QSAR Studies
on
Some Drugs Acting on Central Nervous System

A Thesis

Submitted in Partial Fulfilment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

By

Ranendra Narayan Saha

Pharmacy Group

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE
PILANI (RAJASTHAN)
1990

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

CERTIFICATE

This is to certify that the thesis entitled "QSAR Studies on Some Drugs Acting on Central Nervous System" and Submitted by Mr. Ranendra Narayan Saha, ID No. 87PHXF003, for the award of Ph.D. degree of the Institute, embodies original work done by him under my supervision.

Pilani

Date : 22.8.1990

(S. P. Gupta)
Professor
Chemistry Group

PREFACE

This dissertation submitted by the Author is in partial fulfilment of his Ph.D. degree. It presents an account of quantitative structure-activity relationship (QSAR) studies on some drugs acting on central nervous system (CNS), which includes drugs binding to the benzodiazepine-receptors (BZR)—pyrazolo[4,5-c] quinolines, N-(indol-3-ylglyoxylyl)amino acid derivatives, substituted 9-benzyl-9H-purines, oxadiazolylimidazobenzodiazepines—, some potential neuroleptics, cholecystokinin antagonists etc. It also includes QSAR studies of some local anesthetics, as local anesthetics are also broadly classified as miscellaneous drugs acting on CNS. Most of the work of the thesis is either published or under consideration for publication by international journals of repute.

The thesis as such contains four chapters. Chapter I presents a brief introduction of drugs acting on CNS and QSAR study. Chapter II describes the importance and methods of calculation of various parameters used in the correlation study. Chapters III & IV contain the QSAR studies made by the author on drugs acting on CNS and local anesthetics, respectively.

The author is an M.Pharm in pharmaceutics and is at staff of BITS. It was after joining BITS that the author came in contact with Dr. S.P. Gupta, Professor in Chemistry, BITS, who was involved in theoretical aspect of drug design, and developed interest in that field. This area deals with one of the important aspects of research in Medicinal Chemistry. It was then Dr. Gupta's affectionate and efficient guidance that led the author to produce the work that could be published in journals of international repute. The author therefore expresses his deep gratitude and regards to Dr. Gupta for his guidance.

The author also takes this opportunity to express his sincere gratitude to his parents who continuously encouraged and motivated him from his early childhood, for higher education. The author also expresses his gratitude to his parents-in-law for their continuous encouragement during the whole work.

The author sincerely thanks Dr. C.R. Mitra (Ex-Director) and Dr. S.Venkateswaran, the present Director of the Institute, for permitting him and providing the necessary facilities for the work. He also expresses his sincere thanks to Dr. L.K. Maheshwari, Dean, Research & Consultancy, for his continuous encouragement. The author is also thankful to

Dr. S.S. Mathur, Group Leader, Pharmacy,& Dr. S.K. Ray for showing interest and encouragement. Thanks are also due to Dr. PSVSK Raju, Chief IPC and Prof.R.K. Mittal, Chief, CAHU and staff of these two units for providing computer facilities for the work.

The author is also thankful to some of his students and friends namely A.K. Samantha, N. Agarwal, Ms. L. Kiran, Ms J. Meera, Ms. Sudha Pamidi, J.K. Gupta, C.S. Rao for their help at different level. He also express his thanks to Mr. Ramesh for neatly typing this thesis.

Lastly the author puts on record the help and cooperation provided by his wife Dipa and the pleasant atmosphere provided by his little son Soumik.

(R. N. Saha)

Pharmacy Discipline

Date : 20.8.90

List of Publications

1. A QSAR Study on Some Pyrazolo [4,5-c] quinolines Acting as Inhibitors of Benzodiazepine-Receptor Binding Res. Commun., Chem. Pathol. Pharmacol., 65. 119 (1989).

2. A QSAR Study on Some Potential Neuroleptic Agents. Drugs Design and Delivery; 6 ,41 (1990)

3. A QSAR Study on Some Cholecystokinin Antagonists. In "QSAR in Design of Bioactive Compounds", Ed., M. Kuchar, to be published.

4. A QSAR Study for Local Anesthetic Activity of Mono-and Diaryl-2-quinuclidinylcarbinols. Res. Commun.,Chem. Pathol. Pharmacol. 67, 297(1990).

5. A QSAR Study on Inhibitory Effects of Local Anesthetics on Batrachotoxin-Elicitied Sodium Flux and Phosphoinositide Breakdown and Batrachotoxin Binding to Sodium Channels. Drug Design and Delivery, in press.

6. QSAR Studies on Some Drugs Binding to Benzodiazepine Receptors In preparation.

CONTENTS

CHAPTER I

Introduction

A. Drugs Acting on Central Nervous System :

The most widely utilised group of pharmacologically active substances are the drugs which primarily exert their effects upon the central nervous system. They are the most important group of biologically active substances. One way or other they influence the life of almost each and everyone everyday. These agents are therapeutically invaluable because they can produce specific physiological and psychological effects. Drugs that affect the CNS may selectively relieve pain or fever, suppress disorders of movement or prevent seizures, induce sleep or arousal, reduce the desire to eat or allay the tendency to vomit. They may be used to treat anxiety, mania, depression or schizophrenia without altering consciousness. The introduction of potent psychotherapeutic agents over the past several years has had a dramatic impact on the basic concepts and treatment of mental illness. Socially acceptable stimulants and antianxiety agents produce stability, relief, and even pleasure for many. However, the execessive use of these and other drugs can also adversely affect lives when their use leads to physical dependence or addiction on the drug or to toxic side effects which may lead to the fatal effect.

To understand the cellular and molecular basis of the varied functions of the enormously complex human brain, pharmacologists are using the unique but different quality of drugs that affect the central nervous system and behavior. In this complicated effort, pharmacolgists have two major goals: firstly to use drugs to dissect the mechanisms that operate in the normal CNS and secondly to know the characteristics of the receptors and to develop appropriate drugs to correct pathophysiological events in the abnormal CNS.

Approaches to the elucidation of the sites and mechanisms of action of CNS drugs demand understanding of the cellular and molecular biology of the brain. Although knowledge of the anatomy, physiology and chemistry of the nervous system is far from complete, the acceleration of interdisciplinary research on the CNS has led to remarkable progress. The success of these studies is based on the knowledge of the sites and mechanisms of actions of different CNS drugs, which requires a thorough study on their structure activity relationships. A detailed knowledge of receptors and mechanisms of actions of different CNS

drugs will lead to design and develop new potent drugs of specific action, devoid of side effects. As such in recent times pharmacologists and medicinal chemists are making great efforts in that direction.

B. Classification of CNS Drugs :

For an extensive and systematic study of any kind on CNS drugs, it is essentially required to classify them on some particular basis. The most appropriate way would obviously be according to their modes of action. However, till now there is no adequate and authentic knowledge about their exact modes of action; hence it would not be possible to classify them on this basis.

CNS drugs basically can be classified under two broad headings; specific and nonspecific depending upon the way they produce pharmacological actions. The effect of drug is considered to be specific when it affects an identifiable molecular mechanism unique to target cells that bear receptors for the drug. On the otherhand, a drug is regarded as nonspecific when it produces effects on many different target cells and acts by diverse molecular mechanisms. This terminology

thus distinguishes broad actions at many levels of the CNS through effects on specific molecular mechanisms (e.g., atropine blockade of muscarinic receptors) from nonspecific actions. But obviously a drug that is highly selective when tested at a low concentration may exhibit nonspecific actions at substantially higher doses.

As the number of putative neurotransmitters has increased and as techniques have been evolved for the analysis of the actions of drugs upon specific target neurons, the list of drugs that have been regarded as having general actions has become considerably shorter. Thus, more and more drugs exhibit actions that can be related to specific mechanisms.

Drugs whose mechanisms currently appear to be general or nonspecific are classed according to whether they produce behavioral depression or stimulation, while specifically acting CNS drugs can be classified more definitively according to their locus of action or specific therapeutic usefulness.

(i) General(Nonspecific) CNS Depressants: The agents under this category are having the ability to depress excitable tissue at all levels of the CNS by stabilization of neuronal membranes, leading to a decrease in amount of transmitter released by the nerve impulse as well as to general depression of postsynaptic responsiveness and ion movement. The anesthetic gases and vapors, aliphatic alcohols, and some hypnotic-sedative drugs are considered in this category.

(ii) General(Nonspecific) CNS Stimulants: Under this category are those drugs which are capable of powerful excitation of the CNS and the agents which have high stimulant actions. Stimulation may be accomplished by either of two general mechanisms: by blockade of inhibition or by direct neuronal excitation that may be due to increased transmitter release, more prolonged transmitter action, labilization of the postsynaptic membrane or a decrease in synaptic recovery time. Cerebral stimulants (xanthines), brainstem stimulants (picrotoxin, pentylenetetrazole), and spinal cord stimulants (strychnine)can be put in this category.

(iii) Drugs that Selectively Modify CNS Function: The agents in this group may cause depression or excitation or both simultaneously on different systems. Some agents in this category may have little effect upon the level of excitability in doses that are used therapeutically. Drugs under this category are anticonvulsants, antiparkinsonism drugs, neuroleptics\antidepressants and antimanic and antipsycnotic agents), tranquilizers, sedative and hypnotics, narcotic analgetics and analgetic antipyretics.

(iv) Miscellaneous : There are certain drugs which are not considered to be acting centrally but may sometimes produce profound effects on the CNS as part of their pharmacological actions. Many drugs administered for their peripheral action also produce side effects or toxic reactions that can be referred to the CNS. All such drugs can be placed in a seperate class designated as miscellaneous.

It is now generally accepted that the CNS has excitatory and inhibitory chemical transmitters. The most probable of them are[1] acetylchol ne, adrenaline, noradrenaline, dopamine, serotonine, histamine, L-glutamic acid and related amino acids, γ-aminobutyric acid(GABA), and glycine. Many drugs that modify the functions of the CNS

have been demonstrated to affect concentration of one or more of these substances in the central as well as peripheral nervous systems. Apart from these neurochemicals, several endogenous peptides have been discovered in the brain[1]. They include enkephalin, endorphines, somatostatins, thyrotropin-releasing hormone(TRH), luteinizing hormone releasing hormone(LHRH), gastrin, cholecystokinin, oxytocin and substance P. Drugs interfering with the release and inhibitory action of these endogenous peptides may produce certain CNS effects.

The present dissertation embodies a quantitative structure-activity relationship (QSAR) study on certain catagories of CNS drugs. Mainly the stress has been given to the study of benzodiazepines(BZs) and other agents which act at or bind to benzodiazepine receptors(BZR), some neuroleptics, anticonvulsants and local anesthetics.

C. Quantitative Structure-Activity Relationship (QSAR) Study:

Long ago it was proposed that the biological activity of a compound is a function of its chemical structure. Today biological or therapeutic activity is considered to be a function of physicochemical properties. With this concept, structure-activity relationships are developed when a set of

physicochemical properties of a group of congeners is found to explain the variations in biological responses of those compounds. This has resulted in the discovery, examination, and interpretation of structure-activity relationships in a more systematic way, which has led to the introduction of quantitative structure-activity relationships (QSAR).

The correlation of molecular structure with biological activity is at the heart of modern medicinal chemistry, being fundamental both to our understanding of how drugs act, and to the rational design of more effective analogues. Over the last decade, considerable advances have been made in studies of structure-activity relationship, largely because of the trend towards expressing all aspects of "structure" in quantitative terms relative to some standard. The most significant contributions to this endeavour have been made by an organic chemist, Prof. C. Hansch and co-workers.[2]

The quantitative approach to understanding drug action depends upon the ability to express structure by numerical values and then to relate these values to corresponding changes in activity.

The response is going to be determined by the structure, that is by the physicochemical properties of the compound, and within a closely related or so-called congeneric series of compounds, changes in structure can be related to changes in biological activity.

The QSAR study tries to explain the reasons of observed variations in biological activities of a group of congeners in terms of molecular modifications or variations caused by a change of the substituents. QSAR studies generally have two important aspects: the predictive aspect and the diagnostic aspect. The predictive aspect, as the name implies, deals with the extrapolation and interpolation of a correlation study. The diagnostic aspect, on the other hand, answers mechanistic aspects of the reaction. Results of both these aspects can lead to tailor-made design of new drug of better activity with lesser or no sideeffects.

Several approaches are there for QSAR studies. Some important approaches of them are the nonparametric methods developed by Free and Wilson,[3] or by Fujita and Ban,[4] the parametric method developed by Hansch,[2] discriminant analysis,[5] and the pattern recognition technique.[6] Various factors, such as quality of the biological data, number of compounds tested, degree of variance in the results, and

ratio of the time required for synthesis and biological testing dictate the choice of approach for the QSAR study.

