±0.0156	0.72±0.0145	**1.88**
CA-24	0.22±0.0121	0.11±0.0142	0.16±0.0168	0.28± 0.0170	**0.77**
Aspirin	0.05± 0.0112	-0.02±0.0131	0.03±0.0124	0.11±0.0115	**0.17**
Indomethacin	0.25±0.0118	0.29± 0.0108	0.38±0.0125	0.59±0.0110	**1.51**
Control	0.32± 0.0179	0.71±0.0156	0.96±0.0166	1.22±0.0143	**3.21**

Species used: Female Wistar rats (170±20g); Dose: 100mg/kg for the test compounds and aspirin and 10mg/kg for the standard indomethacin was administered orally after three hours of intraperitoneal administration of LPS at 100µg/kg. Each value represents the mean ± SEM (n=6)

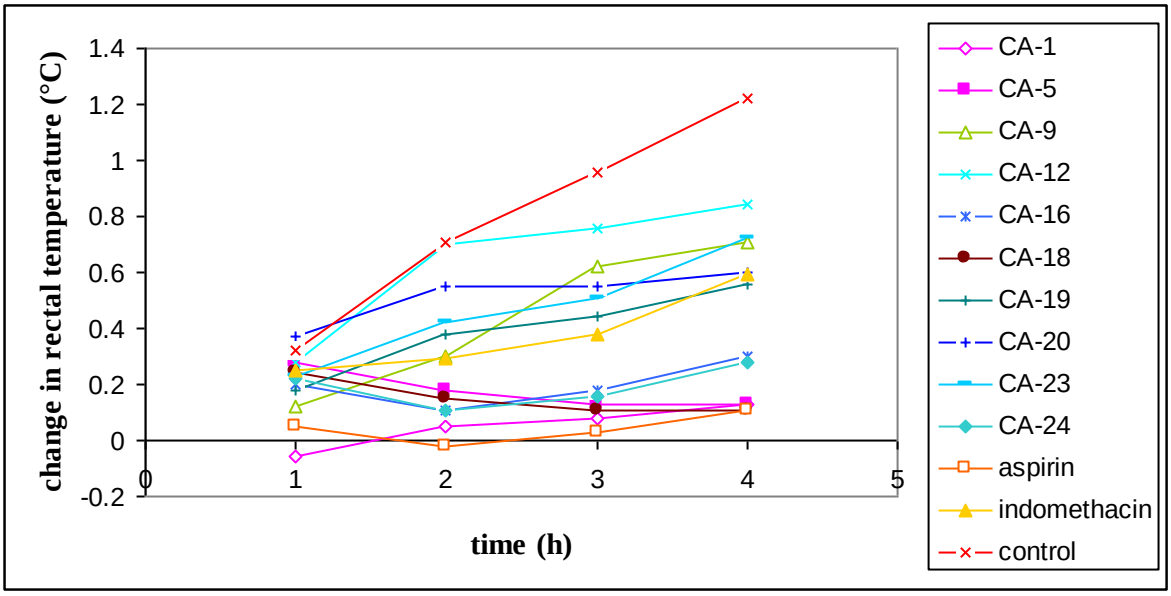


Figure 6.9. **A**ntipyretic activity profile of 6-chloroindan-1-acetic acid amides

6.3.4. Screening of 6-chloroindan-1-acetic acid amides for anti-arthritic activity

Compounds were selected for adjuvant–induced arthritis assay based on the inhibition of carrageenan-induced edema, analgesic and antipyretic activity profile. Results of this test summarized in Table 6.10 show that compound **CA-23** has the best profile in this assay. Compound **CA-1**and **CA-23** was found to be active and the animals did gain more weight than those treated with indomethacin indicating a better toxicity profile compared to the reference drug. The m- chloro substituted derivative though showed good anti-inflammatory activity it was inactive in the arthritis model and failed to inhibit the development of secondary lesions. In this biomodel though compounds **CA-1**and **CA-23** significantly reduced the primary inflammation of the right hind paw but as with indomethacin these were not able to reduce the secondary inflammation of the left hind paw.

Table 6.10. Anti-arthritic activity profile of 6-chloroindan-1-acetic acid amides against adjuvant-induced arthritis

COMPOUND	INCREASE IN PAW VOLUME (ML) ± SEM [% INHIBITION OF EDEMA]				SECONDARY LESIONS	WEIGHT CHANGE
	3rd DAY	8th DAY	13th DAY	13th DAY#		(g)
CA-1	1.65±0.0142 [30.38]*	1.56±0.0155 [26.76]*	1.45±0.0163 [35.84]	1.09±0.0164 [33.54]	Moderate	6.67±0.144
CA-9	2.09±0.0162 [11.81]*	1.47±0.0182 [30.99]*	1.89±0.0171 [16.37]*	1.41±0.0158 [14.02]*	Severe	4.33±0.150
CA-16	1.46±0.0175 [38.40]*	1.55±0.0167 [27.23]*	2.21±0.0154 [2.21]*	1.60±0.0160 [2.44]*	Severe	3.58±0.169
CA-17	2.23±0.0156	2.03±0.0148	2.19±0.0175	1.61±0.0163	Severe	3.25±0.181

	[5.91]*	[4.69]*	[3.10]*	[1.83]*		
CA-20	1.82±0.0158 [23.21]*	1.79±0.0162 [15.96]*	1.56±0.0188 [30.97]*	1.21±0.0171 [26.22]*	Moderate	5.98±0.142
CA-23	1.68±0.0176 [29.11]*	1.14±0.0153 [46.48]*	1.38±0.0144 [38.93]*	1.06±0.0157 [35.37]*	Moderate	6.92±0.158
Indomethacin	1.48±0.0154 [37.55]*	1.16±0.0176 [45.53]*	1.23±0.0187 [45.57]*	1.07±0.0172 [34.76]*	Moderate	5.42±0.133
Control	2.37±0.0170	2.13±0.0194	2.26±0.0173	1.64±0.0166	Severe	3.14±0.141

Species used: Female Wistar rats (160±10g); Dose: 100mg/kg for the test compounds and 1mg/kg for the standard indomethacin was administered orally for 14 days; 0.1ml of adjuvant administered was injected in sub plantar region of the paw on the second day. Each value represents the mean ± SEM (n=6).Data was analyzed by one way ANOVA followed by Post-hoc test. * Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$. # uninjected left paw

6.3.5. Evaluation of ulcerogenic potential of 6-chloroindan-1-acetic amides

Some selected compounds (based on anti-inflammatory and analgesic activity profile) were tested for their ulcerogenic potential. The tested compounds developed much lesser number of ulcerogenic lesions as indicated by ulcer index in comparison to the control (Table 6.11). The nitro derivative (**CA-23**) showed no ulcer at tested dose level of 100mg/kg p.o. It may, however, be noted that the administration of the test compounds at the dose level of 100mg/kg p.o. for 14 days also did not cause any ulceration of the gastric mucosa as revealed in the post mortem studies of sacrificed animals at the end of the adjuvant-induced arthritis study.

Table 6.11. Evaluation of ulcer index of 6-chloroindan-1-acetic acid amides

COMPOUND	DOSE (mg/kg)	TIME	ULCER INDEX	COMPOUND	DOSE (mg/kg)	TIME	ULCER INDEX
CA-1	100	6h	2.75±0.48	**CA-20**	100	6h	8.0±0.82
CA-15	100	6h	11.00±1.29	**CA-23**	100	6h	0
CA-16	100	6h	1.75±0.48				
Indomethacin	30	6h	31.83 ± 2.07	**Control**	-	6h	0
CA-1	100	14days	0	**CA-23**	100	14days	0
Indomethacin	1	14days	21.00 ± 1.96	**Control**	-	14days	0

The compounds were given orally to 24 h fasted male Wistar rats (200±20g);
Each value represents the mean ± SEM (n=4)

6.3.6. Acute Toxicity Studies: The rats employed in anti-inflammatory screening were observed during 24 h. No mortality occurred with any of these compounds except **CA-25** at the end of observation period. Compounds **CA-1** and **CA-16** were also studied for their toxicity profile at higher dose levels. No toxicity was observed with these compounds up to the highest tested dose of 1000mg/kg.

6.4. PHARMACOLOGICAL SCREENING OF 5,6-DIMETHOXYINDAN-1-

CARBOXYLIC ACID AMIDES

6.4.1. Anti-inflammatory screening of 5,6-dimethoxyindan-1-carboxylic acid amides

The anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema model. Examination of Table 6.12 reveals that the carboxylic acid series have low activity profile in comparison to the acetic acid series. Not much correlation was seen between the activity and lipophilicity of the molecules as was observed in acetic acid series (DA and CA series).

Table 6.12. Anti-inflammatory screening of 5,6-dimethoxyindan-1-carboxylic acid amides against carrageenan induced edema

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]					
	1H	2H	3H	4H	6H	24H
DC-1	0.20±0.0135 [25.92]*	0.27±0.0151 [25.0]*	0.42±0.0148 [28.81]*	0.46±0.0126 [29.23]*	0.38±0.0155 [29.63]*	0.23±0.0131 [28.12]*
DC-2	0.20±0.0128 [25.92]*	0.27±0.0133 [25.0]*	0.39±0.0176 [33.90]*	0.45±0.0157 [30.78]*	0.39±0.0114 [27.77]*	0.22±0.0098 [30.62]*
DC-3	0.23±0.0215 [14.81]*	0.29±0.0185 [19.41]*	0.42±0.0178 [28.81]*	0.49±0.0208 [24.62]*	0.44±0.0193 [18.52]*	0.24±0.0135 [25.0]*
DC-4	0.21±0.0118 [22.22]*	0.28±0.0153 [22.22]*	0.41±0.0147 [30.59]*	0.47±0.0165 [27.69]*	0.42±0.0172 [22.22]*	0.24±0.0123 [25.0]*
DC-5	0.22±0.0141 [18.52]*	0.29±0.0137 [19.44]*	0.43±0.0125 [27.12]*	0.49±0.0114 [24.62]*	0.44±0.0136 [18.52]*	0.23±0.0138 [28.12]*
DC-6	0.23±0.0115 [14.81]*	0.30±0.0126 [16.67]*	0.42±0.0134 [28.81]*	0.48±0.0127 [26.15]*	0.44±0.0144 [18.51]*	0.20±0.0108 [37.5]*
DC-7	0.23±0.0146 [14.81]*	0.29±0.0128 [19.44]*	0.43±0.0155 [27.11]*	0.51±0.0134 [21.5]*	0.47±0.0141 [12.96]ns	0.25±0.0125 [21.88]*
DC-8	0.21±0.0136 [22.22]*	0.24±0.0147 [33.33]*	0.40±0.0133 [32.20]*	0.48±0.0145 [26.15]*	0.43±0.0115 [20.37]*	0.20±0.0117 [37.5]*
DC-9	0.19±0.0130 [29.63]*	0.29±0.0138 [19.44]*	0.47±0.0154 [20.33]*	0.49±0.0141 [24.62]*	0.41±0.0137 [24.07]*	0.22±0.0133 [31.25]*
DC-10	0.23±0.0145 [14.81]*	0.29±0.0178 [19.44]*	0.45±0.0167 [23.72]*	0.51±0.0153 [21.54]*	0.44±0.0148 [18.51]*	0.26±0.0136 [18.75]*
DC-11	0.18±0.0117 [33.33]*	0.24±0.0134 [33.33]*	0.38±0.0126 [35.59]*	0.43±0.0108 [33.85]*	0.35±0.0143 [35.19]*	0.16±0.0123 [50.0]*
DC-12	0.19±0.0109 [29.63]*	0.27±0.0157 [25.0]*	0.39±0.0187 [33.90]*	0.45±0.0173 [30.77]*	0.39±0.0125 [27.78]*	0.23±0.0128 [28.12]*

Cont

d…

Table 6.12. Contd.