The most widely used approach continues to be the so called Hansch approach.[2] In Hansch approach, the variance in biological activity (BA) is explained by the variance of certain physicochemical and structural properties of molecules. The physicochemical properties include electronic characteristics, steric factors, and the solvent-partitioning or the hydrophobic effects. The structural properties include van der Waals volume for size and shape and molecular connectivity index for topography of molecules. Thus the Hansch model proposes the dependence of the biological activity of drugs on their physicochemical and structural properties to be in the fashion as shown by eqn.1.1, where π or log P is a hydrophobic parameter, σ an electronic parameter, E_s a steric factor, and S a structural

$$BA = a + b\pi(\text{or log P}) + c\pi^2(\text{or log P})^2 + d\sigma + eE_s + fS - \quad (1.1)$$

parameter defining the shape, size or topography of the molecule. All these parameters are briefly described in Table 1.1 in accordance with various sources.[7-17]

TABLE 1.1 Linear Free Energy Related Parameters

Parameter	Name	Description
P	Partition coefficient	log P taken as a measure of the hydrophobicity, of the molecule; for measuring P, an octanol-water system preferred.[7,8]
π	Hydrophobic constant	$x = \log P_x - \log P_H$, where p_x is the partition coefficient of the substituted compound and P_H that of the unsubstituted reference compound.[8]
R_M	hydrophobic constant from chromatography	log P linearly related[9] to R_M as $\log P = R_m$ + constant
σ (σ_m, σ_p)	Hammett constant	Defined[10] only for meta and para substituents to represent electronic character; positive value of σ denotes electron-withdrawing character and negative value of σ denotes electron-donating character; parameter may represent effects of ionization, hydrogen bonding, and charge-charge or charge-dipole interactions of compounds with the receptor.

Table 1.1 contd..

Parameter	Name	Description
σ^+, σ^-	Hammett constants	Used, respectively, when substituents donate electrons to a positive site or withdrawn from a negative site by direct resonance interaction[11,12]
E_s	Taft steric constant	related to the acid-catalyzed hydrolysis[13] of α-substituted acetates (XCH_2COOR) and represents the steric effect affecting intramolecular and intermolecular hindrance to the reaction or binding.
MR	Molar refractivity	$MR=[(n^2-1)/(n^2+2)]\ MW/d$, where n is the refractive index for the sodium D line, MW is the molecular weight, and d is the density of the compound; MR can be used[14] as a steric parameter in the absence of E_s, measures the electronic effect also[14] and may reflect the dipole-dipole interaction at the active site[15]

Table 1.1 contd..

Parameter	Name	Description
V_w	van der Waals volume	V_w,[16] has also been used as steric parameter.
	Molecular connectivity index	topological parameter[17] defined to account for the effects of kinds of atoms, bonding type, adjacency environment, branching pattern , unsaturation, and heteroatom content in a molecule on its reactivity or activity.

Equation 1.1 shows a nonlinear, i.e., a parabolic, dependence of activity on the hydrophobic character of molecules. Hansch, in fact, had assumed a "random walk" of the molecules, where hydrophilic molecules tend to remain in aqueous phase, hydrophobic molecules tend to go into lipid phase, and only molecules with an optimal hydrophilic/hydrophobic balance tend to reach their goal in reasonable time and concentration. For in vivo systems, the nonlinear dependence of the rate constants of drug transport through aqueous and bioorganic phases on lipophilicity seems to be the most reasonable explanation for the nonlinear dependence of activity

on π or log P. For simple in vitro systems, e.g., enzyme inhibition or drug-binding inhibition, such nonlinear relationships result from equilibrium distribution of the drug toward different areas at the receptor surface, from limited binding space at the active site, or from limited solubility of more lipophilic congeners.

However, in many cases the relationships between activity and lipophilicity were found to be strictly linear,[2] and although the parabolic model proved to be extremely useful for practical purposes, there was an inconsistency between it and the linear model. Although much less is known about the dependence of biological activities on lipophilic character beyond the point of optimal lipophilicity (log P_o or π_o), most often a linear relationship is observed with a negative slope beyond it.

To overcome such inconsistencies between the linear and nonlinear models, a number of different models[18-24] were proposed, out of which Kubinyi's bilinear model was found, after Hansch's parabolic model, to be the most useful[25-31] model to describe the nonlinear relationships.

D. Limitations of QSAR :

While QSAR studies are successfully utilized to predict the biological activity of new analogues and discuss the mechanism of drug-receptor interactions, they have many limitations and drawbacks.[32]

The substituent effect on hydrophobicity is characterized by log P based on an octanol-water system; hence, even a very significant correlation can not represent a true model for hydrophobic interaction between a drug molecule and the receptor. The value of log P also dependson the electronic characters and the hydrogen-bonding properties of the substituents.[33,34] Thus if one gets a correlation with log P only, one can not conclude that there is only hydrophobic interaction between drug and receptor and that no electronic interaction or hydrogen bonding takes place. Another factor that may influence log P is the steric effect that can prevent the access of water to a hydrophilic group.[35] Also values determined for one system can not be universally used for every system.

A more serious problem arises with the electronic parameters. The Hammett constants do not reflect which portion

of the drug molecule would be actually involved in the interaction with the receptor. Quantum mechanical calculations do provide some help in this, but they are time consuming and expensive. Similarly steric factors, MR, MW etc. do not give any idea in what way steric effects would affect the drug-receptor interaction.

Even a successful QSAR study will provide only indirect information about the three-dimensional aspects of the drug-biomolecule interaction. Regarding receptor mapping, most of the QSAR studies have been made with the assumption that drug receptors are relatively rigid molecules.[36,37] In many cases, it is difficult to know the exact dimensions of the active site of a drug.

Although molecules are usually represented on paper as rigid structures, they may infact be quite different in solution and their dynamic nature should be recognized. There is considerable evidence that macromolecules - even in the crystalline state - exhibit a wide spectrum of motion.[38-42] These motions may be involved in some molecular conformational changes on drug binding.

Many structural features that affect the activity but can not be quantitized by the usual variables such as π, σ, E_s etc. are accounted for by the use of indicator variables. These indicator variables are arbitrarily assigned two values: one to indicate the presence of the specific structural features and the other to indicate its absence. If the entire series of congeners is divided into two sets, one with and one without the specific structural feature, one would obtain two equations almost parallel, with a difference in their intercepts only. An indicator variable thus can be pictured simply as a constant that adjusts two parallel equations into one. If two sets are far apart in data space described by the usual parameters, one builds in a large amount of variance with the indicator variable, leading to a much higher correlation coefficient (r).[43] Despite the better r, the new correlation may be a poorer one, and thus, one can be misled if other statistical parameters are not available.

Another serious problem in QSAR analysis is the problem of collinearity.[44] For example, π and MR most often turn out to be so collinear that it becomes impossible to tell whether one or both are involved in SAR.

Over and above all, a QSAR study may be incorrectly interpreted if the biological property of interest is not correctly measured. A measured biological response may be a complex result of several processes, and an in vitro model of drug-receptor intraction does not always represent the true in vivo model.

References:

1. Bloom, F.E., in "The Pharmacological Basis of Therapeutics", Gilman, A.G., Goodman, L.S., Gilman, A., Eds., MackMillan, New York, p 235 (1980).

2. (a) Hansch, C., Fujita, T., J. Am. Chem. Soc. 86, 1616(1964).
(b) Hansch, C., Acc. Chem. Res.2, 232 (1969).
(c) Hansch, C., in 'Drug Design', Ariëns, E.J., Ed., Academic: New York, Vol. I, p 271 (1971).

3. Free, S.M. , Jr., Wilson, J.W., J. Med. Chem., 7, 395(1964).

4. Fujita, T., Ban, T., J. Med. Chem., 14, 148 (1971).

5. Martin, Y.C., Holland, J.B., Jarboe, C.H., Plotnikoff, N., J. Med. Chem., 17, 409 (1974).

6. (a) Kowalski, B.R., Bonder, C.F., J. Am. Chem. Soc., 94, 5632 (1972). (b) Chu, K.C., Anal. Chem. 46, 1181 (1974).

7. Levitan, H., Barker, J.L., Science (Washington, D.C.) 176, 1423 (1972).

8. Leo, A., Hansch, C., Elkins, D., Chem. Rev., 71, 525(1971).

9. Martin, A.J. P., Biochem. Soc. Symp., 3 (1949).

10. Hammett, L.P., Physical Organic Chemistry, McGraw-Hill, New York (1940).

11. Jaffe, H.H., Chem. Rev. 53, 191 (1953).

12. Ritchie, C.D., Sager, W.F., Prog. Phys.Org.Chem., 2, 323 (1964).

13. Taft, R.W., in 'Steric Effects in Organic Chemistry', Newman, M.S., Ed., Wiley, New York, p 556 (1956).

14. Hansch, C., Leo, A., Unger, S.H., Kim, K.H., Nikaitani, D., Lien, E.J., J. Med. Chem. 16, 1207 (1973).

15. Dunn, W.J., III Eur. J. Med. Chem., 12, 109(1977).

16. Gupta ,S.P., Prabhakar, Y.S., J.Sci. Ind.Res. 44, 189(1985).

17. Kier, L.B., Hall, L.H., in 'Molecular Connectivity in Chemistry and Drug Research', Academic, New York, (1976).

18. MacFarland, J.W., J. Med. Chem., 13, 1192(1970).

19. Penniston, J.T., Beckett, L., Bentley, D.L., Hansch, C., Mol. Pharmacol,, 5, 333 (1969).

20. Higuchi, T., David, S.S., J. Pharm. Sci., 59, 1376 (1970).

21. Hyde, R.M., J. Med. Chem., 18, 231 (1975).

22. Franke, R., Schmidt, W., Acta. Biol. Med. Ger. 31, 273 (1973).

23. Yalkowsky, S.H.,Flynn, G.L., J. Pharm. Sci., 62, 210 (1973).

24. Kubinyi, H., Arzneim. -Forsch., 26, 1991 (1976).

25. Kubinyi, H., Prog. Drug. Res., 23, 97 (1979).

26. Kubinyi, H., Arzneim. -Forsch., 29, 1067 (1979).

27. Seydel, J.K., In 'Strategy in Drug Research', Buisman, J.A.K., Ed., Elsevier; Amsterdam, p 179 (1982).

28. Seydel, J.K., Schaper, K.J., Pharmacol. Ther., 15, 131 (1982).

29. Kubinyi, H., J. Med. Chem., 20, 625 (1977).

30. Li, R.L., Hansch, C., Mathews, D., Blaney, J.M., Langridge, R. Delcamp, T.J., Susten, S.S., Freisheim, J.H., Quant. Struct. -Act. Relat . Pharmacol. Chem. Bio., 1, 1 (1982).

31. Kubinyi, H., in 'QSAR in Design of Bioactive Compounds', Kuchar, M., Ed., J.R. Prous Science, Barcelona, Spain, P 321 (1984).

32. Martin, Y.C., J. Med. Chem., 24, 229 (1981).

33. Leo, A., J. Chem. Soc., Perkin Trans., 2, 825 (1983).

34. Fujita, T., J. Pharm. Sci., 72, 285 (1983).

35. Martin, Y.C., Quantitative Drug Design, Marcel Dekker, New York , Basel, p 74 (1978).

36. Dietrich, S.W., Smith, R.N., Brendler, S., Hansch, C., Arch. Biochem. Biophys., 194, 612 (1979).

37. Hansch, C., Dietrich, S., Smith, R.N., Dev. Biochem. 4, 425 (1979).

38. (a) McCammon, J.A., Gelin, B.R., Karplus, M., Wolynes, P.G., Nature (London), 262, 325 (1976). (b) McCammon, J.A., Gelin, B.R., Karplus, M., Nature (London), 267, 585 (1977). (c) McCammon, J.A., Karplus, M., Nature (London), 268, 765(1977). (d) Karplus, M., Ann. N.Y. Acad. Sci., 367, 407 (1981), 439, 107 (1985).

39. Matson, G.B., J. Chem. Phys., 65, 4147 (1976).

40. Wuthrich, K., Wagner, G., Demarco, A., Experientia, 32, 815 (1976).

41. Hallenga, K., Koenig, S.H., Biochemistry, 15, 4255 (1976).

42. Baldwin, R.L., Annu. Rev. Biochem., 44, 453 (1975).

43. Hansch, C., Calef, D.E., J. Org. Chem., 41, 1240 (1976).

44. Hansch, C., Leo, A.J., 'Substituent Constants for Correlation Analysis in Chemistry and Biology', Wiley, New York, (1979).

CHAPTER II

Significance of Different Parameters and Their Calculations.

It has been found that van der Waals volume (V_w), hydrophobic parameter or partition coefficient (log P) for the whole compound or hydrophobic constant (π) for the substituents, molecular connectivity index, electronic parameter, steric parameter etc. are important controlling factors of biological activity. These parameters have been reported to be influencing the therapeutic activity of different compounds.

A. van der Waals Volume :

The van der Waals volume (V_w) has been found to be one of the most fundamental characteristics of the drug structure controlling biological activity. This determines the molecular size and shape of the compounds which are very important in the aspect of drug receptor interaction.

To calculate V_w of molecules, spherical shapes are assumed for all atoms according to Bondi[1] because of the absence of generally accepted pear shapes. The values of the van der Waals radii used and calculated volume of atoms are listed in Table 2.1. Since van der Waals radii are greater than covalent radii, a correction

Table 2.1 van der Waals redius and volume of atoms.*

Atom		Radius (A°)	Sphere volume, (10^2 Å^3)
C		1.7	0.206
H		1.1	0.056
H		1.5	0.141
O		1.4	0.115
S		1.8	0.244
F		1.4	0.115
Cl	Aliphatic	1.7	0.206
	Aromatic	1.8	0.244
Br	aliphatic	1.8	0.244
	aromatic	1.9	0.287
I	aliphatic	2.0	0.335
	aromatic	8.1	0.383
B		2.1	0.383
He		1.2	0.072
Ne		1.6	0.171
Ar		1.9	0.287
Ar		2.0	0.335
Xe		2.2	0.446

* Taken from reference 10.

for sphere overlapping due to covalent bonding between atoms is needed for the calculation of V_w of polyatomic molecules. The covalent bond lengths and correction factors are tabulated in Table 2.2. A correction for branching in the molecules has also been included in the 'V_w' calculation. Such correction factors are also mentioned in the Table 2.2.

B. Hydrophobic Parameter (log P) :

The fragment method suggested by Hansch and Leo[2] for calculating log P, where P is the partition coefficient of the solute in octanol/water system, is known as constructionist or synthetic approach. Experimentally determined log P values can often be reproduced or approached theoretically with the help of this approach. The basic assumption of this approach is: the log P of a solute can be expressed as a linear sum of fundamental structural constants known as fragments (f) and factors (F) that affect the partitioning equilibrium.

$$\log P = \sum_{1}^{n} a_n f_n + \sum_{1}^{m} b_m F_m \qquad (2.1)$$

Table 2.2 Correction values of van der Waals volume, for sphere overlapping due to Covalent Bonding, and Branching*

Bond	Bond length (Å)	Correction value (10^2 Å^3)	Bond	Bond length (Å)	Correction value (10^2 Å^3)
C-C	1.5	-0.078	O-H	1.0	-0.034
C-H	1.1	-0.043	O-B	1.5	-0.079
C-N	1.4	-0.065	S-H	1.3	-0.040
C-O	1.4	-0.056	S-S	2.0	-0.062
C-S	1.8	-0.066	S-F	1.6	-0.052
C-F	1.4	-0.056	C=C	1.3	-0.094
C-Cl (aliphatic)	1.8	-0.058	C=N	1.3	-0.072
C-Cl (aromatic)	1.8	-0.066	C=O	1.2	-0.068
C-Br (aliphatic)	1.9	-0.060	C=S	1.6	-0.081
C-Br (aromatic)	1.9	-0.068	N=N	1.2	-0.061
C-I (aliphatic)	2.1	-0.063	N=O	1.2	-0.053
C-I (aromatic)	2.1	-0.072	S=O	1.5	-0.057
C-B	1.6	-0.113	C≡C	1.2	-0.101
H-H	0.7	-0.030	C≡N	1.2	-0.079
N-H	1.0	-0.038	C=C (aromatic)	1.4	-0.086
N-N	1.4	-0.050			

...contd

Table 2.2 contd..

Bond	Bond length (Å)	Correction value (10^2 Å^3)
N-O	1.4	-0.042
N-S	1.6	-0.061

* Taken from ref. 10

Bond	Bond length (Å)	Correction value (10^2 Å^3)
Branching for Saturated bond except bonding with H		-0.050

Carefully conducted partitioning experiments and statistical survey of the then available partitioning data have been used in assigning values to the fragments and Factors. The working principle is summerised in the following paragraphs.

In this approach carbon atoms are divided into two categories: isolating carbons (IC) and nonisolating carbons (NIC). ICs have either four single bonds (two of which are at least connected to nonheteroatoms) or are multiply bonded to other carbon atoms. NIC atoms are carbon atoms multiply bonded to hetero atoms. For example $-\overset{|}{C}=$ in $CH_2 = CH_2$ is an IC but not in $H_2C=O$. Fragments are two types: (1) fundamental fragments defined as fragments whose free valency will lead to isolating carbons; (2) derived fragments, a derivative of fundamental fragments (e.g. $-CF_3$). A fundamental fragment can be either a single-atom or a multiple-atom (e.g. -C=O, -C≡N etc.) fragment. A single-atom fundamental fragment can be either an isolating carbon atom or a hydrogen or a hetero atom all of which are bonded to ICs. Depending on its nature a fragment will come under one of the following classes: (1) non polar fragments - these are simple ICs and hydrogen attached

to ICs; (2) H-pollar fragments - a fragment that can be expected to form H-bonds either by accepting or donating electron pair (e.g. -OH, -COOH, $-NH_2$ etc.); and (3) S-polar fragments - a fragment that is strong electron withdrawing with little tendency to form H-bonds (halogens). In expressing fragments, the structural formulae (or WLN code) of the respective fragments will be written as subscripts of 'f', for example as $f_{-NH-CO-NH}$ for expressing the fragment -NH-CO-NH- present in $CH_3NHCONHCH_3$. Various factors (F) are designed to account for the intramolecular forces and factors that affect the partitioning equilibrium of the solute. All these 'F's are identified with the help of different subscripts and superscripts. The subscripts are mentioned in the Factors table. The superscripts are applicable also to fragments. They are listed as:

(1) None = aliphatic structural attachment

(2) $\emptyset$ = attachment to the aromatic ring; if bivalent the attachment is from left as written

(3) $1/\emptyset$ = as 2 but attachment from right as written

(4) $\emptyset\emptyset$ = two aromatic attachments

(5) X = aromatic attachment, value enhanced by second, electron-withdrawing substituent ($\sigma I \geqslant \pm 0.35$)

and (6) IR = benzyl attachment.

Underlining any symbol means it is present in a ring system. Whenever halogens and H-polar fragment are seperated by only one IC an additional Factor will come into operation.

In calculating the log P of any compound, the first step is dividing that compound into 'well defined' fragments based on the above discussion and then searching for different Factors operating in between the fragments within the structure of the molecule. Now the sum of all these fragments and Factors will give the calculated log P of that compound. It is always safe to break any compound, especially compounds containing hetero atoms, into fundamental fragments rather than into derived fragments. Some important fragment values and Factor values are listed in Tables 2.3 and 2.4 respectively. A few simple example calculations are shown below.

Table 2.3 Some Common Fragment Constants.*

without carbon	f	$f^{\emptyset}$	$f^{\emptyset\emptyset}$	With Carbon	f	$f^{\emptyset}$	$f^{\emptyset\emptyset}$
-Br	0.20	1.09		C	0.20	0.20	
-Cl	0.06	0.94		$-CF_3$[a]		1.11	
-F	-0.38	0.37		-CN	-1.37	-0.34	
-I	0.59	1.35		-CON<	-3.04	-2.80	-1.93
-N<	-2.18	-0.93	-1.13	-C(O)-	-1.90	-1.09	-0.50
$-NO_2$	-1.16	-0.03		$-CO_2-$	-1.49	-0.56	-0.09
-O-	-1.82[b]	-0.61	0.53	$-CO_2^{(-)}$	-5.19	-4.13	
-H	0.23	0.23		-COH	-1.10	-0.42	
-NH-	-2.15	-1.03	-0.09	$-CO_2H$	-1.11	-0.03	
$-NH_2$	-1.54	-1.00		$-CONH_2$	-2.18	-1.26	
-OH	-1.64	-0.44		-CONH	-2.71	-1.81	-1.06
-SH	-0.23	0.62		-NHCONH-	-2.18	-1.57	-0.82

Fused in Aromatic Ring

Without Carbon	$f^{\emptyset}$	Without Carbon	$f^{\emptyset}$	With Carbon	$f^{\emptyset}$	with carbon	$f^{\emptyset}$
-N=	-1.12	-N=N-	-2.14	C	0.13	-CH-	0.35_5
-N<	-1.60	-O-	-0.08	C	0.22_5[c]	$-\overset{O}{\overset{\|}{C}}-$	-0.59
-N< (∅)	-0.56	-NH-	-0.65	C	0.44[d]	$-O\overset{O}{\overset{\|}{C}}-$	-1.40

* Taken from reference 2. [a] Derived fragment. [b] For methyl ethers and ethylene oxide, use -1.54. [c] For ring fusion carbon.
[d] For ring fusion - hetero.

*Table 2.4 Some Factors**

Involving BONDS

Unsaturation		Geometric	
Double	Triple	Proportional to length: x(n-1)	Short chains: 1-time
Normal $F(=) = -0.55$	$F(\equiv) = -1.42$	Chain : $F_b = -0.12$	Alkane chain: $F_{cBr} = -0.13$
Conjugate to Ø: $F^{Ø}(=) = -0.42$		Ring[a] : $F_b = -0.09$	H-polar fragment: $F_{gBr} = -0.22$
Conjugate to 2Ø: $F^{ØØ}_{(=)} = 0.0$	$F^{ØØ}_{(\equiv)} = 0.0$	Branching: $F_{bYN} = -0.20$[c]	Ring cluster: $F_{rCl} = -0.45$
		$F_{bYP} = -0.31$[d]	

Involving MULTIPLE HALOGENATION[e]

On same Carbon (geminal) $(n=2) = 0.30$
$(n=3) = 0.53$
F_{mhGm} $(n=4) = 0.72$

On adjacent Carbon (vicinal) $F_{mhVn} : 0.28\ (n-1)$

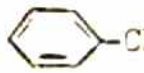
Involving H-POLAR PROXIMITY

Chain :	$F_{p1} = -0.42 \sum f_1+f_2$	Aliphatic ring :	$F_{p1} = -0.32 \sum f_1+f_2$	Aromatic: $F^{Ø}_{p1} = -0.16 \sum f_1+f_2$
	$F_{p2} = -0.26 \sum f_1+f_2$		$F_{p2} = -0.20 \sum f_1+f_2$	$F^{Ø}_{p2} = -0.08 \sum f_1+f_2$
	$F_{p3} = -0.10 \sum f_1+f_2$			

Involving INTRAMOLECULAR H-BOND

$F_{HBN} = 0.60$ for nitrogen $F_{HBO} = 1.0$ for oxygen.

* Taken from ref.2 [a] aromatic rings are excluded [c] for amine, [d] for phosphorus esters, [e] value per halogen atom.

Example 1. Toluene (): This can be treated as a compound consisting of six aromatic carbons, one aliphatic carbon and eight hydrogens. The fragments can be expressed as

$$6f_{C}^{\phi} + f_{C} + 8f_{H} = \log P \text{ (Toluene)}$$

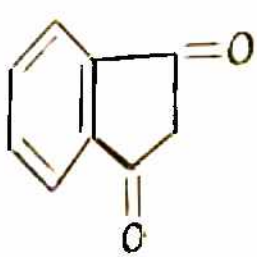
$$6(0.13) + 0.20 + 8(0.23) = 2.82 \text{ (Cald.)}, 2.80 \text{ (Obsd.)}$$

Since aromatic rings are excluded from bond Factor, there is no F term in the above equation. And here aliphatic chain length is one (CH_3), so (n-1)F is equal to zero (C-H bonds are excluded from Factors). The log P of this compound can also be calculated from two derived fragments as:

$$f_{C_6H_5}^{\phi} + F_{CH_3} = \log P \text{ (Toluene)}$$

$$1.90 + 0.89 = 2.79 \text{ (Cald.)}$$

Example 2. 1,3-indanedione : This can be considered as two fused rings (one six membered aromatic and one five membered saturated ring). This compound can be fragmented in the following way:



(1,3-indanedione)

$$4f_{\underline{CH}} + 2f_{\underline{C}} + f_{CH_2} + 2\,f_{C=O}^{\phi} + 3F_{\underline{\ell}}$$

$$4(0.35) + 2(0.13) + 0.66 + 2(-1.09) + 3(-0.09)$$

$$F_{\underline{P_1}} \qquad +F_{\underline{P_2}}^{\phi}$$

$$0.32(2x1.09) + 0.08(2x1.09)$$

$$= 0.76 \text{ (cald.)}, \quad 0.61 \text{(obsd.)}$$

Here the five membered ring is not aromatic, so two carbonyls are considered as aromatic-attached fragments. The ring fusion carbon atoms do not join two aromatic ring systems and therefore they are given the lowest carbon fragment value (0.13). There are only four bonds to consider in the saturated ring since the fusion bond is accounted for in the fusion carbon constants and so n-1=3. To be consistent, two proximity factors, an aliphatic P_1 and an aromatic P_2, must also be included, although the later appears superfluous in this instance.