DC-13	0.24±0.0145 [11.11]ns	0.33±0.0130 [8.33]ns	0.51±0.0177 [13.36]ns	0.55±0.0095 [15.38]*	0.50±0.0171 [7.41]ns	0.25±0.0196 [21.88]*
DC-14	0.23±0.0084 [14.81]*	0.30±0.0114 [16.67]*	0.41±0.0182 [30.51]*	0.50±0.0123 [23.08]*	0.44±0.0174 [18.51]*	0.19±0.0109 [40.62]*
DC-15	0.19±0.0115 [29.63]*	0.25±0.0133 [30.56]*	0.39±0.0106 [33.90]*	0.47±0.0130 [27.69]*	0.43±0.0149 [20.37]*	0.13±0.0145 [59.38]*
DC-16	0.18±0.0125	0.24±0.014	0.38±0.0126	0.44±0.0176	0.41±0.0156	0.24±0.0176

	[33.33]*	[33.33]*	[35.59]*	[32.31]*	[24.07]*	[25]*
DC-17	0.21±0.0108 [22.22]*	0.32±0.0207 [11.11]ns	0.47±0.0167 [20.33]*	0.46±0.0130 [29.23]*	0.38±0.0128 [29.63]*	0.19±0.0092 [40.62]*
DC-18	0.19±0.0123 [29.63]*	0.30±0.0105 [16.67]*	0.42±0.0167 [28.81]*	0.44±0.0148 [32.31]*	0.43±0.0117 [20.37]*	0.25±0.0130 [21.88]*
DC-19	0.26±0.0136 [3.70]ns	0.34±0.0130 [5.50]ns	0.53±0.0145 [10.17]ns	0.60±0.0155 [7.69]ns	0.52±0.0176 [3.84]ns	0.25±0.0123 [21.88]*
DC-20	0.20±0.0128 [25.92]*	0.28±0.0114 [22.22]*	0.45±0.0186 [23.72]*	0.51±0.0138 [21.54]*	0.43±0.0134 [20.37]*	0.26±0.0145 [18.75]*
DC-21	0.22±0.0138 [18.52]*	0.30±0.0154 [16.67]*	0.41±0.0174 [30.51]*	0.46±0.0165 [29.23]*	0.38±0.0144 [29.63]*	0.25±0.0130 [21.88]*
DC-22	0.23±0.0087 [14.81]*	0.32±0.0099 [11.11]ns	0.42±0.0141 [28.81]*	0.46±0.0095 [29.23]*	0.39±0.0098 [27.78]*	0.26±0.0076 [18.75]*
DC**	0.24±0.0138 [11.11]ns	0.30±0.0145 [16.67]*	0.47±0.0132 [20.33]*	0.53±0.0156 [18.46]*	0.46±0.0141 [14.81]*	0.26±0.0121 [18.75]*
Indomethacin	0.16±0.0145 [40.74]*	0.18±0.0125 [50]*	0.23±0.0131 [61.02]*	0.24±0.0113 [63.08]*	0.26±0.0154 [51.85]*	0.28±0.0141 [12.5]ns
Control	0.27±0.0176	0.36±0.0145	0.59±0.0158	0.65±0.0189	0.54±0.0167	0.32±0.0145

Species used: Female Wistar rats (160±20g); Dose: 100mg/kg for the test compounds and 10mg/kg for the standard indomethacin was administered orally, 1 h before the carrageenan injection (0.1ml of 1%solution in saline).
Each value represents the mean ± SEM (n=6)
Data was analyzed by one way ANOVA followed by Post-hoc test
* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$
DC refers to the 5,6-dimethoxyindan-1-carboxylic acid (compound **V-XXI)

The lower members of the aliphatic amides exhibited mild activity and there was no enhancement in the activity with the increase in chain length as seen for the amyl (**DC-6**) and hexyl (**DC-7**) derivatives of the acetic acid series. Among the cyclic amine derivatives cyclopentyl (**DC-8**) derivative was comparatively more active than the cyclohexyl derivative (**DC-9**). The piperazine derivative (**DC-11**) exhibited the best activity profile in this series (Figure 6.10). The benzyl derivative (**DC-12**) was found to be more active than the aniline (**DC-13**) derivative emphasizing the importance of the benzylic methylene carbon in the structure. The electron withdrawing group at para position enhanced activity while the electron withdrawing group at meta position decreased activity. The p-chloro (**DC-15**) and p-bromo (**DC-16**) derivatives were found to be more active than the m-chloro derivative (**DC-14**). The electron donating group at para and at meta position did not improve the activity profile. The p-anisidine derivative (**DC-19**) was found to be least active among the aromatic amides (Figure 6.10). However there was an overall slight enhancement in the activity in comparison to the parent acid. Though these compounds were much less active than the standard drug indomethacin but these compounds exhibited better residual activity at the 24^{th} hour in comparison to indomethacin. The p-chloro derivative (**DC-15**) was found to have maximum inhibition of 59% at 24^{th} hour.

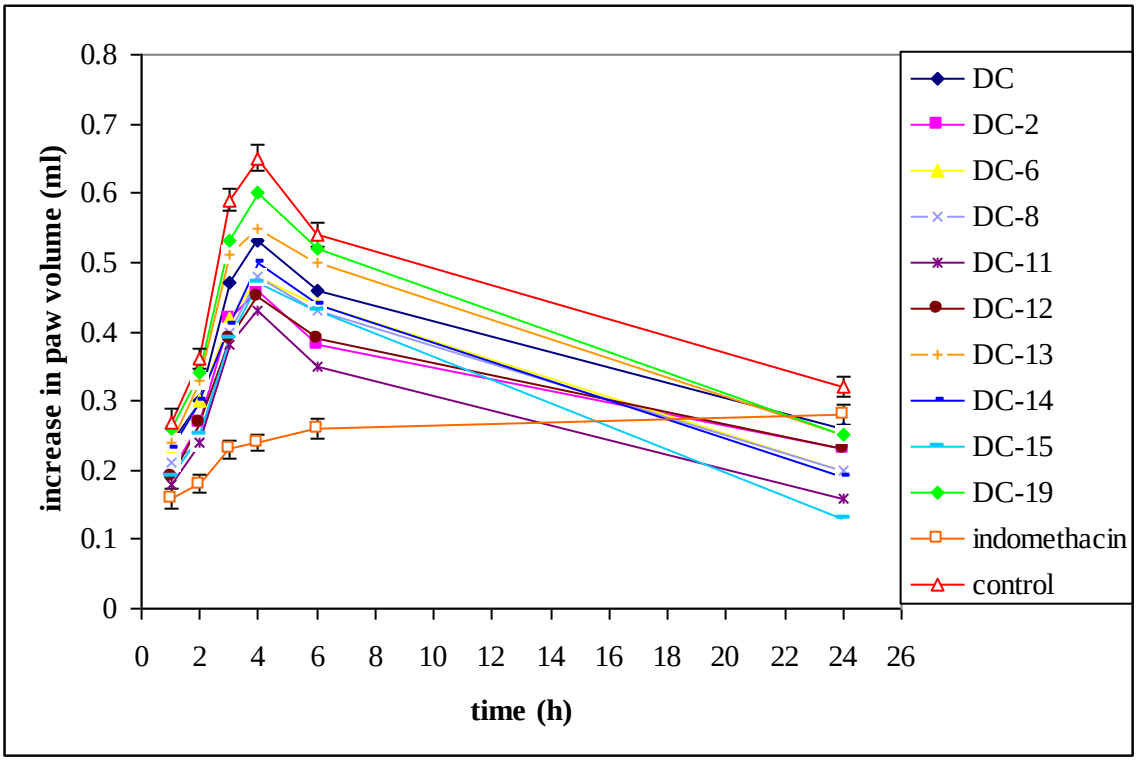


Figure 6.10. Anti-inflammatory activity profile of 5,6-dimethoxyindan-1-carboxylic acid amides

6.4.2. Screening of 5,6-dimethoxyindan-1-carboxylic acid amides for analgesic activity

The analgesic activity was determined by acetic acid induced-writhing assay. The results (Table 6.13) reveal that the activity cannot be correlated with logP values as in the case of anti-inflammatory screening. The lower members (**DC-1, DC-2**) showed better activity as compared to long chain amide (**DC-6, DC-7**) derivatives. It can be possible that for lower members because of low logP values they become bioavailable in the acute phase. The cyclopentyl (**DC-8**) derivative showed intermediate activity while the cyclohexyl (**DC-9**) derivative showed very mild activity.

The piperazine derivative (**DC-11**) and the p-chloro derivative (**DC-15**) which showed good anti-inflammatory activity were comparatively less active in analgesic screening. The p-bromo derivative (**DC-16**) was the most active compound with 71.83% inhibition of acetic acid induced writhing and was more active than the standard drug aspirin. The p-toluidine (**DC-18**) derivative showed much higher analgesic activity as compared to m-toluidine (**DC-17**) and p-anisidine (**DC-19**) derivatives. The 3-amino (**DC-21**) and 4-aminopyridyl (**DC-22**) derivative also showed moderate analgesic activity (Figure 6.11).

Table 6.13. Analgesic activity profile of 5,6-dimethoxyindan-1-carboxylic acid amides against acetic acid-induced writhing

COMPOUND	WRITHING ± S.E.M	INHIBITION %	COMPOUND	WRITHING ± S.E.M	INHIBITION %
DC-1	18.17±1.22	53.18*	**DC-12**	27.17±1.14	25.91*
DC-2	14.00±0.67	61.82*	**DC-13**	28.17±0.79	23.18*
DC-3	21.83±1.19	40.46*	**DC-14**	18.83±0.95	48.65*
DC-4	16.00±1.24	56.37*	**DC-15**	27.00±1.06	26.37*
DC-5	18.67±0.56	49.09*	**DC-16**	10.33±0.42	71.83*
DC-6	30.00±1.65	18.79*	**DC-17**	32.17±1.25	12.27^{ns}
DC-7	28.17±0.65	23.18*	**DC-18**	13.83±1.30	62.29*
DC-8	21.17±0.48	42.27*	**DC-19**	28.17±0.79	23.18*
DC-9	31.83±1.58	13.19^{ns}	**DC-20**	27.17±1.14	25.91*
DC-10	20.33±0.82	44.57*	**DC-21**	18.17±1.22	50.45*
DC-11	23.33±1.94	36.38*	**DC-22**	16.00±0.93	56.36*
DC	24.17±1.74	34.08*	**Aspirin**	13.67±1.25	62.72*
Control	36.67±1.69		**Indomethacin**	17.17±1.44	53.18*

Species used: Female Swiss albino mice (20±5g); Dose: 100mg/kg for the test compounds and aspirin and 10mg/kg for the standard indomethacin were administered orally, 1 h before the intraperitoneal injection of acetic acid injection (0.1ml/10g of 1% v/v acetic acid.)
Data was analyzed using student's t test. * Represents data significantly different from control at $p < 0.05$ and ns represents not significant at $p < 0.05$
Each value represents the mean ± SEM (n=6)