Sometimes calculated log P values of compounds deviate very much from the experimentally determined values. For example, observed log P of 1,2-methylene-dioxybenzene is 2.08, but the calculated value comes out to be 1.34 only. This large difference may be due to Factors beyond the control of this method. However,

since it is an additive model, it will serve the purpose of drug-design when used in a congeneric series of compounds. Further details are given in the monograph by Hansch and Leo.[2]

C. Hydrophobic Constant (π) of Substituents:

Although log P can be used as a measure of the hydrophobicity of a whole molecule, one often works with a set of derivatives of a parent compound in which a large portion of the structure remains constant. In such a case, knowing the relative hydrophobicity of substituents can be sufficient for correlation analysis. Sometimes it has been found that only substituents in certain positions interact hydrophobically with a given biosystem.[3,4] To enable one to work with the relative hydrophobicity of substituents and in this way separate hydrophobic character from electronic and steric effects of substituents, the parameter π has been defined analogous to σ as

$$\pi_X = \log P_X - \log P_H \qquad (2.22)$$

In this expression, P_X is the partition coefficient of a derivative and P_H that of the parent compound, for example,

$$\pi_{Cl} = \log P_{C_6H_5Cl} - \log P_{C_6H_6} \qquad (2.3)$$

$$2.84 \quad - \quad 2.13 \quad = 0.71$$

A positive value for π means that relative to H the substituent favours the octanol phase. A negative value indicates its hydrophilic character relative to H. The values of π vary somewhat from system to system. Certain π values are given in Table 2.5.

D. Electronic Parameter (σ) :

The development of electronic parameter is one of the most important breakthroughs for mechanistic organic chemistry which came in 1935 when L.P. Hammet proposed[7] the following equation to define

$$\sigma = \log K_X - \log K_H \qquad (2.4)$$

In eq. 2.4, K_H is the ionization constant of benzoic acid in water at 25°C and K_X is the ionization constant for a meta or para derivative under the same experimental conditions. Positive values of σ represent the

Table 2.5 . Data on Physicochemical parameters of some important Substituents *

No	Substituent	π	σ_m	σ_p	E_s
1	H	0.0	0.0	0.0	0.0
2	CH_3	0.56	-0.07	-0.17	-1.24
3	C_2H_5	1.02	-0.07	-0.15	-1.31
4	n-C_3H_7	1.55	-0.07	-0.13	-1.60
5	i-C_3H_7	1.53	-0.07	-0.15	-1.71
6	n-C_4H_9	2.13	-0.08	-0.16	-1.63
7	F	0.14	0.34	0.06	-0.46
8	Cl	0.71	0.37	0.23	-0.97
9	Br	0.86	0.39	0.23	-1.16
10	I	1.12	0.35	0.18	-1.40
11	OCH_3	-0.02	0.12	-0.27	-0.55
12	NH_2	-1.23	-0.16	-0.66	-0.61
13	OH	-0.67	0.12	-0.37	-0.55
14	COOH	-0.32	0.37	0.45	-
15	$COOCH_3$	-0.01	0.37	0.45	-
16	CF_3	0.88	0.43	0.54	-2.40
17	NO_2	-0.28	0.71	0.78	-2.52
18	CHO	-0.65	0.35	0.42	-
19	C_6H_5	1.96	0.06	-0.01	-3.82
20	CN	-0.57	0.56	0.66	-0.51

* Taken from ref. 2

electron-withdrawing and the negative ones the electron donating character of substituents in the aromatic ring. For certain substituents, σ^- values are given in Table 2.5.

E. Steric Parameter (E_s) :

Steric effect of substituents in organic reactions are very important. The first generally successful numerical definition of steric effects in organic reactions was proposed by Taft,[8,9] Following a suggestion of Ingold Taft defined the steric constant E_s as,

$$E_s = \log \left(\frac{K_X}{K_H} \right)_A \qquad (2.5)$$

where K refers to the rate constant for the acid hydrolysis (denoted by A) of esters of type $X\text{-}CH_2COOR$.

The size of X will affect attainment of the transition state, which is a essential step for acid hydrolysis by water. Some E_s values are also tabulated in Table 2.5.

For QSAR studies in this thesis, standard values for different parameters for various substituents have been taken from literature.[2]

References :

1. Bondi, A., J. Phys. Chem., 68, 441 (1964).

2. Hansch, C., Leo, A., In "Substituent Constants for correlation Analysis in Chemistry and Biology", John Wiley, New York (1979).

3. Hansch, C., Deutsch, F.W., Smith, R.N., J. Amer. Chem.Soc. 87, 2738 (1965).

4. Yoshimoto, Y., Hansch, C., J. Med. Chem., 19. 71 (1976).

5. Fujita, T., Iwasa, J., Hansch, C., J. Amer,Chem. Soc. 86, 5175 (1964).

6. Leo, A., Hansch, C., Elkins, D., Chem. Rev. 71, 525 (1971).

7. Hammet, L.P., Chem. Rev., 17, 125 (1935).

8. Taft, R.W., J. Amer. Chem. Soc., 71, 3120 (1952).

9. Taft, R.W., in "Steric effect in Organic Chemistry", Newman, M.S., Ed., John Wiley, New York, pp 556 (1956).

10. Moriguchi, I., Kanada, Y., Komatsu, K., Chem. Pharm. Bull,, 24, 1799 (1976).

CHAPTER III

QSAR Studies on Some Drugs Acting on CNS.

A. Drugs Binding to the Benzodiazepine Receptors (BZR):

The discovery of Benzodiazepines (BZs) has opened a new era in research of Central Nervous System (CNS) and drugs acting on it. The benzodiazepines are a class of centrally acting drugs with wide range of therapeutic applications. They are used therapeutically as anxiolytics, hypnotics -sedatives, anticonvulsants, muscle relaxants, etc.[1] In central nervous system, high affinity binding sites or receptors for BZs have been identified.[2,3] There is a high correlation between the affinity of various BZs for this receptor and their pharmacological potencies.[3,4] Compounds of diverse structures and natures have been found to bind to the benzodiazepine receptors (BZR).[5] It is well established that benzodiazepines and related ligands interact with a specific site (BZR) that is closely associated with a neuro-inhibitory, postsynaptic GABA receptor and a chloride ionophore channel.[6,7] The efficiency of coupling of the GABA receptor to the chloride ion effector mechanism can be modified by several series of compounds that bind at this site. Uniquely, it has been shown that this receptor can be occupied by ligands having a continuum of intrinsic efficacy, from positive efficacy (anxiolytic, anticonvulsant, and sedative agents),

through nil intrinsic efficacy (recptor antagonists) to negative intrinsic efficacy (proconvulsant, anxiogenic agents).[6,8] The existence of these three categories would also imply that partial agonists and partial inverse agonists exist. Partial inverse agonists may be useful as cognition enhancers.

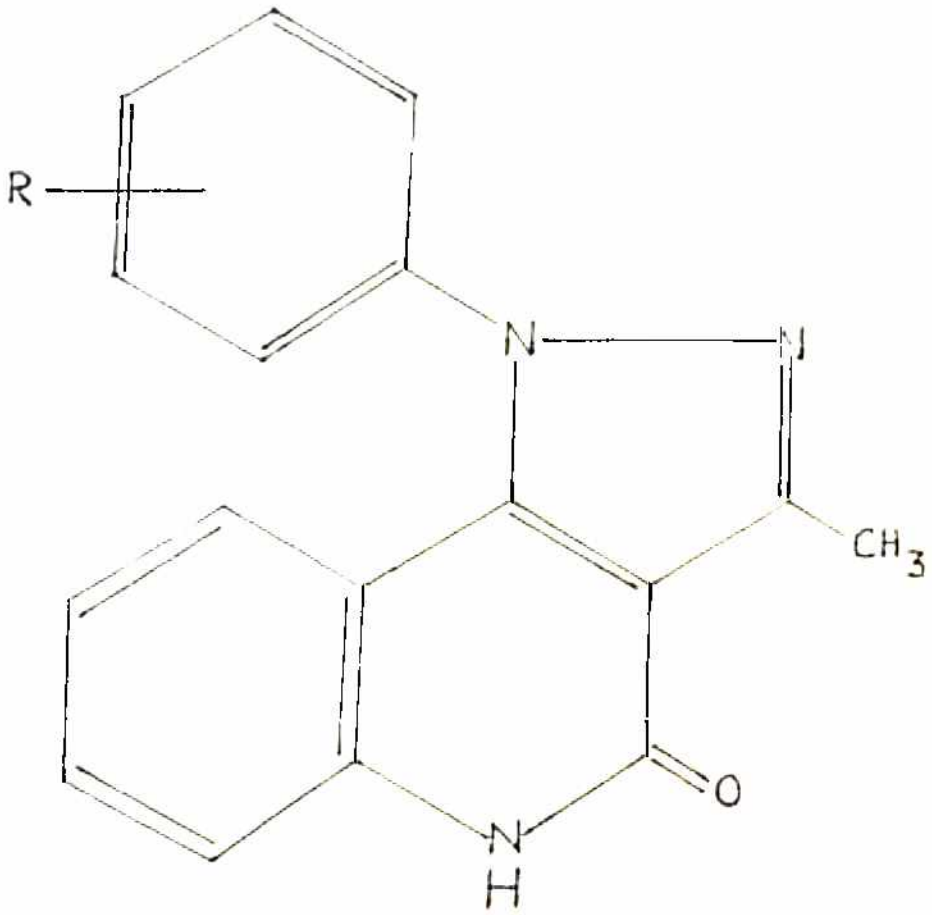
Various compounds have been suggested as possible "endogenous ligands" that physiologically act on these BZR.[9] The initial observations have shown that β-carboline derivatives like harmane and norharmane and esters of β-carboline-3-carboxilic acid,[10] cyclopyrrolones, pyrazoloquinolines etc. bind to the BZR. Also purines, inosin, hypoxanthine, were proposed as possible endogenous ligands.[11,12] The report on imidazobenzodiazepines have shown that a structural modification of benzodiazepines can lead to a change in activity without affecting the receptor binding. All these substances are supposed to act via specific receptor mechanism and therefore much attention has been paid towards the study of the nature of their binding with receptor. An extensive search is going on continuously to find novel compounds with specific binding to BZR.

A quantitative analysis of the correlation of the biological activity and the physicochemical properties of the compounds will precisely determine extent of role played by different physicochemical properties for the BZR binding. Further, the correlation equations may be exploited to design a better compound or to predict the potency of prospective compounds.

QSAR studies have been made on the following categories of drugs binding to BZR.

(i) Pyrazolo[4,5-c]quinolines Acting as Inhibitors of BZR Binding

In a recent study, Cecchi et al.[13-15] prepared a series of 1-aryl-3-methylpyrazolo[4,5-c]quinolin-4-ones(I) and studied their ability to displace specific [^{3}H]-flunitrazepam, a tritiated benzodiazepine, from bovine brain membranes. Melani et al.[15] also made a ^{13}C NMR study on these compounds and found a correlation existing between their binding affinity to BZR and the chemical shift value of a carbon atom of the tricyclic system. In this work we studied the correlation of binding affinity of these compounds with the physicochemical parameters.



(I)

The series of 1-aryl-methylpyrazolo [4,5-c] quinolin-4-ones and their activity data studied by Cecchi et al. are listed in Table 3.1. The physicochemical parameters used in the correlation are also listed in the same Table. A Multiple regression analysis revealed that there existed a significant correlation between the BZR binding affinity of compounds (-log IC_{50}) and the Taft steric constant, E_s, and the Hansch hydrophobic constant, π, of aryl substituents. The correlation equation obtained was as

$$-\log IC_{50} = 0.481(\pm 0.188)E_s(2,6) + 0.606(\pm 0.372)\pi(3,5) + 4.814$$
$$n=20,\ r=0.87,\ s=0.27,\ F(2,17) = 24.76 \quad (3.1)$$

In this equation, n is the number of data points, r is the correlation coefficient, s is the standard deviation, F is the F-ratio between the variances of calculated and observed activities, and the data with $\pm$ sign within the parentheses are the 95% confidence intervals. The term $E_s(2,6)$ refers to E_s value of aryl substituents at 2- or/and 6-position(s), and similarly the term $\pi(3,5)$ to π value of those at 3- or/and 5-position(s). Both of these terms are statistically significant at 95% confidence level in the equation and together account

Table3.1 The Benzodiazepine Receptor Binding Affinity of 1-Aryl-3-methylpyrazolo[4,5-c] quinolin-4-ones (I) and Related Physicochemical Parameters.

S.No.	R	Es(2,6)[a]	π(3,5)[a]	V_w(2,6)[b] (10^2 .A°^{3})	V_w(3,5)[b] (10^2 A°^{3})	-log IC_{50}		
						Obsd[c]	Cald,Eq.3.1	Cald.Eq. 3.4
1.	H	0.00	0.00	0.056	0.056	4.64	4.81	4.81
2.	2-Cl	-0.97	0.00	0.244	0.056	4.40	4.35	4.32
3.	3-Cl	0.00	0.71	0.056	0.244	5.36	5.24	5.11
4.	4-Cl	0.00	0.00	0.056	0.056	4.66	4.81	4.81
5.	2-CH_3	-1.24	0.00	0.245	0.056	4.44	4.22	4.32
6.	3-CH_3	0.00	0.56	0.056	0.245	5.05	5.15	5.12
7.	4-CH_3	0.00	0.00	0.056	0.056	4.68	4.81	4.81
8.	2-OCH_3	-0.55	0.00	0.304	0.056	4.25	4.55	4.16
9.	3-OCH_3	0.00	-0.02	0.056	0.304	5.13	4.80	5.21
10.	4-OCH_3	0.00	0.00	0.056	0.056	4.40	4.81	4.81
11.	2-Br	-1.16	0.00	0.287	0.056	4.70	4.26	4.21
12.	3-Br	0.00	0.86	0.056	0.287	5.60	5.34	5.18
13.	3-F	0.00	0.14	0.056	0.115	4.85	4.90	4.90
14.	4-F	0.00	0.00	0.056	0.056	5.00	4.81	4.81
15.	3,5-$(CH_3)_2$	0.00	1.12	0.056	0.490	5.52	5.49	5.51
16.	2,4-$(CH_3)_2$	-1.24	0.00	0.245	0.056	4.35	4.22	4.32
17.	2,3-$(CH_3)_2$	-1.24	0.56	0.245	0.245	4.03	4.56	4.62
18.	3,4-$(CH_3)_2$	0.00	0.56	0.056	0.245	5.43	5.15	5.12
19.	2,6-$(CH_3)_2$	-2.48	0.00	0.490	0.056	3.66	3.62	3.68
20.	2,5-$(CH_3)_2$	-1.24	0.56	0.245	0.245	4.33	4.56	4.62

[a]Taken from ref. 2; [b]Calculated according to ref 10 [c]Taken from ref. 15
of chapter II; of chapter II;

for 76% (r^2 = 0.76) of the variance in the activity. Further, the F-value of the equation is also significant at 99% level [F(2,17)(0.01) = 6.11]. Thus equation 3.1 represents a very significant correlation and suggests that substituents at 3-and 5-positions of the aryl ring could be involved in strong hydrophobic interaction with some hydrophobic pocket of the receptor and those at 2- and 6-positions would be producing strong steric hindrance to this interaction. No physicochemical or electronic parameter related to the substituent at 4-position was found to be relevant. Electronic parameters related to any position were not found to be effective and nor was the square term of $\pi(3,5)$ of any further consequence.

However, of the two parameters E_s (2,6) and $\pi(3,5)$, which proved to be important for the binding affinity, the former was found to be individually much better related with the activity (Eq.3.2) than the latter (Eq.3.3), suggesting that the steric

$$-\log IC_{50} = 0.553(\pm 0.230)E_s(2,6) + 5.00$$
$$n=20,\ r=0.77,\ s=0.34,\ F(1,18) = 25.49 \quad (3.2)$$

$$-\log IC_{50} = 0.831(\pm 0.576)\pi(3,5) + 4.514$$
$$n=20,\ r=0.58,\ s=0.43,\ F(1,18) = 9.17 \quad (3.3)$$

effects produced by the substituents at 2-and 6-positions were much more dominant than the hydrophobic effects of substituents at 3- and 5-positions. Thus to have the compounds of better activity, the substituents at 2-and 6-positions must be of smaller size. To have an exclusive account for the effect of the size of substituents at these two positions, accounting simultaneously indirectly for the hydrophobic effect of substituents at 3-and 5-positions, on binding affinity, we correlated the activity with van der Waals volume (V_w) of substituents as

$$-\log IC_{50} = 4.864 - 2.60(\pm 1.14)V_w(2,6) + 1.62(\pm 1.15)V_w(3,5)$$
$$n=20,\ r=0.86,\ s=0.28,\ F(2,17) = 22.56 \quad ..(3.4)$$

The $V_w(2,6)$ was well correlated with $E_s(2,6)$ (r=0.96) and $V_w(3,5)$ with $\pi(3,5)$ (r=0.88), The V_w was calculated as described in Chapter II.

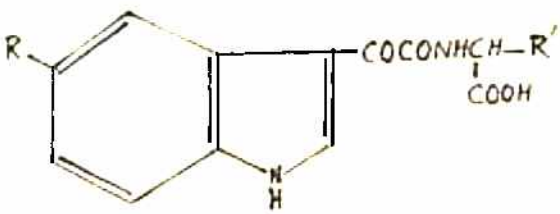
(ii) <u>N-(indol-3-ylglyoxylyl) Amino Acid Derivatives</u>:

Recently Primofiore, et al. have reported[16] synthesis and biological study of BZR binding of some

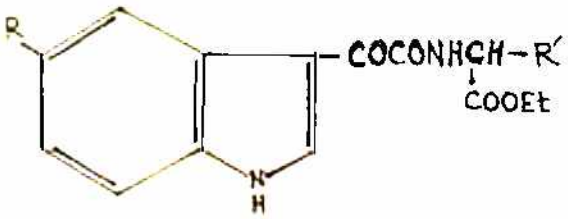
N-(indol-3-ylglyoxylyl) amino acid derivatives that contain an aminoethylindolic flexible structure analogues to that of β-carboline. In receptor binding, stereoselectivity is very important factor and sometimes used as one of criteria for identifying an interaction with the receptor.[17,18] An inhibition of specificity of [^{3}H] diazepam binding, for geometric isomers, was already demonstrated by Cain, et al.[19] and for enantiomeric compounds by Lippke, et al.[20] Different N-(indol-3-yl-glyoxylyl) amino acid derivatives containing optical active amino acid moieties were prepared to study the influence of the amino acid stereochemistry on the affinity to the benzodiazepine receptors.

By their qualitative study Primofiore et al.[16] found that presence of Cl, Br, NO_2 group in 5 position of benzene ring increased the inhibition of [^{3}H] flunitrazepam binding, while hydrogen and methoxy substitution decreased the inhibitory potency. Esterification of the free carboxylic acid increased the receptor binding activity indicating that the masking of the ionizable functions is crucial for the dramatic increase in receptor affinity, as already shown for β-carboline by others.[19] The invivo study of the most potent compounds exhibited that they were inverse agonists to the BZs.

The in vitro BZR binding data of the series of N-[(5-substituted indol-3-yl)glyoxylyl] amino acids (II) and its ethyl esters (III) reported by Pimofiore et al.[16] were considered for QSAR study. In their study, Pimofiore et al. varied the substituent R at position 5 of the benzyl ring and substituent R' at the assymetric



(II)



(III)

carbon atom in both acid and ethyl ester series. The substituent R is of varying nature where as the substituent R' has only two variables (CH_3 or CH_2-C_6H_5). We analysed the in vitro activity data of these compounds for displacing [^{3}H] flunitrazepam in bovine cerebral cortex membrane in relation to van der Waals volume and hydrophobic parameter of the substituent R in both ester (Table 3.2) and acid series (Table 3.3) .As the substituent R' at the assymetric carbon atom has only two variables, an indicator parameter (IR') is taken. IR' = 0 for CH_3 and 1 for CH_2-C_6H_5. The compounds under study exist in L(levo) or D(dextro) isomeric form or as recemic mixtures. So, another dummy parameter

Table 3.2 : N-[(5-Substituted indol3-yl)glyoxyl] amino Acid Esters (III) and Their in vitro BZR Binding Affinity.

No.	R	R'	Amino acid moiety config.	π_R[a]	$V_{w,R}$[b]	-log IC_{50}		
						Obsd[c]	Calcd, Eq. 3.9	Calcd, Eq. 3.12
1	H	CH_3	L,D	0.0	0.056	4.89	4.93	4.85
2	H	CH_3	D	0.0	0.056	5.13	5.62	5.38
3	H	CH_3	L	0.0	0.056	4.77	4.23	4.28
4	Br	CH_3	L,D	0.86	0.287	5.32	4.66	4.87
5	Br	CH_3	D	0.86	0.287	6.74[d]	5.35	5.41
6	Br	CH_3	L	0.86	0.287	4.29	3.96	4.32
7	Cl	CH_3	L,D	0.71	0.244	6.30	5.96	5.83
8	Cl	CH_3	D	0.71	0.244	6.82	6.65	6.37
9	Cl	CH_3	L	0.71	0.244	4.60	5.26	5.28
10	NO_2	CH_3	L,D	-0.28	0.276	6.70	6.52	6.22
11	NO_2	CH_3	D	-0.28	0.276	7.15	7.22	6.76
12	NO_2	CH_3	L	-0.28	0.276	5.52	5.83	5.67
13	OCH_3	CH_3	D	-0.02	0.304	6.05	6.02	5.88
14	OCH_3	CH_3	L	-0.02	0.304	3.96	4.63	4.79

Cont...

Table 3.2 contd..

No.	R	R'	Amino Acid Moiety Config.	π_R [a]	$V_{w,R}$ [b]	-log IC_{50}		
						Obsd[c]	Calcd, Eq. 3.9	Calcd, Eq. 3.12
15	Br	$CH_2C_6H_5$	L,D	0.86	0.287	3.74	4.04	4.41
16	Br	$CH_2C_6H_5$	D	0.86	0.287	4.26	4.73	4.96
17	Br	$CH_2C_6H_5$	L	0.86	0.287	3.66	3.34	3.07
18	Cl	$CH_2C_6H_5$	D	0.71	0.244	4.52[d]	5.03	5.92
19	Cl	$CH_2C_6H_5$	L	0.71	0.244	4.35	4.64	4.83
20	NO_2	$CH_2C_6H_5$	D	-0.28	0.276	7.00	6.59	6.31
21	NO_2	$CH_2C_6H_5$	L	-0.28	0.276	5.55	5.21	5.22

[a] Taken from Ref. 2 of Chapter II; [b] Calculated according to Ref. 10 of Chapter II;

[c] Taken from Ref. 16 ; [d] Not included in the derivation of Eqn. 3.9 and 3.12

Table 3.3: N-[(5-Substituted indol-3-yl)glyoxylyl] amine acids (II) and their in vitro BZ Binding Affinity.

No.	R	R'	Amino acid moiety config.	π_R^a	$V_{w,R}^b$	-log IC_{50} Obsd[c]	Cal., [d]	Cal., [e]
1	H	CH_3	L,D	0.0	0.056	3.98	4.07	4.03
2	H	CH_3	D	0.0	0.056	4.32	4.34	4.37
3	H	CH_3	L	0.0	0.056	4.52	3.93	3.48
4	Br	CH_3	D	0.86	0.287	[illegible]	[illegible]	[illegible]
5	Br	CH_3	D	0.86	0.287	5.16	4.86	4.61
6	Br	CH_3	L	0.86	0.287	4.10	3.80	3.52
7	Cl	CH_3	L,D	0.71	0.244	4.66	4.80	5.03
8	Cl	CH_3	D	0.71	0.244	5.30	5.30	5.57
9	Cl	CH_3	L	0.71	0.244	4.34	4.50	4.48
10	NO_2	CH_3	L,D	-0.28	0.276	5.21	5.11	5.42
11	NO_2	CH_3	D	-0.28	0.276	5.52	5.51	5.96
12	NO_2	CH_3	L	-0.28	0.276	4.72	4.70	4.87
13	OCH_3	CH_3	D	-0.02	0.304	4.66	4.92	5.08
14	OCH_3	CH_3	L	-0.02	0.304	3.77	4.12	3.99

Cont...

Table 3.3 contd...

No.	R	R'	Amino acid moiety Config.	π_R [a]	$V_{w,B}$ [b]	-log $1/C_0$		
						obsd [c]		
15	Br	$CH_2C_6H_5$	L,D	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
16	Br	$CH_2C_6H_5$	D	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
17	Br	$CH_2C_6H_5$	L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
18	Cl	$CH_2C_6H_5$	D	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
19	Cl	$CH_2C_6H_5$	L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
20	NO_2	$CH_2C_6H_5$	D	-0.28	0.276	[illegible]	[illegible]	[illegible]
21	NO_2	$CH_2C_6H_5$	L	-0.28	0.276	[illegible]	[illegible]	[illegible]

[a] Taken from Ref. 2 of Chapter II; [b] Calculated according to Ref. 10 of Chapter II;

[c] Taken from Ref. 16;

(IS) has been included in the study. It is assigned a value of 1 for D isomer, zero for recemic mixture, and -1 for L isomer.