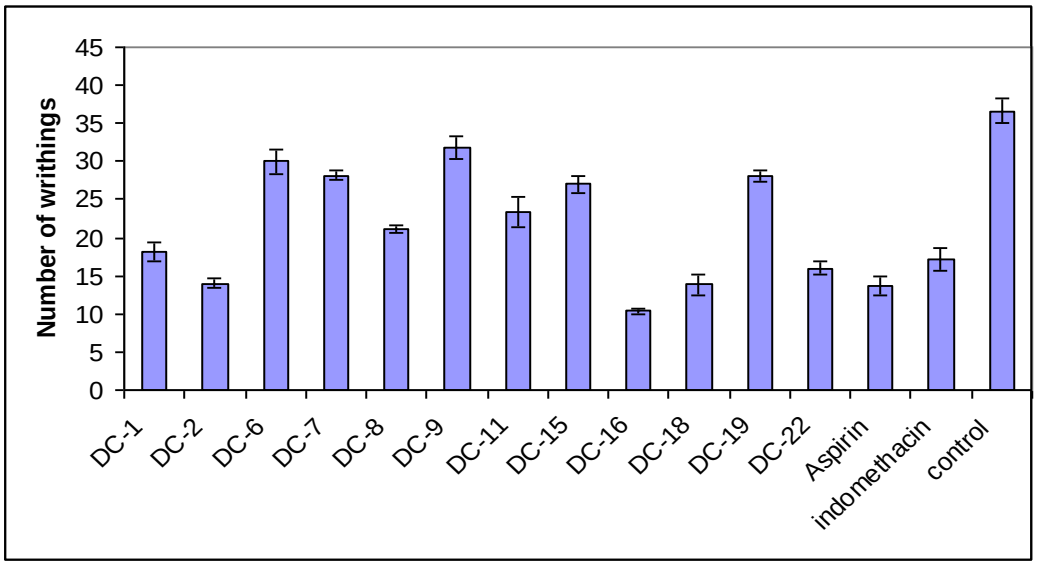


Figure 6.11. Analgesic activity of 5,6-dimethoxyindan-1-carboxylic acid amides

6.4.3. Screening of 5,6-dimethoxyindan-1-carboxylic acid amides for antipyretic activity

Few selected compounds were subjected to lipopolysaccharide (LPS)-induced hyperthermia assay. The initial hypothermic phase of LPS induced pyresis was excluded from the experimental protocol. This change was made seeing the low activity profile of the test compounds. The methyl derivative (**DC-2)** which showed good analgesic activity exhibited poor antipyretic activity as indicated by the high temperature index (Table 6.14 and Figure 6.12). The 3-chloro (**DC-14**), 4-bromo (**DC-16**) and 4-aminopyridyl (**DC-22**) derivatives were also comparatively less active. The p-toluidine (**DC-18**) and p-chloro (**DC-15**) derivative showed intermediate activity profile. The cyclopentyl (**DC-8**) and the piperazine (**DC-11**) derivative showed the best antipyretic activity with temperature index of 0.73 and 0.81. The results obtained indicate that substitution pattern was not the determining factor for antipyretic activity in this series.

Table 6.14. Antipyretic activity profile of 5,6-dimethoxyindan-1-carboxylic acid amides against LPS-induced pyresis

COMPOUND	CHANGE IN RECTAL TEMPERATURE (ºC) AND TEMPERATURE INDEX				
	4H	5H	6H	7H	TI
DC-2	0.22± 0.0133	0.49± 0.0148	0.88±0.0126	1.10±0.0135	2.69
DC-8	0.02± 0.0098	0.12±0.0130	0.18±0.0147	0.41±0.0141	0.73
DC-11	0.07± 0.0158	0.17±0.0167	0.24±0.0155	0.33±0.0173	0.81
DC-14	0.14±0.0203	0.28±0.0180	0.44±0.0156	0.58±0.0158	1.96
DC-15	0.29± 0.0160	0.38±0.0165	0.42±0.0183	0.57±0.0154	1.66
DC-16	0.19±0.0131	0.42± 0.0170	0.64±0.0138	0.79±0.0151	2.04
DC-18	0.23±0.0181	0.30± 0.0167	0.54±0.0148	0.68±0.0143	1.56
DC-22	0.25±0.0117	0.37±0.0137	0.53± 0.0142	0.71±0.0155	1.86
Aspirin	0.09± 0.0128	0.03±0.0116	0.04±0.0123	0.10±0.0135	0.26
Indomethacin	0.21±0.0123	0.28± 0.0145	0.34±0.0137	0.45±0.0126	1.28
Control	0.35±0.0117	0.67±0.0137	0.91± 0.0142	1.18±0.0155	1.86

Species used: Female Wistar rats (170±20g); Dose: 100mg/kg for the test compounds and aspirin and 10mg/kg for the standard indomethacin was administered orally after three hours of the intraperitoneal administration of LPS (100µg/kg); each value represents the mean ± SEM (n=6)

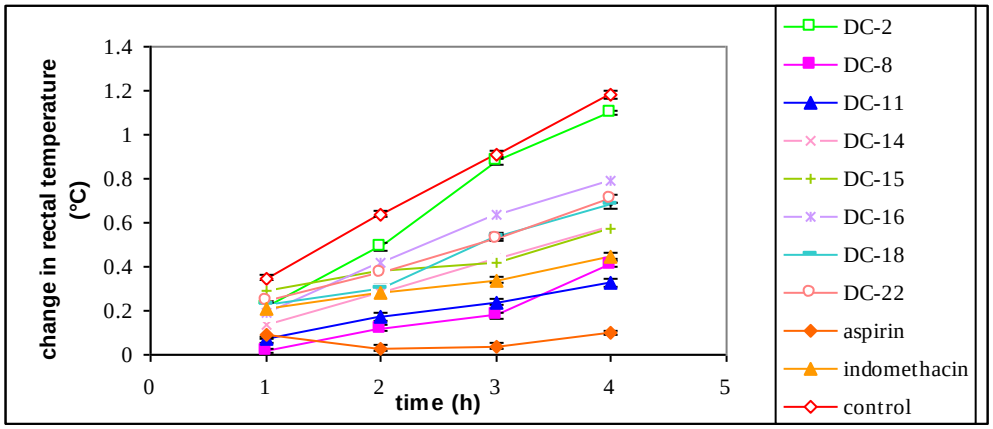


Figure 6.12. Anti-pyretic activity profile of 5,6-dimethoxyindan-1-carboxylic acid amides

6.4.4. Screening of 5,6-dimethoxyindan-1-carboxylic acid amides for anti-arthritic activity

Compounds were selected for adjuvant–induced arthritis assay based on the inhibition of carrageenan-induced edema, analgesic and antipyretic activity profile. The compounds showing good anti-inflammatory activity at 24th hour were studied for the adjuvant assay. As compared to acetic acid series the compounds belonging to carboxylic acid series exhibited better activity profile in adjuvant induced-arthritis. The cyclopentyl derivative (**DC-8**) and the m-chloroaniline derivative (**DC-14**) exhibited excellent activity in this assay. As seen from the Table 6.15 these compounds were even better in preventing the secondary inflammation as compared to the standard indomethacin. Also with these compounds the animals gained more weight than those treated with indomethacin indicating a better toxicity profile compared to the reference drug. The piperazine derivative (**DC-11**) showed good inhibition of inflammation on the 3rd day but failed to inhibit the development of secondary lesions and was comparatively inactive in the latter phase of this assay. For the compounds **DC-8** and **DC-14** experiment was conducted to find out if the inhibition of TNF-α was the underlying mechanism of action of good anti-arthritic activity.

Table 6.15. **A**nti-arthritic activity profile of 5,6-dimethoxyindan-1-carboxylic acid amides against adjuvant induced arthritis

COMPOUND	INCREASE IN PAW VOLUME (ML) ± SEM [% INHIBITION OF EDEMA]				SECONDARY LESIONS	WEIGHT CHANGE (g)
	3rd DAY	8th DAY	13th DAY	13th DAY$^{\#}$		
DC-6	1.43±0.0167 [34.70]*	1.54±0.0185 [20.21]*	1.91±0.0195 [8.17	1.36±0.0171 [7.48]	Severe	3.54±0.142
DC-8	1.20±0.0181 [45.21]*	1.05±0.0177 [45.60]*	1.02±0.0186 [50.96]*	0.78±0.0157 [46.94]*	Moderate	8.46±0.147

DC-11	1.05±0.0155 [52.05]*	0.99±0.0176 [48.70]*	1.96±0.0165 [5.77]*	1.40±0.0167 [4.76]*	Severe	3.33±0.158
DC-14	1.27±0.0148 [42.01]*	0.97±0.0156 [49.74]*	1.04±0.0178 [50.00]*	0.80±0.0145 [45.58]*	Moderate	8.51±0.165
DC-15	1.76±0.0163 [19.63]*	1.47±0.0178 [23.83]*	1.56±0.0181 [25.00]*	1.15±0.0158 [21.77]*	Moderate	5.46±0.167
DC-17	1.40±0.0193 [36.07]*	1.22±0.0185 [36.79]*	1.44±0.0215 [30.77]*	1.08±0.0163 [26.53]*	Moderate	5.67±0.173
Indomethacin	1.39±0.0175 [36.53]*	1.08±0.0169 [44.04]*	1.13±0.0188 [45.67]*	0.93±0.0178 [36.73]*	Moderate	5.45±0.136
Control	2.19±0.0163	1.93±0.0189	2.08±0.0156	1.47±0.0155	Severe	2.67±0.145

Species used: Female Wistar rats (160±10g); Dose: 100mg/kg for the test compounds and 1mg/kg for the standard indomethacin was administered orally for 14 days; 0.1ml of adjuvant administered was injected in sub plantar region of the paw on the second day. Each value represents the mean ± SEM (n=6)
Data was analyzed by one way ANOVA followed by Post-hoc test
* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$
# uninjected left paw

6.4.5. Evaluation of ulcerogenic potential of 5,6-dimethoxyindan-1-carboxylic acid amides

Some selected compounds (based on anti-inflammatory and analgesic activity profile) were tested for their ulcerogenecity potential. The tested compounds developed much less number of ulcerogenic lesions as indicated by ulcer index in comparison to the control (Table 6.16). The p-bromo derivative (**DC-16**) showed lowest ulcerogenecity at the tested dose level of 100mg/kg p.o. The ulcers developed in all cases were seen as pin points. It may, however, be noted that the administration of the test compounds at the dose level of 100mg/kg p.o. for 14 days also did not cause any ulceration of the gastric mucosa as revealed in the post mortem studies of sacrificed animals at the end of the adjuvant-induced arthritis study.

Table 6.16. Evaluation of ulcer index of 5,6-dimethoxyindan-1-carboxylic acid amides

COMPOUND	DOSE (mg/kg)	TIME	ULCER INDEX
DC-2	100	6h	8.75±0.75
DC-8	100	6h	5.50±0.65
DC-11	100	6h	4.50±0.65
DC-16	100	6h	2.25±0.48
DC-18	100	6h	3.75±0.48
Indomethacin	30	6h	28.17 ± 1.35
Control	-	6h	0
DC-8	100	14days	0
DC-14	100	14days	0
Indomethacin	1	14days	23.75±1.75
Control	-	14days	0

The compounds were given orally to 24 h fasted male Wistar rats (200±20g);

Each value represents the mean ± SEM (n=4)

6.4.6. Acute Toxicity Study: The rats employed in anti-inflammatory screening were observed for 24 h. No mortality occurred with any of these compounds at the end of observation period. Compounds **DC-11** and **DC-16** were also studied for their toxicity profile at higher dose levels. No toxicity was observed with these compounds up to the highest tested dose of 1000 mg/kg p.o.

6.4.7. Evaluation of inhibition of TNF-α through LPS induced pyresis.

Two active compounds each from chloro series and carboxylic acid series were studied using LPS-induced pyresis model for evaluation of inhibition of TNF-α by these compounds. The data reveals (Table 6.17) that the test compounds exhibited significant antipyretic property, but these compounds did not exhibit antagonism of the initial LPS-induced hypothermia as indicated by the first temperature index in the Table 6.17. This indicates that the antagonism of TNF- α is not possibly involved in the mechanism of action of these compounds. Though DC-8 and DC-14 showed best anti-arthritic activity profile among all the tested compounds but inhibition of TNF- α was not the underlying mechanism.

Table 6.17. Antipyretic activity of 5,6-dimethoxyindan-1-carboxylic acid amides against LPS-induced pyresis

COMPOUND	CHANGE IN RECTAL TEMPERATURE (ºC) AND TEMPERATURE INDEX								
	1H	2H	3H	TI	4H	5H	6H	7H	TI
CA-1	-0.51±0.013	-1.37±0.011	-0.47±0.012	**-2.35**	0.03±0.011	0.11±0.014	0.19±0.016	0.27±0.015	**0.60**
CA-23	-0.49±0.017	-1.25±0.014	-0.35±0.011	**-2.09**	0.18±0.013	0.40±0.015	0.59±0.012	0.71±0.013	**1.88**
DA-8	-0.46±0.011	-1.23±0.015	-0.37±0.014	**-2.06**	0.11±0.016	0.23±0.012	0.36±0.013	0.43±0.016	**1.13**
DA-14	-0.54±0.014	-1.39±0.016	-0.49±0.021	**-2.42**	0.15±0.017	0.33±0.016	0.49±0.014	0.68±0.012	**1.65**
Aspirin	-0.43±0.013	-1.31±0.014	-0.43±0.012	**2.17**	0.01±0.015	0.09±0.011	0.18±0.16	0.31±0.014	**0.59**
Indomethacin	-0.36±0.015	-1.03±0.016	-0.11±0.014	**1.50**	0.17±0.014	0.28±0.012	0.41±0.015	0.55±0.013	**1.41**
Control	-0.49±0.012	-1.12±0.020	-0.14±0.018	**1.75**	0.23±0.015	0.63±0.014	0.94±0.017	1.18±0.011	**2.98**

Species used: Female Wistar rats (170±20g); Dose: 100mg/kg for the test compounds and aspirin and 10mg/kg for the standard indomethacin was administered orally, half an hour before the intraperitoneal administration of LPS 100µg/kg; Each value represents the mean ± SEM (n=6)

6.5. METABOLISM INHIBITION STUDY USING SKF-525A IN CARRAGEENAN INDUCED EDEMA

We hypothesized that the high residual anti-inflammatory activity of the test compounds could be due to higher protein binding of its active metabolite(s). These metabolites may arise via their hydrolytic metabolism to demethylated and/or deamidated product. We, therefore, studied the anti-inflammatory activity of few compounds in carrageenan induced rat paw edema model using SKF-525A, a standard hepatic microsomal enzyme inhibitor[12], pretreated rats (Table 6.18)

Table 6.18. Study with Cytochrome P450 enzyme inhibitor

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]					
	1H	2H	3H	4H	6H	24H
SKF-525A & DA-11	0.10±0.0168 [52.38]	0.26±0.0154 [36.58]	0.34±0.0147 [43.33]	0.35±0.0127 [38.60]	0.28±0.0145 [40.42]	0.12±0.0161 [45.45]
DA-11	0.11±0.0124 [47.62]	0.24±0.0172 [41.46]	0.33±0.0144 [45.0]	0.33±0.0179 [42.10]	0.29±0.0159 [38.29]	0.115±0.0153 [47.73]
SKF-525A & DA-23	0.075±0.0138 [64.29]	0.18±0.0151 [56.10]	0.26±0.0183 [56.67]	0.24±0.0169 [57.89]	0.19±0.0135 [59.57]	0.07±0.0114 [68.18]
DA-23	0.07±0.0147 [66.7]	0.17±0.0176 [58.53]	0.24±0.0154 [60.0]	0.23±0.0143 [59.65]	0.185±0.0161 [60.63]	0.07±0.0125 [68.18]
SKF-525A & CA-1	0.16±0.0135 [23.81]	0.23±0.0147 [43.90]	0.32±0.0132 [46.67]	0.34±0.0151 [40.35]	0.30±0.0142 [36.17]	0.11±0.0133 [50.00]
CA-1	0.17±0.0141 [19.05]	0.22±0.0138 [46.34]	0.31±0.0144 [48.33]	0.33±0.0148 [42.10]	0.29±0.0152 [38.29]	0.107±0.0128 [51.36]
SKF-525A & CA-16	0.10±0.0153 [52.38]	0.21±0.0146 [48.78]	0.30±0.0143 [50.00]	0.32±0.0154 [43.85]	0.29±0.0161 [38.30]	0.10±0.0125 [54.54]

Table 6.18. Contd.

CA-16	0.11±0.0147 [47.62]	0.23±0.0148 [43.90]	0.32±0.0139 [46.67]	0.34±0.0147 [40.35]	0.30±0.0158 [36.17]	0.11±0.0131 [50.00]
SKF-525A & DC-11	0.13±0.0136 [38.09]	0.27±0.0144 [34.15]	0.39±0.0154 [35.00]	0.37±0.0141 [35.09]	0.30±0.0153 [36.17]	0.106±0.0123 [51.82]
DC-11	0.14±0.0145 [33.33]	0.28±0.0152 [31.71]	0.40±0.0149 [33.33]	0.39±0.0138 [31.58]	0.32±0.0146 [31.09]	0.115±0.0132 [47.72]
Control	0.21±0.0267	0.41±0.0148	0.60±0.0173	0.57±0.0203	0.47±0.0165	0.22±0.0135

Species used: Female Wistar rats (170±20g); Dose: Rats pretreated with SKF 525A (50 mg/kg i.p.) were dosed orally 100mg/kg with the test compounds; 1 h later the carrageenan injection (0.1ml of 1%solution in saline)was administered in the sub plantar region of the rat paw.
Each value represents the mean ± SEM (n=6)
The results obtained were analyzed by student's t test and the difference between the two groups was not found to be statistically significant.

Examination of Table 6.18 reveals that there is no significant difference between data generated from this test and those generated using standard protocol. This indicates that probably the test compound *per se* is the active species. The higher residual activity may be associated with the high lipophilicity of the amide derivatives in comparison to the parent free acid having lower logP values. Due to high lipophilicity these amide derivatives are likely to

form lipid depots in the body which may be responsible for longer activity profile of these compounds.

6.6. ANTI-INFLAMMATORY SCREENING OF 5-CYCLOPENTYLOXY-6-METHOXY -INDAN-1-ALKANOIC ACIDS

Compounds **V-XXVI** (5-cyclopentyloxy-6-methoxy-indan-1-acetic acid) and **V-XXXI** (5-cyclopentyloxy-6-methoxy-indan-1-carboxylic acid) showed 46.67 and 35.00 percent inhibition respectively (Table 6.19).

Table 6.19. Anti-inflammatory screening of 5-cyclopentyloxy-6-methoxy-indan-1-alkanoic acids against carrageenan induced edema.

COMPOUND	INCREASE IN PAW VOLUME (ml) ± SEM [% INHIBITION OF EDEMA]					
	1H	2H	3H	4H	6H	24H
V-XXVI	0.12±0.0146 [45.45]*	0.19±0.0161 [51.28]*	0.27±0.0137 [48.08]*	0.32±0.0149 [46.67]*	0.30±0.0133 [40.82]*	0.18±0.0125 [40.0]*
V-XXXI	0.14±0.0139 [36.36]*	0.24±0.0148 [38.46]*	0.33±0.0154 [36.54]*	0.39±0.0144 [35.00]*	0.32±0.0141 [34.69]*	0.20±0.0132 [33.33]*
Indomethacin	0.12±0.0151 [45.45]*	0.18±0.0167 [53.85]*	0.19±0.0141 [63.46]*	0.21±0.0136 [65.00]*	0.23±0.0133 [53.06]*	0.26±0.0128 [13.33]ns
Control	0.22±0.0157	0.39±0.0172	0.52±0.0163	0.60±0.0151	0.49±0.0149	0.30±0.0136

Species used: Female Wistar rats (160±20g); Dose: 100mg/kg for the test compounds and 10mg/kg for the standard indomethacin was administered orally, 1 h before the carrageenan injection (0.1ml of 1% solution in saline).
Each value represents the mean ± SEM (n=6). Data was analyzed by one way ANOVA followed by Post-hoc test
* Represents data significantly different from control at $p < 0.05$, ns represents not significant at $p < 0.05$

Since the acetic acid derivative **V-XXVI** was more active than the carboxylic acid derivative **V-XXXI,** it can be said that increase in chain length has a beneficial effect on activity. Also as both of these compounds were more active than other indanyl acids studied, it can be concluded that the bulkier group at C-5 of indan-1-alkanoic acids enhances the activity profile. This isosteric modification (incorporation of cyclopentyloxy for cyclopentylmethyl) expectedly exhibited retention of activity of 6-chloro-(5-cyclopentyl methyl)indan-1-carboxylic acid.

These compounds were also evaluated for their ulcerogenic potentials. Both of the compounds V-XXVI and V-XXVII were not found to be ulcerogenic at 100mg/kg p.o. dose level but showed mild ulcerogenecity at 500mg/kg p.o. dose level as shown in Table 6.20.

Table 6.20. Evaluation of ulcer index of 5-cyclopentyloxy-6-methoxy-indan-1-alkanoic acids

COMPOUND	DOSE (mg/kg)	TIME	ULCER INDEX
V-XXVI	100	6h	0
V-XXVI	500	6h	7.0±0.41
V-XXXI	100	6h	0
V-XXXI	500	6h	7.75±0.63
Indomethacin	30	6h	30.25 ± 1.38
Control	-	6h	0

The compounds were given orally to 24 h fasted male Wistar rats (200±20g);
Each value represents the mean ± SEM (n=4)

CHAPTER 7

MOLECULAR ORBITAL STUDIES

Argus lab 3.1.0 is free software for molecular modeling and drug design supplied by Planaria Software. The molecular orbital studies including highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) surface analysis, electron density, spin density and electrostatic potential can be done by this software. Electronic spectra can also be obtained for a compound from this software. The general procedure for obtaining HOMO and LUMO surfaces and their energies included drawing of 3D structure of the compounds, energy minimization using Hamiltonian PM3 method, single point energy calculation using PM3 Hamiltonian and RHF (closed shell), visualization of the HOMO and LUMO surfaces at the contour value of 0.05 and noting the energies for the molecular orbitals assigned as HOMO and LUMO.

7.1. Terminologies used:

Hamiltonian: Hamiltonian represents the energy of the electrons and nuclei in a molecule. This Hermitian operator and the associated Schrodinger equation play a central role in computational chemistry and physics for computing properties of molecules and aggregates of molecules, such as conductivity, optical and magnetic properties, and reactivity. The molecular Hamiltonian is a sum of several terms: its major terms are the kinetic energies of the electrons and the Coulomb (electrostatic) interactions between the two kinds of charged particles including its shape (three-dimensional structure.