A multiple regression analysis was carried out on the series of 21 compunds of Table 3.2 and initially Eq. 3.5 was obtained correlating the [^{3}H] flunitrazepam binding inhibition (-log IC_{50}) with π . In this equation the correlation coefficient is very low and variables

$$-\log IC_{50} = 5.280 - 2.547(\pm 3.121)\pi_R + 2.578(\pm 4.840)(\pi_R)^2$$

$$n=21,\ r = 0.480,\ s = 1.052,\ F(2,18) = 2.70 \quad ..(3.5)$$

were not statistically significant. Similarly, van der Waals volume (V_w) was also not found to reveal any significant correlation (Eq. 3.6). The correlation was

$$-\log IC_{50} = 5.467 + 8.648(\pm 20.412)V_{w,R} - 32.761(\pm 87.320)(V_{w,R})^2$$

$$n=21,\ r=0.208,\ s= 1.173,\ F(2,18) = 0.41 \quad ..(3.6)$$

however significantly improved when π and V_w were combined and dummy parameters I_s and IR' were introduced (Eq.3.7). In Eq. 3.7, π_R^2

$$-\log IC_{50} = 6.233 + 17.973(\pm 11.280)V_{w,R} - 73.180(\pm 46.121)(V_{w,R})^2$$
$$-3.288(\pm 2.156)\pi_R + 3.678(\pm 3.540)(\pi_R)^2$$
$$+ 0.686(\pm 0.309)I_s + 1.111(\pm 0.626)IR'$$

$$n=21,\ r= 0.906,\ s= 0.576,\ F(6,14) = 9.14 \quad ..(3.7)$$

is, however, only marginally significant, hence if it is dropped the resulting equation is

$$-\log IC_{50} = 6.012 + 20.061(\pm 12.305)V_{w,R} - 70.153(\pm 51.065)(V_{w,R})^2$$
$$-1.194(\pm 0.673)\pi_R + 0.686(\pm 0.343)I_s$$
$$-0.952(\pm 0.672)IR'$$

$$n=21,\ r= 0.872,\ s= 0.643,\ F(5,15) = 9.50 \quad ...(3.8)$$

In Eq. 3.8 two compounds (cmpds. 5 and 18 in Table 3.2) were outlier showing a wide variation between observed and calculated values. When these compounds were eliminated a slightly better correlation (Eq. 3.9) was obtained.

$$-\log IC_{50} = 6.522 + 24.559(\pm 9.779)V_{w,R} - 94.034(\pm 41.519)(V_{w,R})^2$$
$$-1.354(\pm 0.539)\pi_R + 0.694(\pm 0.283)I_s - 0.623)(\pm 0.547)IR'$$

$$n=19,\ r= 0.932,\ s= 0.483,\ F(5,13) = 13.29 \quad .....(3.9)$$

A similar equation (Eq.3.10) was obtained for the compounds of Table 3.3. Eq. 3.10 also expresses a very significant correlation. The use of π and V_w together was permitted because they were not found to be mutually correlated (r=0.232).

$$-\log IC_{50} = 4.744 + 15.190(\pm 4.357)V_{w,R} - 52.531(\pm 18.080)(V_{w,R})^2 - 0.599(\pm 0.238)\pi_R + 0.403(\pm 0.121)I_s - 0.263(\pm 0.238)IR'$$

$$n=21,\ r=0.944,\ s=0.228,\ F(5,15) = 24.47 \quad \ldots\ldots(3.10)$$

As there was only one structural difference between compounds of Tables 3.2 and 3.3 (ethyl esters and acid derivatives), so they were combined together with an indicator parameter IC. The value of this parameter was taken equal to 1 for ethyl esters and zero for acids and the correlation equation obtained for all 42 compounds was as,

$$-\log IC_{50} = 4.980 + 17.625(\pm 6.768)V_{w,R} - 61.342(\pm 28.089)(V_{w,R})^2 - 0.896(\pm 0.370)\pi_R + 0.544(\pm 0.188)I_s - 0.607(\pm 0.370)IR' + 0.797(\pm 0.329)IC$$

$$n=42,\ r=0.870,\ s=0.525,\ F(6,35) = 15.56 \quad \ldots \quad (3.11)$$

Here also the same compounds 5 and 18 of Table 3.2 were outliers showing wide devaiation from observed value. So, these two compounds were eliminated and a new equation

(Eq. 3.12) was obtained with better r value. In Eq. 3.12 F-ratio is also significant at 99% level $[F(6,33)(0.01)=3.40]$

$$-\log IC_{50} = 5.218 + 19.769(\pm 5.792)V_{w,R} - 72.679(\pm 24.286)(V_{w},R)^2 - 0.974(\pm 0.318)\pi_R + 0.546(\pm 0.164)I_s - 0.452(\pm 0.320)IR' + 0.800(\pm 0.284)IC$$

$$n= 40,\ r=0.907,\ s = 0.440,\ F(6,33) = 23.28 \quad \ldots (3.12)$$

All the above equations show that both van der Waals volume and hydrophobic nature of the substituent R at 5-position of the bezene ring are important. Since correlations are parabolic in $V_{w,R}$, a limit on the size of R-substituent is exhibited. The optimum value of $V_{w,R}$ would be 0.136×10^2 Å^3. Upto this value of $V_{w,R}$ the activity will increase and beyond that it would start decreaseing. The negative coefficient of π on the other hand shows that not hydrophobic but hydrophilic group at 5-position will be effective. This suggests that substituent at 5-position is responsible for binding through dispersion interaction with the receptor which is probably surrounded by aqueous media. Receptor binding is also found to be influenced by the substituent R' at the assymetric carbon atom. An alkyl group favors the affinity where as an arylalkyl group decreases the affinity as shown by negative sign of coefficient of IR'. This is

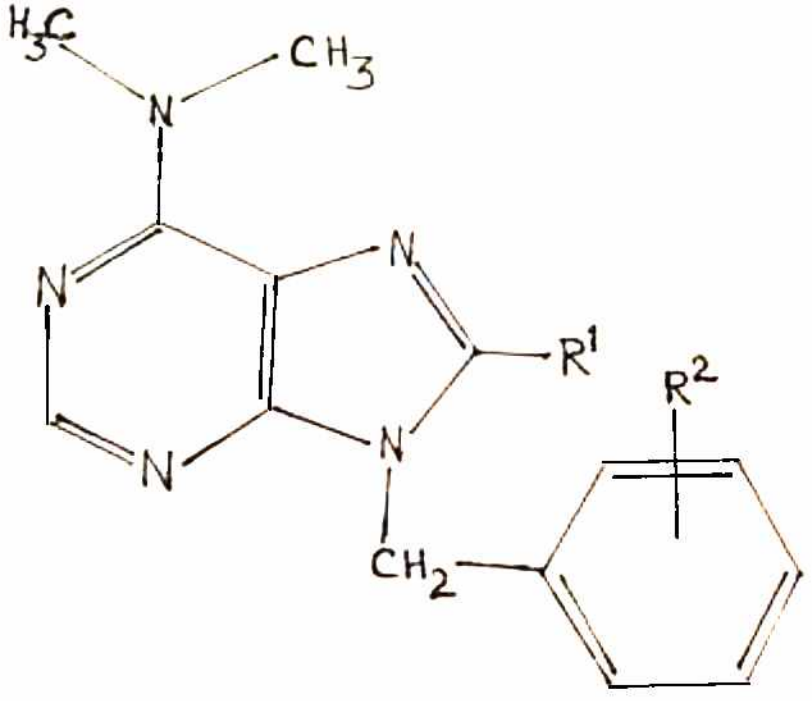
probably due to the steric hinderance of a bulky group like arylalkyl. A bulky group will not be accepted by the receptor. As the coefficient of I_s is positive, it shows 'D' isomer is having more affinity, 'L' isomer will give least affinity. It therefore proves that the receptor binding is stereoselective in nature. Orientation of substituents at assymetric carbon atom is playing an important role in receptor binding. In equation 3.12 the positive sign of the coefficient of IC indicates that the ester derivatives are having more affinity than acid derivatives. Esterification prevents the ionization of the acids and thus increases the affinity. This indicates that this end of the compounds is probably involved in a hydrophobic interaction with the receptors.

(iii) Substituted 9-benzyl-9H-purines:

Kelley et al. in their two recent communications[21,22] reported sunthesis and BZR binding of two series of substituted purines in rat brain tissue. Their study showed that structure requirements for good binding by substituted purines were highly specific. The effect of various 6-substituents, ranging in size from amino to cyclopentylmethyl amino, on BZR binding affinity was found to be very insignificant as observed from their very close range of IC_{50} values. Very good BZR receptor

binding has been found by 6-(dimethylamino)-9-substituted-benzyl purines. Removal of benzene ring resulted in complete loss of receptor affinity. However, 9-unsubstituted purine had weak affinity. This level of activity was comparable to that reported by Davies et al.[23] for 6-(dimethylamino) purine. Substitution of 9-phenyl group or replacement of the one carbon bridge with a two- or three - carbon bridge resulted in no measurable affinity. Also substitution in 8-position has been found to influence affinity. But all these studies were qualitative in nature. Though few of them showed BZR binding affinity comparable to that of diazepam , none showed significant anxiolytic or anticonvulsant activity on a modified Geller-Seifter conflict schedule.[24,25] Their in vivo experimental results supported that these compounds may be antagonists of BZs. Otherwise, also it is possible that the absence of activity may be due to lack of absorption or penetration to CNS or rapid metabolism. Alternatively, it is posible that these compounds bind to a subtype of BZR[26] that is not involved in conflict behavior.

In two consecutive studies,[21,22] Kelley et al. reported two series of substituted 9-benzyl-9H-purines(IV) for their in vitro binding activity to rat brain receptors



(IV)

by inhibiting specific binding of [^{3}H]diazepam, where IC_{50} value refers to the molar concentration of the drug leading to the 50% blockade of the binding of [^{3}H] diazepam. The data of the two studies are compiled in Table 3.4. In their first study, Kelley et al. varied the substituents R^2 at different position of benzyl ring and R at 6-position. Change of R did not show any appreciable change in binding property. But change of R^2 influenced the binding to a great extent. In next study t! ey varied substitutent R^2 at only meta position of the benzyl ring and also the substituent R^1 at 8-position keeping dimethylamino group at 6-position. In first study variation of R^2 was at both meta and ortho positions (compds. 1,7,24-31) where as in second study it was only at meta position (compds. 1-23) with compounds 1 and 7 common in both the studies. The activity was analysed with hydrophobic and electronic parameters of R^2 and R^1 substituents. For electronic effect of ortho substituents, σ_{para} was taken.

With the use of data as given in Table 3.4, the regression analysis revealed Eq.3.13 for all meta substituted compounds (1-28). Eq. 3.13 expresses that neither the electronic nor the hydrphobic character of R^1-substituent

Table 3.4 : 8-Substituted-9-benzyl-9H-purines (IV) and Their in vitro BZR Binding Affinity

No.	R^1	R^2	π_{R^2}[a]	σ_{R^2}[a]	σ_{R^1}[a]	$-\log IC_{50}$		
						Obsd[b]	Calcd. Eq. 3.14	Calcd. Eq. 3.15
1	H	H	0.0	0.0	0.0	4.89	5.00	5.03
2	Br	H	0.0	0.0	0.39	5.52	5.58	5.64
3	CH_3	H	0.0	0.0	-0.07	5.07	4.90	4.93
4	OCH_3	H	0.0	0.0	0.12	5.27	5.18	5.25
5	$N(CH_3)_2$	H	0.0	0.0	-0.15	5.24	4.78	4.86
6	$NHCH_3$	H	0.0	0.0	-0.30	4.77	4.56	4.63
7	H	3-NH_2	-1.23	-0.16	0.0	6.05	6.03	6.05
8	Br	3-NH_2	-1.23	-0.16	0.39	6.96	6.61	6.63
9	Cl	3-NH_2	-1.23	-0.16	0.37	6.72	6.58	6.60
10	OCH_3	3-NH_2	-1.23	-0.16	0.12	6.30	6.21	6.24
11	$N(CH_3)_2$	3-NH_2	-1.23	-0.16	-0.15	6.22	5.81	5.85
12	$NHCH_3$	3-NH_2	-1.23	-0.16	-0.30	5.14	5.59	5.63
13	OH(Oxo)	3-NH_2	-1.23	-0.16	0.12	5.74	6.21	6.24
14	H	3-NHCHO	-0.98	0.19	0.0	6.47	6.72	6.77
15	Br	3-NHCHO	-0.98	0.19	0.39	7.96	7.34	7.29

..cont.

Table 3.4 contd..