PM3: Parameterized Model is a semi-empirical method for the quantum calculation of molecular electronic structure in computational chemistry. It is based on the Neglect of Differential Diatomic Overlap Integral Approximation.

RHF: Restricted Hartree-Fock, when system has multiplicity of zero, which means all the electrons are paired, then the electrons may be restricted to moving in pairs.

Eigenvalue is synonymous with Molecular Orbital Energy and **Eigenvector** is synonymous with Molecular Orbital (MO).

Contour Value: The Contour Value is the absolute value of the molecular orbital (MO) where the surface will be generated. As we get further away from the molecule, the value of the MO at that point in space becomes smaller and smaller. Hence putting in smaller contour values will generate "larger" surfaces. Generally 0.05 is a good place to start with a MO.

7.2. Results and Discussion:

The concept of quantum mechanical modeling was applied to all the synthesized compounds. The parameters which were studied includes

- Single point energy calculation of HOMO, LUMO energies (E_{HOMO}, E_{LUMO})and difference (ΔE) in E_{HOMO} and E_{LUMO}
- HOMO and LUMO surfaces of all the compounds were compared with the surfaces generated for the standard non-steroidal anti-inflammatory drugs.

7.2.1. HOMO and LUMO surface analysis of anti-inflammatory drugs

It was found that the HOMO surface for benzoic acid, salicylic acid, aspirin, paracetamol, ibuprofen, naproxen, naphthyl acetic acid, flurbiprofen, indomethacin, indomethacin debenzoylated, indomethacin-hexylamide, indomethacin-phenethylamide, clidanac, diclofenac, meclofenamic acid, and sulindac, is spread on the aromatic region of the molcecule and was similar to that of benzene (Figure 7.1). In case of meloxicam and piroxicam the HOMO region was spread on hydroxyl group, C-1 and C-2 and on the ring nitrogen. As shown in the Figure 7.1 for COX-2 selective compounds (celecoxib, nimesulde, rofecoxib, flosulide, lumaricoxib, valdecoxib and etoricoxib) the HOMO surface was spread either on the five membered heterocycle or on the aromatic ring. However, by simple observation of the surfaces and their energies (Table 7.1) one cannot explain the structure activity relationship. The benzoyl group has been reported to be important for the activity of indomethacin, but the HOMO surfaces for both indomethacin and debenzoylated indomethacin were found to be same. The HOMO and LUMO surfaces were also same for indomethacin and their amide derivatives, hence no relation between the surface and the activity could be developed supporting the fact that amidation will increase COX-2 selectivity.

The LUMO surfaces (Figure 7.2) for all the acidic NSAIDs studied was found to be spread on the carbonyl group of the carboxylic acid functionality (except indomethacin and sulindac). In case of indomethacin and its amides the LUMO surface was found to spread on C=C of the indole ring. For COX-2 inhibitors the LUMO surface was spread on the aromatic part of the structure. The difference in the energies of HOMO and LUMO of all the studied NSAIDs was lesser than that of benzene (Table 7.1). Though no generalized pattern was observed but the COX-2 specific inhibitors showed lower energy differences (celecoxib having the eigen value

of 0.203). Sulindac, indomethacin and piroxicam also showed much lower energy differences than other nonspecific NSAIDs. Therefore, the lower energy difference cannot be correlated with better COX-2 selectivity. Hence from this study correlation between surfaces and their energies can not be generalized with the anti-inflammatory activity or the selectivity.

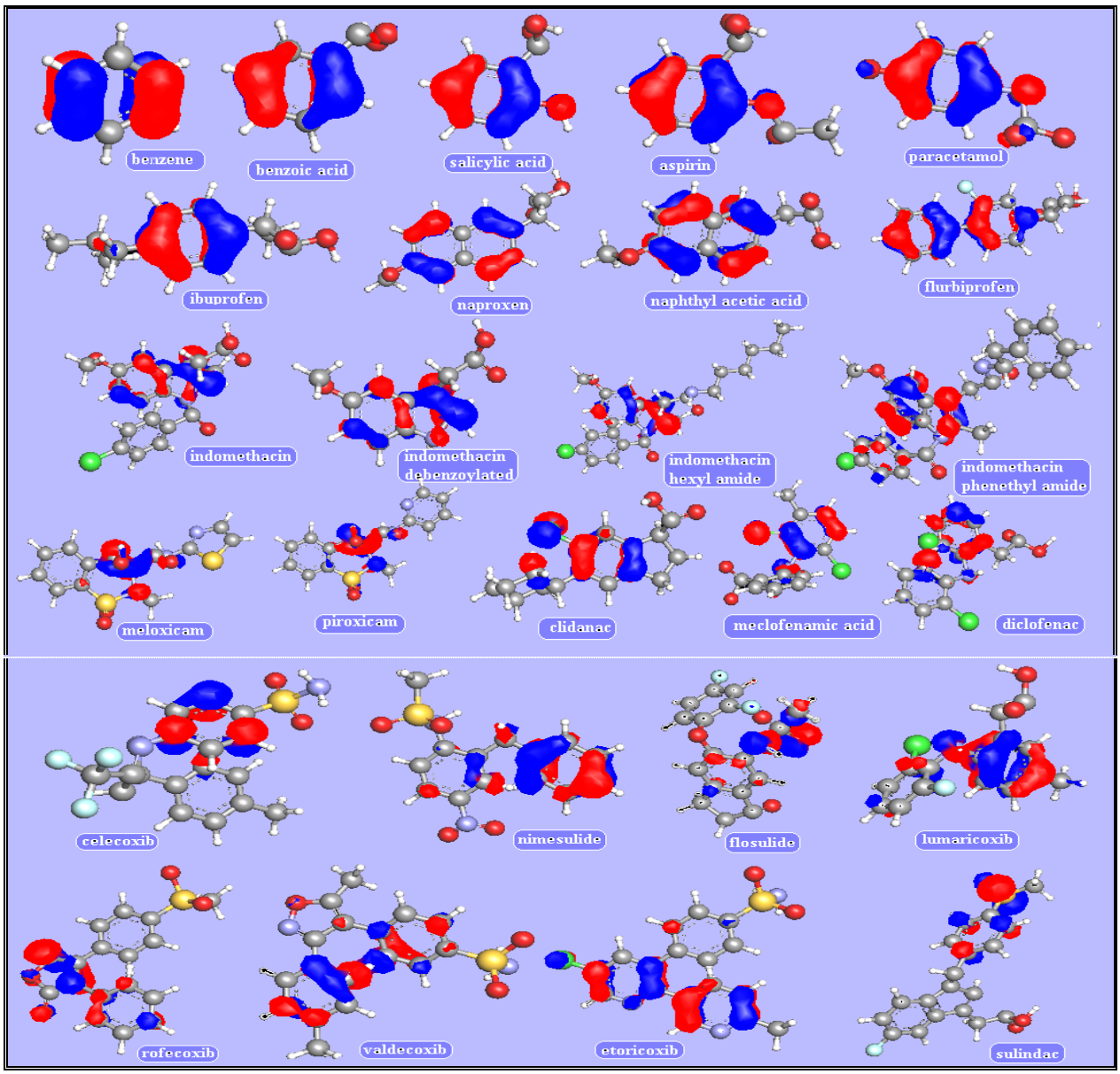


Figure 7.1. The HOMO surface visualization of some NSAIDs. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative).

Table 7.1. E_{HOMO}, E_{LUMO} and ΔE of some NSAIDs

COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)	COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)
Benzene	-0.357	0.013	0.37	Sulindac	-0.249	-0.061	0.188

Benzoic acid	-0.368	-0.034	0.334	Meloxicam	-0.337	-0.113	0.224
Salicylic acid	-0.350	-0.026	0.324	Piroxicam	-0.331	-0.106	0.225
Aspirin	-0.355	-0.045	0.31	Meclofenamic acid	-0.349	-0.028	0.321

Contd….

Table 7.1 Contd.

Paracetamol	-0.355	-0.042	0.313	Diclofenac	-0.347	-0.041	0.306
Ibuprofen	-0.347	-0.031	0.316	Lumaricoxib	-0.340	-0.043	0.297
Naproxen	-0.319	-0.052	0.267	Flosulide	-0.309	-0.046	0.263
Nabumetone	-0.317	-0.028	0.289	Clidanac	-0.348	-0.036	0.312
Naphthyl acetic acid	-0.318	-0.038	0.280	Nimesulide	-0.358	-0.062	0.296
Flurbiprofen	-0.333	-0.039	0.294	Celecoxib	-0.317	-0.114	0.203
Indomethacin	-0.338	-0.075	0.263	Rofecoxib	-0.316	-0.076	0.240
Indomethacin db	-0.323	-0.059	0.26	Valdecoxib	-0.343	-0.063	0.280
Indomethacin ha	-0.333	-0.073	0.26	Etoricoxib	-0.353	-0.069	0.284
Indomethacin pea	-0.334	-0.055	0.279				

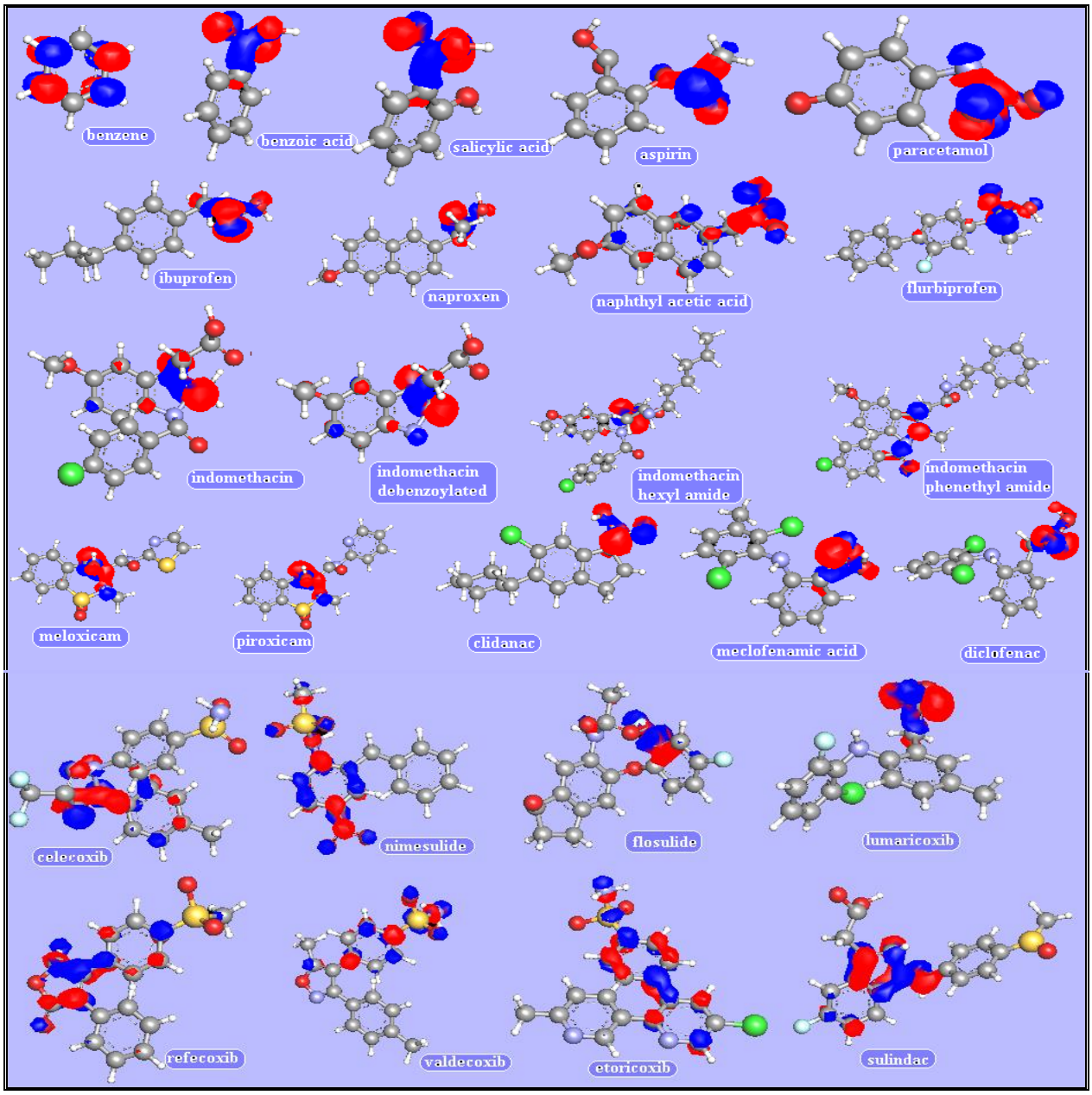


Figure 7.2. The LUMO surface visualization of some NSAIDs. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative).

7.2.2. HOMO and LUMO surface analysis of 5, 6-dimethoxyindan-1-acetic acid amides.

In 5, 6-dimethoxyindan-1-acetic acid series though the energy differences between the HOMO and LUMO was in range that matched with the existing NSAIDs (Table 7.1); their activity profile could not be explained (Table 7.2). The HOMO surfaces of the free acid best matched with that of ibuprofen. This surface was spreading over the aromatic part of indan ring moiety. In case of amides some variations were found depending on the aliphatic and aromatic nature of the amide derivative. In the aliphatic amides the HOMO surface was as

that of free acid but in case of aromatic amide it shifted to aromatic part of amide side chain (Figure 7.3). It was found that those aromatic amide derivatives which showed more delocalization of the HOMO surface had shown better activity in anti-inflammatory screening. The most active compound, the chloro derivative, **DA-23**, showed best delocalization of the HOMO surface and was found to be most active in the series.

The LUMO surface of all the compounds were delocalized over the carbonyl group and matched with that of the existing acidic NSAIDS. Both acid and their amide derivatives showed LUMO surfaces at same positions as shown in Figure 7.4. However the effect of substituents on the anti-inflammatory activity could not be explained. Moreover it can be seen that the energy difference between HOMO and LUMO surfaces of aromatic amides were more close to COX-2 inhibitors (Table 7.2).

Table 7.2. E_{HOMO}, E_{LUMO} and ΔE of 5,6-dimethoxyindan-1-acetic acid derivatives

COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)	COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)
DA	-0.347	-0.037	0.310	**DA-18**	-0.346	-0.035	0.311
DA-1	-0.345	-0.030	0.315	**DA-19**	-0.345	-0.031	0.314
DA-2	-0.345	-0.029	0.316	**DA-20**	-0.337	-0.027	0.310
DA-3	-0.344	-0.028	0.316	**DA-21**	-0.337	-0.042	0.295
DA-4	-0.345	-0.029	0.316	**DA-22**	-0.344	-0.046	0.298
DA-5	-0.343	-0.029	0.314	**DA-23**	-0.338	-0.047	0.291
DA-6	-0.345	-0.030	0.315	**DA-24**	-0.342	-0.047	0.295
DA-7	-0.345	-0.029	0.316	**DA-25**	-0.332	-0.051	0.281
DA-8	-0.345	-0.030	0.315	**DA-26**	-0.333	-0.043	0.290
DA-9	-0.345	-0.026	0.319	**DA-27**	-0.329	-0.043	0.286
DA-10	-0.345	-0.030	0.315	**DA-28**	-0.337	-0.028	0.309
DA-11	-0.345	-0.030	0.315	**DA-29**	-0.334	-0.048	0.286
DA-12	-0.345	-0.029	0.316	**DA-30**	-0.334	-0.053	0.281
DA-13	-0.345	-0.029	0.316	**DA-31**	-0.354	-0.061	0.293
DA-14	-0.338	-0.037	0.301	**DA-32**	-0.343	-0.027	0.316
DA-15	-0.343	-0.027	0.316	**DA-33**	-0.348	-0.057	0.291
DA-16	-0.344	-0.027	0.317	**DA-34**	-0.350	-0.049	0.301
DA-17	-0.345	-0.033	0.312				

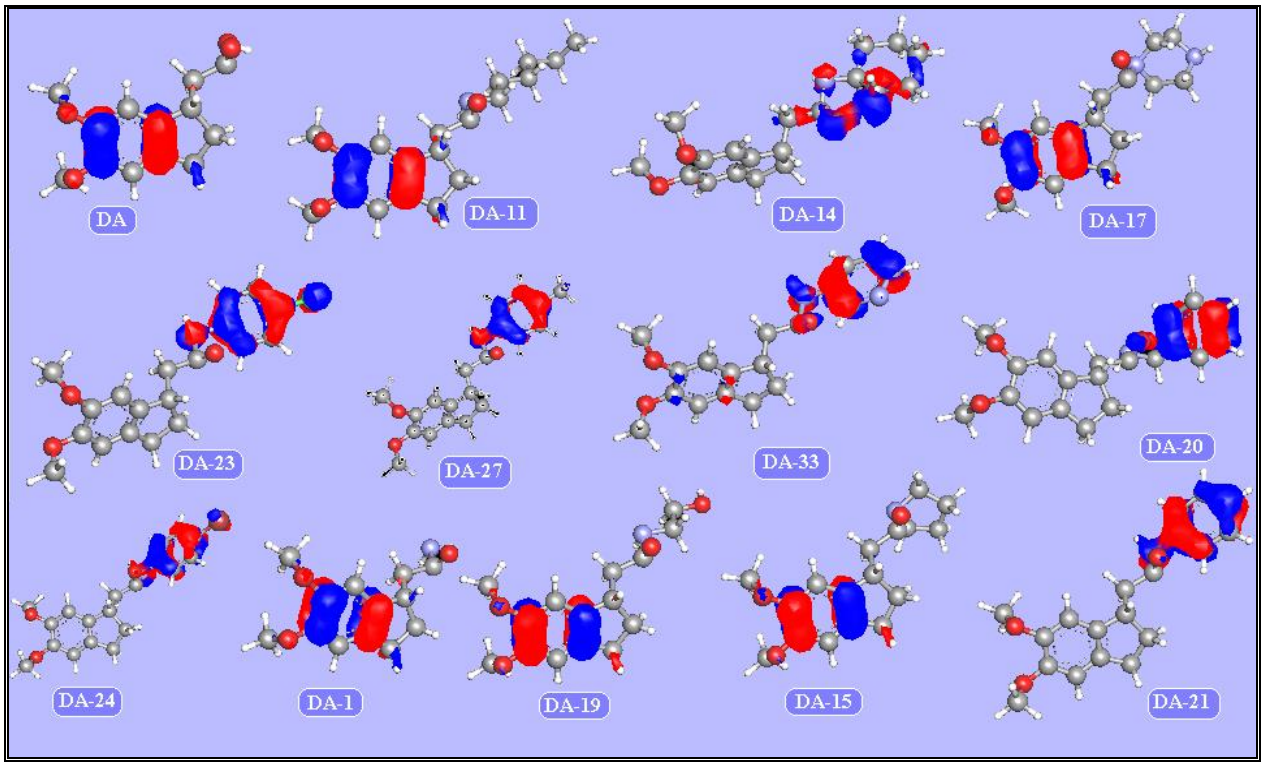


Figure 7.3. The HOMO surface visualization of 5,6-dimethoxyindanacetic amides. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative).

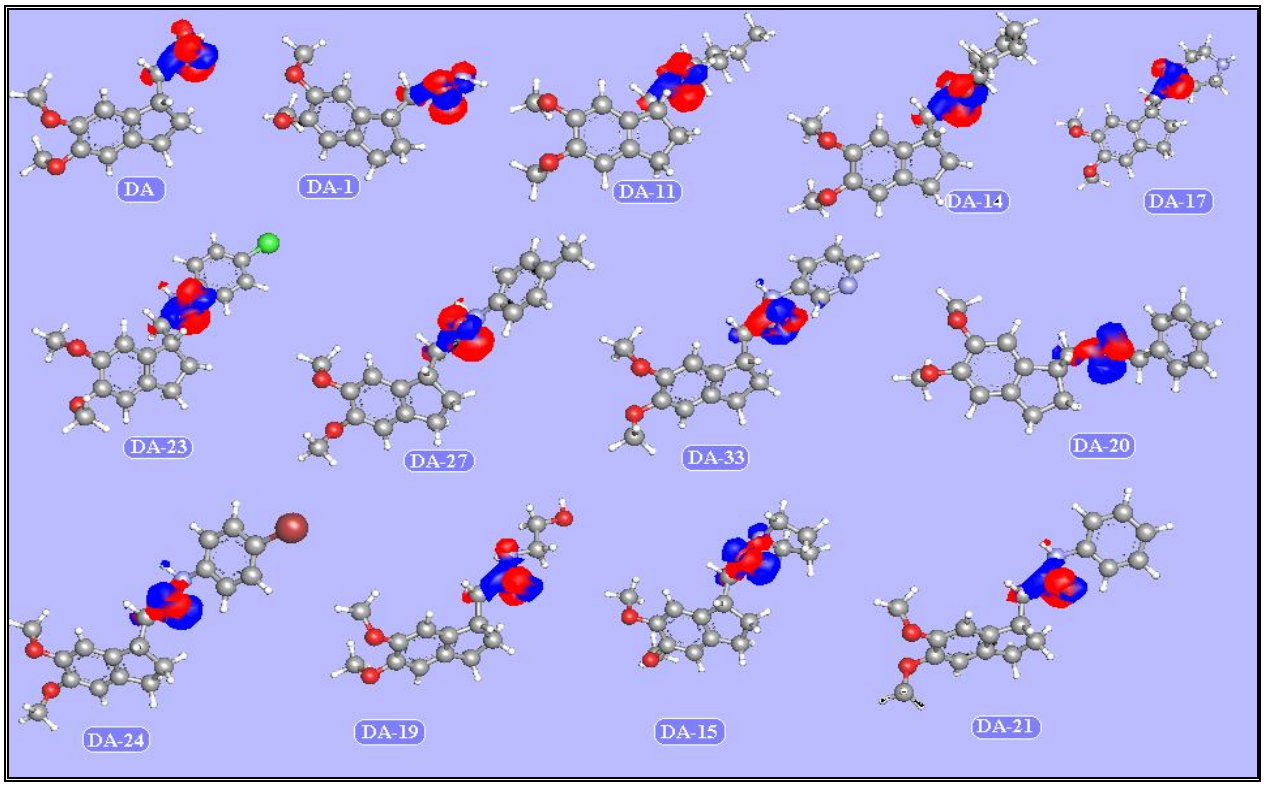


Figure 7.4. The LUMO surface visualization of 5,6-dimethoxyindanacetic amides. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative).