No.	R^1	R^2	π_{R^2} [a]	σ_{R^2} [a]	σ_{R^1} [a]	-log IC_{50} Obsd. [b]	Calcd. Eq. 3.14	Calcd. Eq. 3.15
16	$N(CH_3)_2$	3-NHCHO	-1.98	0.19	-0.15	6.96	6.54	6.51
17	$NHCH_3$	3-NHCHO	-0.92	0.19	-0.30	6.44	6.32	6.30
18	OH(OXO)	3-NHCHO	-0.98	0.19	0.12	6.89	6.94	6.90
19	Br	3-$NHCOCH_3$	-0.97	0.21	0.39	6.08 [c]	7.39	7.33
20	Br	3-$NHCOC_6H_5$	0.49	0.02	0.39	5.10	5.05	5.13
21	Br	3-$NHCOOCH_3$	-0.37	0.07	0.39	5.28	6.34	6.26
22	Br	3-$NHCONH_2$	-1.30	-0.03	0.39	7.60	7.08	7.06
23	Br	3-$NHSO_2CH_3$	-1.18	0.20	0.39	5.82 [c]	7.62	7.55
24	H	3-F	0.14	0.34	0.0	5.14	5.84	5.84
25	H	3-CH_2OH	-1.03	0.0	0.0	5.30	6.26	6.27
26	H	3-OH	-0.67	0.12	0.0	5.92	6.18	6.18
27	H	3-$OCOC(CH_3)_2$	0.69	0.37	0.0	5.80	5.26	5.29
28	H	3-$OCOCH_3$	-0.64	0.39	0.0	6.36	6.94	6.89
29	H	2-F	0.14	0.06	0.0	5.27	-	5.08
30	H	2-Cl	0.71	0.23	0.0	4.89	-	4.88
31	H	2-OCH_3	-0.02	-0.27	0.0	4.70	-	4.37

[a] Taken from Ref. 2 of Chapter II; [b] Taken from Ref. 21 for compds 1,7,24-31 and from Ref. 22 for compds 1-23;

[c] Not included in the derivation of both the Eqs. 3.14 and 3.15 ;

$$-\log IC_{50} = 5.092 - 1.048(\pm 0.436)\pi_{R}^{2} + 2.119(\pm 1.538)\sigma_{R}^{2} + 0.520(\pm 1.659)\sigma_{R}^{1} + 0.227(\pm 0.763)\pi_{R}^{1}$$

$$n=28,\ r=0.758,\ s=0.616,\ F(4,23)=6.75 \quad \ldots(3.13)$$

has any importance in the binding of the compounds with the BZR. However, elimination of two compounds 19 and 23 whose calculated values were found to largely differ from the observed ones, led to Eq. 3.14 where σ_{R}^{1} was shown to be statistically significant. Thus Eq. 3.14 exhibits the

$$-\log IC_{50} = 5.004 - 1.221(\pm 0.365)\pi_{R}^{2} + 2.961(\pm 1.341)\sigma_{R}^{2} + 1.487(\pm 0.906)\sigma_{R}^{1}$$

$$n=26,\ r=0.856,\ s=0.499,\ F(3,22)=19.15 \quad \ldots\ (3.14)$$

importance of electronic character of R^1 substituent, along with the role played by hydrophobic and electronic character of R^2 substituent. Since the coefficient of π_{R}^{2} is negative, a hydrophilic not the hydrophobic group would be desired at R^2 -position for the better binding. Because of the positive influence of hydrophilic and electronic nature of R^2-substituents, a strong polar interaction can be expected between the latter and the active site of the receptor.

When ortho - compounds (compds. 29-31) were also included in the analysis, using the σ_{para} for ortho substituents , Eq. 3.15 was obtained with exclusion of compounds 19 and 23 again.

$$-\log IC_{50} = 5.078 - 1.157(\pm 0.303)\pi_R 2 + 2.719(\pm 1.140)\sigma_R 2 + 1.441(\pm 0.854)\sigma_R 1$$

$$n=29,\ r= 0.871,\ s= 0.477,\ F(3,25) = 25.12 \quad ..(3.15)$$

Thus Eq. 3.15 leads to the same conclusion as Eq. 3.14, that both hydrophobic nature as well as electronic character of R^2 substituent at 9-benzyl ring and electronic characterstic of the substituent at 8-position are contributing to the receptor binding affinity. Though the benzyl group is essential for strong affinity, the negative sign of the coefficient of $\pi_R 2$ indicates that a hydrophobic group will not be tolerated by the receptor, instead a hydrophilic group would be preferred. Between meta- and ortho- substitution, meta- substitution can produce better effect as it has larger σ value than ortho-substituent. Positive coefficients of σ for both R^2 and R^1 substituents indicates that electron withdrawing group (positive σ value) at both positions will give better

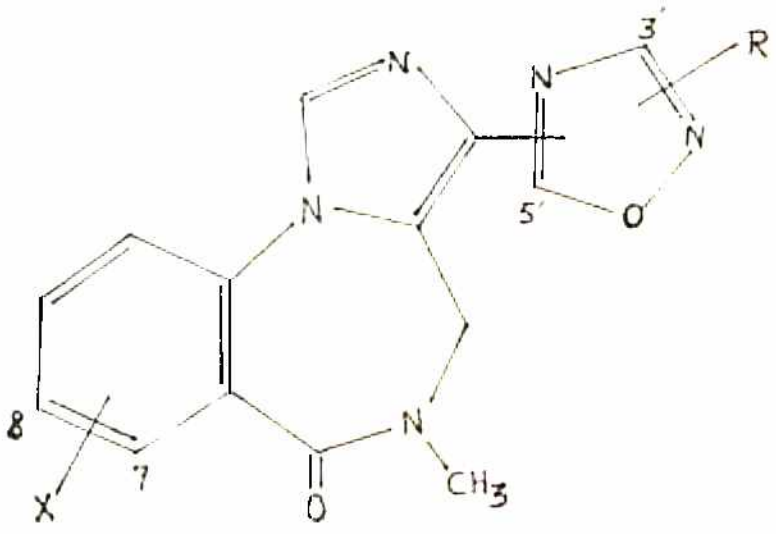
affinity to compounds for the receptor. The analysis gives an overall idea that a hydrophilic substituent of electron withdrawing nature will increase the affinity. This is probably due to the presence of some aqueous media surrounding the receptor molecules. Also electron withdrawing substituent at 8-position will potentiate the affinity, however, the size or hydrophobicity of this substituent was not found to influence the receptor binding.

(iv) Oxadiazolylimidazobenzodiazepines: A Novel BZR Partial Agonists:

Watzen et al.[27] recently reported synthesis and biochemical activity of oxadiazolylimidazobenzodiazepines as novel BZR partial agonists. Classical full agonists (such as diazepam and nitrazepam) at the BZR continue to be widely prescribed as anxiolytics, anticonvulsants, sedatives-hypnotics and muscle relaxants. These latter two properties, however, are often seen as unwanted side effects and are believed to be related to the high level of receptor stimulation achieved by full agonists. Partial agonists are of interest since they are proposed to be

capable of selectively eliciting the lower efficacy responses such as anxiolysis.[28] Several carboxylic acid derivatives of the imidazobenzodiazepines have been reported to act as partial agonists at the BZR; at least three (Ro 16-6028), Ro 17-1812, and Ro 23-0364) have been evaluated in humans.[29] In the β-carbolines, it has been demonstrated[30] that replacement of the carboxyl derivative at the 3-position by a 1,2,4-oxadiazole moiety led to a series of highly active benzodiazepine receptor ligands. Major chemical similarities exist between the imidazobenzodiazepine framework and the β-carboline framework. It was anticipated therefore that this substitution should provide novel partial agnoists with a favourable seperation between anxiolytic and sedative properties.

In their study Watzen et al. synthesized various derivatives of 6-oxo-oxadiazolylimidazobenzodiazopines and measured their biochemical activity by its ability to displace the specific binding of the radiolabeled antagonist [^{3}H]Ro 15-1788.[31] The work of Watzen et al. was basically a qualitative structure activity relationship study. In order to have a better picture of the mechanism of binding of these compounds with the receptor, a QSAR study is presented.



(V)

The data of 6-oxo-oxadiazolylimidazobenzodiazepines (V) are listed in Table 3.5 (33 compounds). In their study Watzen et al. varied the substituent X at 7-and 8-position of the fused benzene ring and substituent R at 3' or 5' position of oxadiazolyl ring. This ring is attached to imidazobenzodiazepine via either 3' or 5' position having substituent R at other position. The activity was analysed with π, σ and V_w of the substituents X and R at different positions. For σ value of X at 7-position we took σ_{-meta} and at 8-position σ_{-para}, as according to the numbering system 7-is becoming meta to C_1 and 8-position para. Similarly to differentiate substituent R at two different positions (3' and 5') we incorporated a dummy parameter, IR, with a value of zero for 3'-position and 1 for 5'-position.

A multiple regression analysis of the data of Table 3.5 revealed Eq. 3.16 for the inhibition of binding of [^{3}H] Ro 15-1788. In Eq. 3.16, the $V_{w,R}$ is not found to be statistically significant, hence it could be safely

$$-\log IC_{50} = 7.138 + 0.422(\pm 0.577)\pi_x + 1.358(\pm 0.998)\sigma_x - 0.357(\pm 0.999)V_{w,R} + 0.463(\pm 0.232)IR$$

$$n=33,\ r=0.791,\ s=0.325,\ F(4,28) = 11.72 \quad ..(3.16)$$

Table 3.5: Oxadiazolylimidazobenzodiazepines (V) and their in vitro BZR binding affinities

No.	X	R	π_X [a]	σ_X [a]	-log IC_{50} Obsd [b]	-log IC_{50} Cald. Eq. 3.18
1	H	3'-CH_3	0.0	0.0	7.17	7.05
2	H	3'-C_2H_5	0.0	0.0	7.29	7.05
3	H	3'-n-C_3H_7	0.0	0.0	6.92	7.05
4	H	3'-i-C_3H_7	0.0	0.0	6.98	7.05
5	H	5'-CH_3	0.0	0.0	7.57	7.43
6	H	5'-C_2H_5	0.0	0.0	7.66	7.43
7	H	5'-n-C_3H_7	0.0	0.0	7.47	7.43
8	H	5'-i-C_3H_7	0.0	0.0	7.28	7.43
9	8-Cl	3'-C_2H_5	0.71	0.23	6.22 [c]	7.89
10	7-Cl	3'-CH_3	0.71	0.37	8.09	8.01
11	7-Cl	3'-C_2H_5	0.71	0.37	8.01	8.01
12	7-Cl	3'-n-C_3H_7	0.71	0.37	8.22	8.01
13	7-Cl	3'-i-C_3H_7	0.71	0.37	7.85	8.01
14	7-Cl	5'-CH_3	0.71	0.37	8.47	8.39
15	7-Cl	5'-C_2H_5	0.71	0.37	8.19	8.39
16	7-Cl	5'-n-C_3H_7	0.71	0.37	8.42	8.39
17	7-Cl	5'-i-C_3H_7	0.71	0.37	8.44	8.39
18	7-F	3'-CH_3	0.14	0.34	7.66	7.46
19	7-F	3'-C_2H_5	0.14	0.34	7.38	7.46
20	7-F	3'-n-C_3H_7	0.14	0.34	7.62	7.46
21	7-F	3'-i-C_3H_7	0.14	0.34	7.17	7.46

Contd...

Table 3.5 contd...

No.	X	R	π_x [a]	σ_x [a]	-log IC_{50}	
					Obsd[b]	Cald.Eq.3.18
22	7-F	5'-CH_3	0.14	0.34	7.96	7.84
23	7-F	5'-C_2H_5	0.14	0.34	7.82	7.84
24	7-F	5'-n-C_3H_7	0.14	0.34	7.85	7.84
25	7-F	5'-i-C_3H_7	0.14	0.34	7.72	7.84
26	8-F	3'-CH_3	0.14	0.06	7.30	7.23
27	8-F	3'-C_2H_5	0.14	0.06	6.98	7.23
28	8-F	3'-n-C_3H_7	0.14	0.06	7.38	7.23
29	8-F	3'-i-C_3H_7	0.14	0.06	7.00	7.23
30	8-F	5'-CH_3	0.14	0.06	7.68	7.61
31	8-F	5'-C_2H_5	0.14	0.06	7.57	7.61
32	8-F	5'-n-C_2H_5	0.14	0.06	7.57	7.61
33	8-F	5'-i-C_2H_5	0.14	0.06	7.37	7.61

[a] Taken from Ref. 2 of Chapter II ; [b] Taken from Ref.27;

[c] Not included in the derivation of Eq. 3.18

$$-\log IC_{50} = 6.987 + 0.424(\pm 0.571)\pi_x + 1.355(\pm 0.0989)\sigma_x + 0.462(\pm 0.230)IR$$

$$n=33,\ r=0.787,\ s=0.323,\ F(3,29) = 14.62 \quad ..(3.17)$$

dropped to have Eq. 3.17. However, neither Eq. 3.16 nor Eq. 3.17 represents any satisfactory correlation. But when compound 9, for which there was a large difference between the calculated and observed activities, was excluded, a highly significant correlation was obtained (Eq. 3.18). Compound 9 possesses very low observed activity as compared to the predicted one. Since it is the only

$$-\log IC = 7.052 + 0.918(\pm 0.301)\pi_x + 0.829(\pm 0.501)\sigma_x + 0.376(\pm 0.115)IR$$

$$n=32,\ r=0.939,\ s=0.159,\ F(3,28) = 67,24 \quad .. \quad (3.18)$$

compound which has Cl group at its 8-position, the reason of its low activity may be assumed due to intolerance of a bulky group like Cl at the receptor site.