7.2.3. HOMO and LUMO surface analysis of 6-chloroindan-1-acetic acid derivatives

The HOMO and LUMO surfaces for the 6-chloroindan-1-acetic acid and their amide derivatives matched with the HOMO and LUMO surfaces of clidanac. The HOMO surface was spread on the aromatic part of the indan ring and on the attached chloro group (Figure 7.5). The LUMO surface was present on the carbonyl group as in case of most NSAIDs (Figure 7.6). Also the energy difference between the HOMO and LUMO (Table 7.3) matched with that of the clidanac in most of the cases. However no explanation can be offered regarding the effect of the substituents on the activity profile.

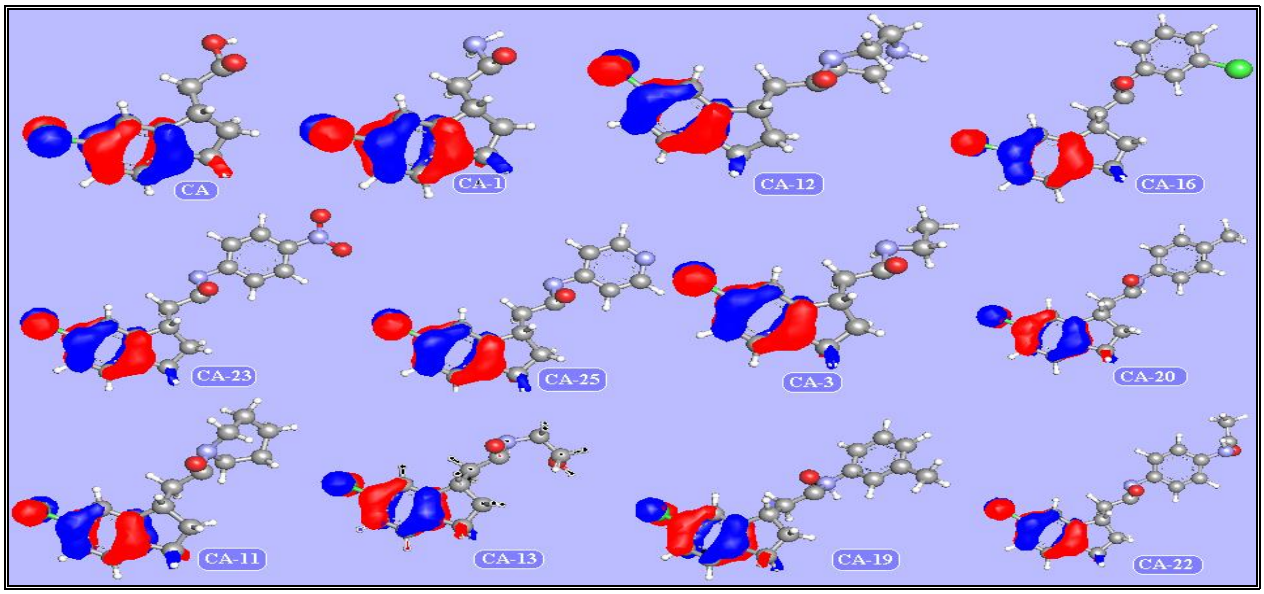


Figure 7.5. The HOMO surface visualization of 6-chloroindan-1-acetic acid amides. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative)

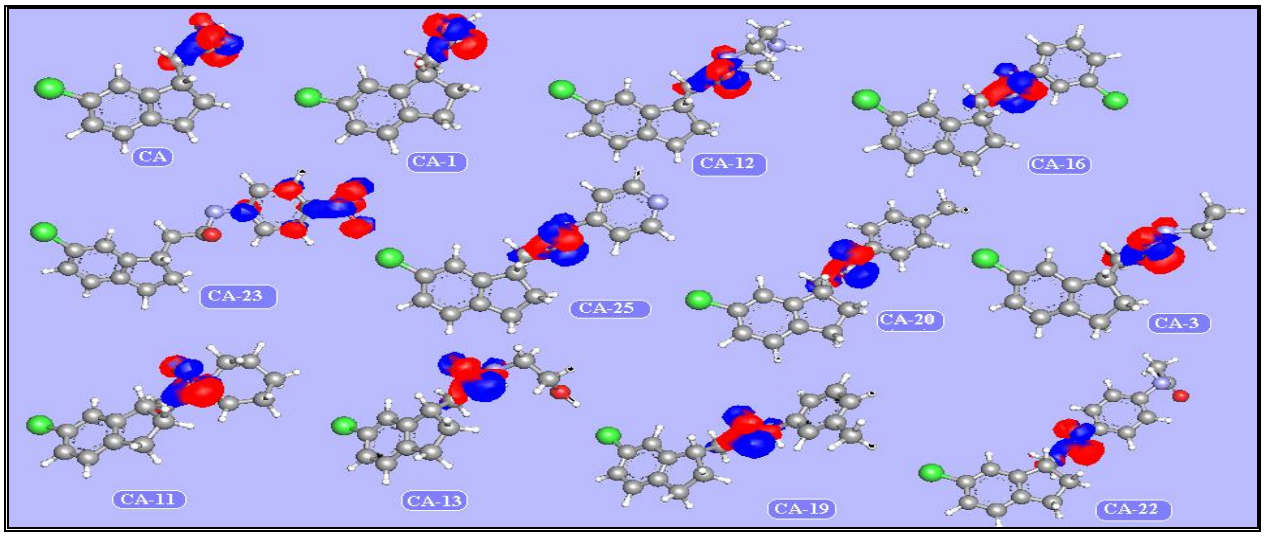


Figure 7.6. The LUMO surface visualization of 6-chloroindan-1-acetic acid amides. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative)

Table 7.3. E_{HOMO}, E_{LUMO} and ΔE of 6-chloroindan-1-acetic acid derivatives

COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)	COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)
CA	-0.347	-0.037	0.310	**CA-13**	-0.344	-0.026	0.318
CA-1	-0.344	-0.030	0.314	**CA-14**	-0.345	-0.030	0.315
CA-2	-0.345	-0.030	0.315	**CA-15**	-0.344	-0.029	0.315
CA-3	-0.344	-0.029	0.315	**CA-16**	-0.345	-0.034	0.311
CA-4	-0.344	-0.029	0.315	**CA-17**	-0.346	-0.034	0.312
CA-5	-0.344	-0.027	0.317	**CA-18**	-0.346	-0.034	0.312
CA-6	-0.344	-0.029	0.315	**CA-19**	-0.344	-0.028	0.316
CA-7	-0.344	-0.029	0.315	**CA-20**	-0.344	-0.029	0.315
CA-8	-0.344	-0.029	0.315	**CA-21**	-0.345	-0.032	0.313
CA-9	-0.344	-0.027	0.317	**CA-22**	-0.345	-0.034	0.275
CA-10	-0.344	-0.027	0.317	**CA-23**	-0.350	-0.075	0.311
CA-11	-0.342	-0.026	0.316	**CA-24**	-0.346	-0.037	0.311
CA-12	-0.344	-0.032	0.312	**CA-25**	-0.347	-0.036	0.311

7.2.4. HOMO and LUMO surface analysis of 5,6-dimethoxyindan-1-carboxylic acid amide derivatives

The HOMO surfaces in most of the aliphatic amides (except the cyclohexyl amide derivative) were on the aromatic part of indan ring moiety. In case of aromatic amides the HOMO surfaces were also delocalized on the aromatic part the of side chain. In p-chloro derivative the HOMO surfaces was completely on the aromatic part and the chloro group (Figure 7.7). As in other cases the LUMO surfaces in carboxylic acid series was also on the carbonyl group of amide group (Figure 7.8). Though the energy differences (Table 7.4) were matching with that of the standard compounds but these compounds were found to have low anti-

inflammatory activity profile. In comparison to other series these compounds showed higher energy differences which can be partially correlated with their low activity profile. However, no explanation can be offered regarding the effect of the substituents on the activity profile.

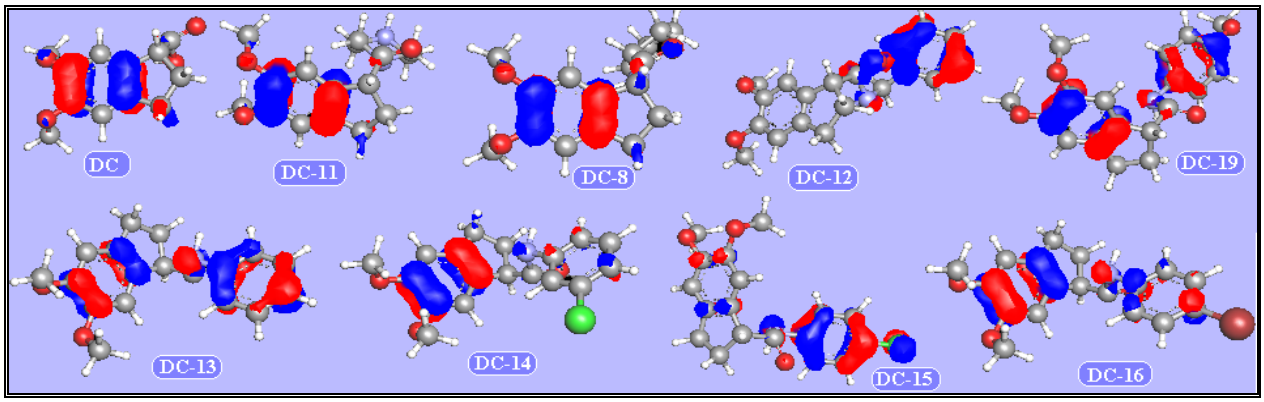


Figure 7.7. The HOMO surface visualization of 5, 6-dimethoxyindan-1-carboxylic acid amides. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative).

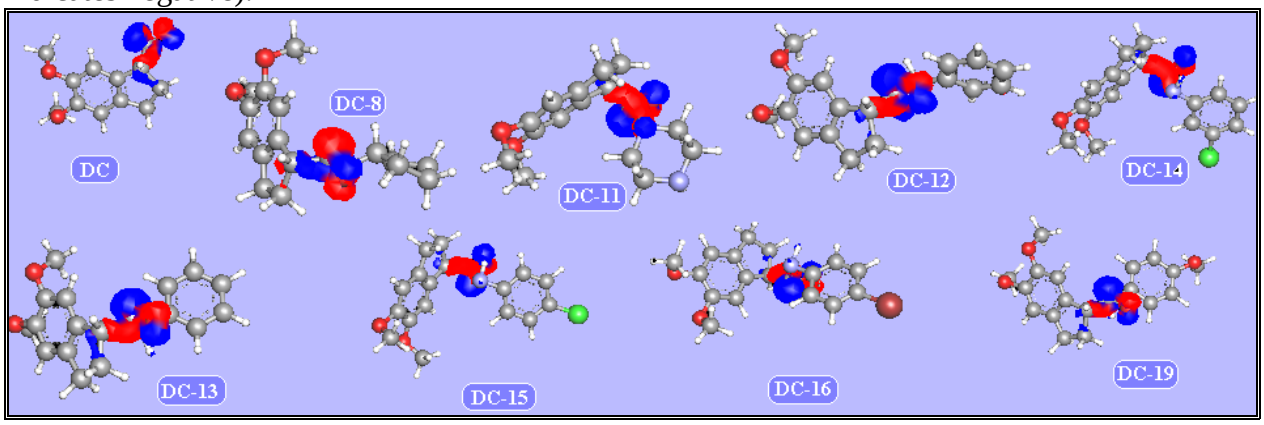


Figure 7.8. The LUMO surface visualization of 5, 6-dimethoxyindan-1-carboxylic acid amides. The colors indicate the phase of orbital in space (blue indicates positive and red indicates negative).

Table 7.4. E_{HOMO}, E_{LUMO} and ΔE of 5, 6-dimethoxyindan-1-carboxylic acid derivatives

COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)	COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)
DC	-0.352	-0.036	0.316	**DC-12**	-0.320	-0.036	0.284
DC-1	-0.351	-0.030	0.321	**DC-13**	-0.345	-0.039	0.306
DC-2	-0.351	-0.029	0.322	**DC-14**	-0.348	-0.044	0.304
DC-3	-0.351	-0.029	0.322	**DC-15**	-0.345	-0.044	0.301
DC-4	-0.351	-0.029	0.322	**DC-16**	-0.348	-0.044	0.304
DC-5	-0.351	-0.029	0.322	**DC-17**	-0.344	-0.037	0.307
DC-6	-0.352	-0.029	0.323	**DC-18**	-0.338	-0.038	0.300
DC-7	-0.355	-0.031	0.324	**DC-19**	-0.347	-0.040	0.307
DC-8	-0.352	-0.032	0.320	**DC-20**	-0.355	-0.046	0.309
DC-9	-0.267	-0.006	0.261	**DC-21**	-0.349	-0.045	0.304
DC-10	-0.345	-0.029	0.316	**DC-22**	-0.350	-0.046	0.304

DC-11	-0.348	-0.037	0.311				

7.2.5. HOMO and LUMO surface analysis of 5-cyclopenyloxy-6-methoxyindan-1-alkanoic acids

The HOMO and LUMO surface analysis was also done for compounds **V-XXVI, V-XXXI** and the lead compound **II-XXXVII.** The HOMO surface of the compound **V-XXVI** and **V-XXXI** were similar to each other but slightly different from **II-XXXVII** (Figure 7.9). The HOMO surface was more spread on the chlorine as compared to the aromatic part of indan ring in **II-XXXVII**. However, it was much more concentrated on the aromatic part of indan ring as compared to the methoxyl group in compounds **V-XXVI** and **V-XXXI**. The LUMO surfaces in all the three compounds were present on the carboxylic group. It was found that in case of carboxylic acid compounds it was also spreading on the C-C bond of indan ring. The energy difference was found to be similar for **V-XXVI** and **V-XXXI** but higher as compared to the compound **II-XXXVII** (Table 7.5).

Table 7.5. E_{HOMO}, E_{LUMO} and ΔE for compounds **V-XXVI, V-XXXI** and **II-XXXVII.**

COMPOUND	E_{HOMO} (au)	E_{LUMO} (au)	ΔE (au)
II-XXXVII	-0.175	-0.016	0.159
V-XXVI	-0.347	-0.038	0.306
V-XXXI	-0.344	-0.036	0.311

Though the HOMO surfaces and the energy differences (ΔE) of the compounds **V-XXVI** and **V-XXXI** did not match with that of **II-XXXVII** but 5-cyclopenyloxy-6-methoxyindan-1-alkanoic acids showed moderate anti-inflammatory activity. No conclusions can be drawn regarding the activity profile after the isosteric modification of cyclopentyl methyl group in **II-XXXVII** to cyclopentyloxy group in **V-XXVI** and **V-XXXI**.

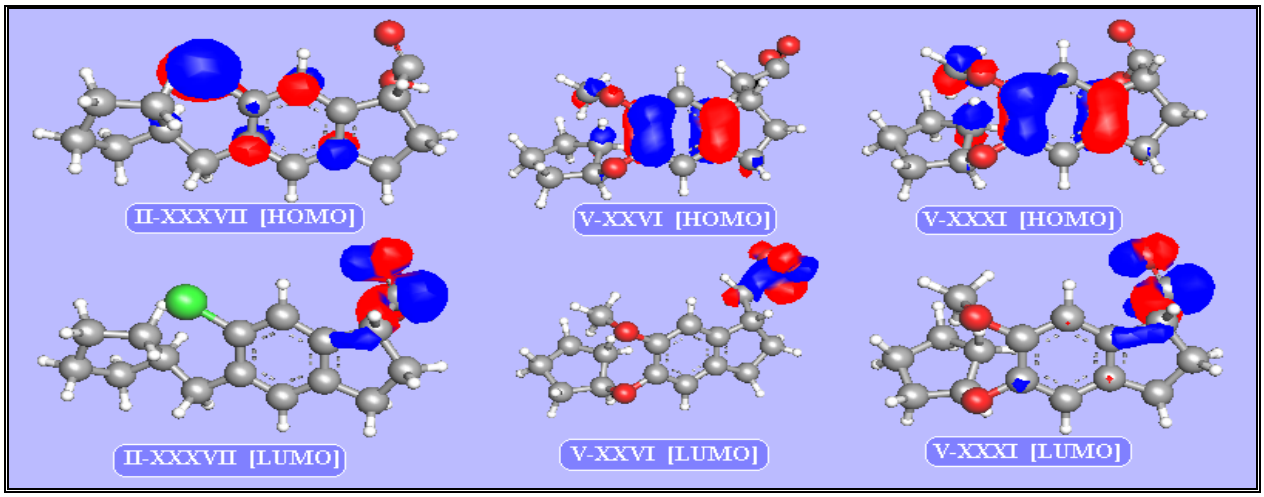


Figure 7.9. The HOMO and LUMO surface analysis for compounds **V-XXVI, V-XXXI** and **II-XXXVII.**

CHAPTER 8

CONCLUSIONS

It can be said that the objectives stated in chapter 3 of this thesis have been mostly attained. These attainments are detailed below:

- A total of 83 new compounds belonging to 4 series were designed based on the available literature and were synthesized using standard reaction schemes. The compounds were variously substituted [(5, 6-dimethoxy-2, 3-dihydro-1*H*-inden-1-yl)acetic acid amides, (6-chloro-2, 3-dihydro-1*H*-inden-1-yl)acetic acid amides, (5, 6-dimethoxy-2, 3-dihydro-1*H* -inden-1-yl)carboxylic acid amides and 5-(cyclo pentyloxy)-6-methoxy-indan-1-alkanoic acids].
- Purity of the synthesized compounds was ascertained by thin layer chromatography and melting point. Their structures were confirmed by spectral (IR, ^{1}H-NMR, Mass) and elemental analysis.
- The synthesized compounds were screened for their anti-inflammatory activity against carrageenan induced edema and analgesic activity against acetic acid induced writhing. Few selected compounds were also screened for their antipyretic activity against LPS-induced pyresis, anti-arthritic activity against adjuvant induced arthritis and for their ulcerogenic potentials.
- Some of the synthesized compounds exhibited significant anti-inflammatory, analgesic and antipyretic activities. These compounds were found to be long acting and their residual anti-inflammatory activity at 24th hour exceeded that of Indomethacin. However these compounds showed poor anti-arthritic activity profile except few belonging to the dimethoxycarboxylic acid series.
- Compounds **DA-23** and **CA-25** were among the most active compounds in anti-inflammatory screening with 60.94 % and 81.54 % of inhibition respectively against carrageenan induced rat paw edema. The compound **DA-11** and **CA-24** showed maximum inhibition of 81% and 94% respectively in acetic acid induced writhing assay. The compound **DA-17** and **CA-1** showed best profile in antipyretic screening. In adjuvant induced arthritis assay **DC-8** and **DC-14** were the most potent compounds.
- These compounds showed much less gastrointestinal ulcerogenecity as compared to the standard indomethacin. Compound **DA-23** did not show any gastrointestinal toxicity at the highest tested dose of 500mg/kg p.o. Their low toxicity further warrants

determination of their COX selectivity profile. Hence, these compounds carry the potential to turn out as nonsulfonyl COX-2 inhibitors.

- The compounds were not toxic and no mortality was found in anti-inflammatory screening (except one) at the end of 24 hrs. Few of the tested compounds did not show any toxicity at highest tested dose of 1000mg/kg p.o.
- It was found that increase in side chain length enhances the activity profile (acetic acid derivatives were more active than the carboxylic acid derivatives). Also it was seen that presence of lipophilic group at C-5 of the indan nucleus improved the activity. However these relationships have been elucidated working with racemates, therefore, may not hold good, repetition of pharmacological experiments with pure enantiomers is needed for validation.
- The LPS induced pyresis model for antipyretic screening also showed that inhibition of TNF-α is not involved in the mechanism of action of these compounds.
- The studies with the enzyme inhibitors also revealed that probably the test compound *per se* is the active species.
- The molecular orbital study establishes the fact that HOMO and LUMO surfaces and their energies are not an important factor for determining the biological activity of these compounds.
- Compound CA-25 emerged as the most active and interesting of all the compounds synthesized. This compound showed an ED_{30} value of 6.45mg/kg p.o. in rats and exhibited a unique toxicity profile in mice and rats. This compound was able to accelerate the rate of protein denaturation after death of the experimental animals. We feel that this compound *per se* or on suitable chemical transformation and necessary testing can be used as a drug or a pharmacological tool. This requires further study of this compound.

5.5.2. NOMENCLATURE AND PHYSICAL DATA OF (5,6-DIMETHOXY-2,3-DIHYDRO-1*H*-INDEN-1- YL)CARBOXYLIC ACID AMIDES

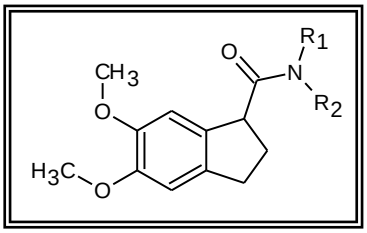


Table 5.9. Nomenclature of (5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxylic acid amides

COMPOUND	R'	R''	NOMENCLATURE (IUPAC)
DC-1	H	H	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -2	H	Me	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-methylcarboxamide
DC -3	H	Et	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-ethylcarboxamide
DC -4	H	n-Pr	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-propylcarboxamide
DC -5	H	n-Bu	*N*-butyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -6	H	n-amyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pentylcarboxamide
DC -7	H	n-hexyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-hexylcarboxamide
DC -8	H	cyclopentyl	*N*-cyclopentyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -9	H	cyclohexyl	*N*-cyclohexyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -10	--piperidino--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carbonyl]piperidine
DC -11	--piperazine--		1-[(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carbonyl]piperazine
DC -12	H	Benzyl	*N*-benzyl-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -13	H	Phenyl (C_6H_5)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-phenylcarboxamide
DC -14	H	C_6H_4(m-Cl)	*N*-(3-chlorophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -15	H	C_6H_4(p-Cl)	*N*-(4-chlorophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -16	H	C_6H_4(p-Br)	*N*-(4-bromophenyl)-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)carboxamide
DC -17	H	C_6H_4(m-CH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3-methylphenyl)carboxamide
DC -18	H	C_6H_4(p-CH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-methylphenyl)carboxamide
DC -19	H	C_6H_4(p-OCH_3)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(2-hydroxyethyl)carboxamide
DC -20	H	C_6H_4(p-NO_2)	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-(4-nitrophenyl)carboxamide
DC -21	H	3-pyridyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-3-ylcarboxamide
DC -22	H	4-pyridyl	(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)-*N*-pyridin-4-ylcarboxamide