Above study suggests that the hydrophobic nature of the substituent at 7-or 8-position is important for the activity. As σ_{meta} is always higher than σ_{para} for any substituent, the positive nature of coefficient of σ

suggests that substitution of any group at 7-position would lead to better activity than at 8-position. The hydrophobic nature or size of the substituent R at oxadiazolyl ring was found to be of no consequence, but positive nature of the coefficient of IR signifies that the substituent R at 5'-position gives better binding activity than at 3'-position. However, this can also be explained in otherway that when the oxadiazolyl ring is attached to imidazobenzodiazepine through 3'-position, the compound exhibits more activity.

B. Some Potential Neuroleptic Agents:

Neuroleptic drugs are thought to modulate catecholamine functions in central nervous system (CNS) by blocking dopamine receptors.[32] A series of 2,6-dialkoxybenzamide derivatives(VI) have been recently characterized as potent and selective dopamine blocking agents,[33-35] and among them, the remoxipride (VI; R=CH_3, X=Br was found to be a potent inhibitor of the apomorphine syndrome in rat without an accompanying ability to cause catalepsy. In continuation to their studies, Ogren et al. reported the synthesis and antidopamine activity of some alkoxybenzamide derivatives with alterations at many points (VII).[36,37] All these derivatives alongwith remoxipride are listed

in Table 3.6. Very recently Högberg et al.[38] has reported the syntheiss and biological activity of some more alkoxybenzamide derivatives of two isomeric forms (S & R) with alterations at different points (VIII). These compounds are listed along with compounds FLA 797, raclopride, eticlopride in Table 3.7 (24 compounds). They were tested for antidopamine activity in vivo by their ability to inhibit the apomorphine syndrome in rat and in vitro by their ability to displace [^{3}H] spiperone from striatal preparations of the rat brain. An analogue of haloperidol, the spiperone is one of the most potent neuroleptics discovered so far.

In rat the apomorphine induces the motoric hyperactivity and stereotypic behaviours (sterotypies), such as sniffing and chewing/licking/biting. The ED_{50} in Table 3.6 refer to doses (mole/Kg) of compounds that reduce the hyperactivity or stereotypies by 50% over the total observation period. For in vitro activity, the IC_{50} refers to the molar concentration of the drug, leading to 50% blockade of [^{3}H]spiperone binding. Both ED_{50} and IC_{50} parameters were qualitatively observed[36,37] to depend upon the lipophilic character of the substituent at 3-position of the phenyl ring (X' in VII). Further,

Table 3.6 : 6-Methoxybenzamide analogues(VII) and their in vitro and in vivo Antidopamine Activities.

No.	X^1	X^2	R^1	R^2	-log IC_{50} (in vitro) Obsd[a]	Calcd. Eq. 7.31	-log D_{50} (anti-apomorphine activity) Obsd[a]	Calcd. Eq. 7.32	-log D_{50} (anti-stereotype) Obsd[a]	Calcd. Eq. 7.33
1	Br	H	CH_3	C_2H_5	5.80	5.52	6.06	6.15	5.19	5.30
2	H	H	CH_3	C_2H_5	4.82	4.62	4.20	4.10	4.07	4.03
3	I	H	CH_3	C_2H_5	5.56	5.70	5.95	6.11	5.03	5.51
4	C_2H_5	H	H	C_2H_5	6.04	6.21	6.02	6.11	4.95	5.43
5	H	H	H	C_2H_5	6.50	6.55	6.40	6.75	5.11	5.42
6	F	H	H	C_2H_5	6.44	6.75	-	6.87	-	5.53
7	Cl	H	H	C_2H_5	7.41	7.65	6.70	6.42	6.06	5.85
8	Br	H	H	C_2H_5	7.92	7.76	7.26	6.97	6.49	6.10
9	I	H	H	C_2H_5	8.47	7.94	6.82	6.52	6.27	6.30
10	NO_2	H	H	C_2H_5	5.52	5.16	-	5.46	-	5.20
11	CH_3	H	H	C_2H_5	7.72	8.03	5.91	6.23	6.02	5.86
12	C_2H_5	H	H	C_2H_5	8.33	8.44	6.92	6.72	6.50	6.22
13	n-C_3H_7	H	H	C_2H_5	8.32	8.39	6.80	7.04	6.10	6.64
14	n-C_4H_9	H	H	C_2H_5	7.59	7.85	-	7.71	-	7.03
15	Br	H	CH_3CO	C_2H_5	5.46	5.52	6.52	6.05	6.34	5.30

Contd....

Table 3.6 Contd...

No.	X^1	X^2	R^1	R^2	-log IC_{50} (in vitro) Obsd[a]	Cald.Eq.3.21	-log ED_{50} (antinociceptive activity) Obsd[b]	Cald. Eq. 3.22	-log ED_{50} (antiheterotropic) Obsd[c]	Cald. Eq. 3.25
16	Br	H	CH_3	C_3H_7	6.01	5.52	5.47	5.85	4.21	5.30
17	Cl	H	CH_3	C_2H_5	5.50	5.41	5.20	5.71	4.03	5.19
18	Br	H	H	C_3H_7	8.20	7.76	6.37	6.57	6.22	6.10
19	H	Br	H	C_2H_5	6.22	6.85	-	5.73	-	5.42
20	H	Cl	H	C_2H_5	7.19	6.85	6.11	5.73	5.60	5.42
21	H	Br	H	C_2H_5	7.25	6.85	5.21	5.73	5.18	5.42
22	H	C_2H_5	H	C_2H_5	6.96	6.85	6.33	5.73	6.10	5.42
23	Br	H	H	C_2H_5	7.68	7.76	6.78	6.57	6.53	6.10
24	Br	H	C_2H_5	C_2H_5	4.89	5.52	6.33	5.85	5.78	5.30
25	Cl	Cl	H	C_2H_5	7.50	7.65	6.39	6.42	5.77	5.98
26	Cl	Br	H	C_2H_5	7.24	7.65	-	6.42	-	5.98
27	Cl	CH_3	H	C_2H_5	7.96	7.65	-	6.42	-	5.98
28	Cl	C_2H_5	H	C_2H_5	7.92	7.65	5.60	6.42	6.82	5.98
29	Cl	C_3H_7	H	C_2H_5	6.96	7.65	-	6.42	-	5.98
30	Br	F	H	C_2H_5	8.18	7.76	6.80	6.57	6.38	6.10

Contd....

Contd... (Table 3.6)

No.	X^1	X^2	R^1	R^2	$-\log IC_{50}$ (in vitro)		$-\log ED_{50}$ (anti-Mycoactivity)		$-\log El_{50}$ (antiinflammatory)	
					Obsd[a]	Cald. Eq. 3.21	Obsd[b]	Cald. Eq. 3.22	Obsd[c]	Cald. Eq. 3.23
31	Br	Cl	H	C_2H_5	7.77	7.76	6.02	6.27	6.27	6.10
32	Br	Br	H	C_2H_5	7.59	7.76	6.19	6.27	6.03	6.10
33	Br	NO_2	H	C_2H_5	6.73	7.76	-	6.27	-	6.10
34	Br	CH_3	H	C_2H_5	7.96	7.76	-	6.27	-	6.10
35	Br	C_2H_5	H	C_2H_5	7.77	7.76	6.06	6.27	6.34	6.10
36	NO_2	Br	H	C_2H_5	5.20	5.16	-	5.46	-	5.30
37	CH_3	Cl	H	C_2H_5	8.50	8.03	6.13	6.27	5.88	5.96
38	CH_3	Br	H	C_2H_5	8.26	8.03	-	6.27	-	5.96
39	CH_3	CH_3	H	C_2H_5	8.11	8.03	5.73	6.27	5.51	5.96
40	CH_3	C_3H_7	H	C_2H_5	7.82	8.03	-	6.27	-	5.96
41	C_2H_5	F	H	C_2H_5	8.82	8.44	7.19	6.72	6.51	6.22
42	C_2H_5	Cl	H	C_2H_5	9.04	8.44	7.49	6.72	6.72	6.22
43	C_2H_5	Br	H	C_2H_5	8.64	8.44	7.07	6.72	6.33	6.22
44	C_2H_5	C_2H_5	H	C_2H_5	8.75	8.44	7.12	6.72	6.34	6.22

Contd...

Table 3.6 contd..

No.	X^1	X^2	R^1	R^2	-log IC_{50} (in vitro) Obsd[a]	-log IC_{50} (in vitro) Calcd. Eq. 3.21	-log ED_{50} (antinociceptivity) Obsd[a]	-log ED_{50} (antinociceptivity) Calcd. Eq. 3.22	-log D_{50} (antistereotype) Obsd[a]	-log D_{50} (antistereotype) Calcd. Eq. 3.25
45	C_3H_7	Cl	H	C_2H_5	8.52	8.39	7.00	7.24	7.46	7.64
46	C_3H_7	CH_3	H	C_2H_5	8.30	8.39	-	7.24	-	7.64
47	Br	Br	CH_3	C_2H_5	5.47	5.52	6.36	6.85	5.60	5.30

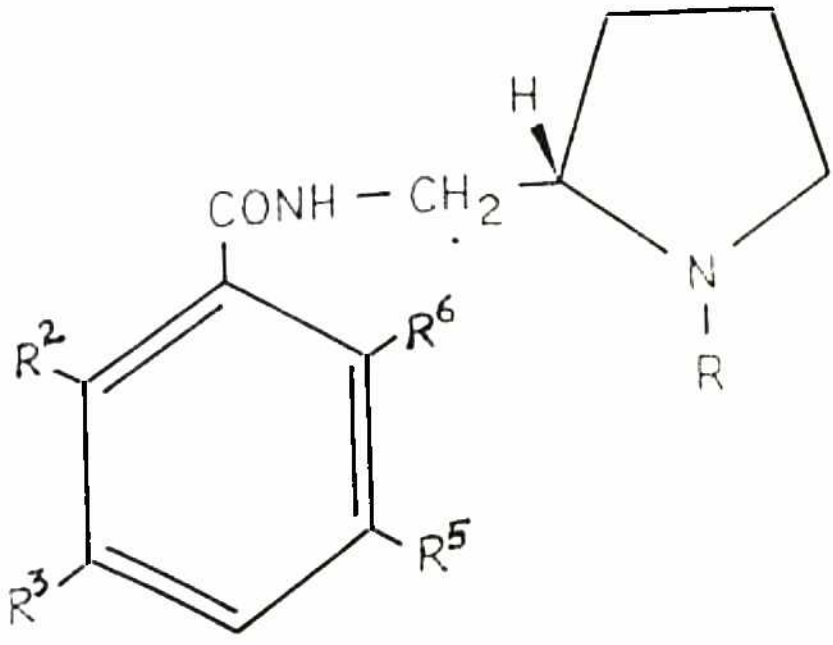
[a] Taken for compounds 1-24 from ref.36 and for compounds 25-47 from ref.37

Values of hydrophobic and electronic constants used for X^1- and X^2 - substituents in compounds of Table 3.6

X^1/X^2	π[a]	σ[a,b]
H	0.00	0.00
F	0.14	0.34
Cl	0.71	0.37
Br	0.86	0.39
I	1.12	0.35
NO_2	-0.28	0.71
CH_3	0.56	-0.07
C_2H_5	1.02	-0.07
n-C_3H_7	1.55	-0.07
n-C_4H_9	2.04	-0.08

[a] Taken from ref. 2 of Chapt.II

[b] For meta to amide moiety.



(VIII)

Table 3.7 : Benzamide derivatives (VIII) and Their in vitro Activity.

No	R^2	R^3	R^5	R^6	R	Stereochem.	$\pi_R{}^3$ [a]	-log IC_{50} Obsd [b]	-log IC_{50} Calcd. Eq. 3.20	-log IC_{50} Calcd. Eq. 3.21
1	OCH_3	Cl	OCH_3	OCH_3	C_2H_5	S	0.71	6.37	5.75	5.74
2	OCH_3	Br	OCH_3	OCH_3	C_2H_5	S	0.86	5.76	5.84	5.83
3	OCH_3	C_2H_5	OCH_3	OCH_3	C_2H_5	S	1.02	6.09	5.93	5.93
4	OCH_3	C_3H_7	OCH_3	OCH_3	C_2H_5	S	1.55	5.53	6.04	6.06
5	OH	H	OCH_3	OCH_3	C_2H_5	S	0.0	8.06	8.01	7.99
6	OH	Cl	OCH_3	OCH_3	C_2H_5	S	0.71	8.77	.40	8.33
7	OH	Br	OCH_3	OCH_3	C_2H_5	S	0.86	8.85	.51	8.43
8	OH	C_2H_5	OCH_3	OCH_3	C_2H_5	S	1.02	9.11	.61	.53
9	OH	C_3H_7	OCH_3	OCH_3	C_2H_5	S	1.55	8.62	.91	8.86
10	OH	Br	OCH_3	OCH_3	C_2H_5	R	0.86	7.10	6.78	6.74
11	OH	Br	OCH_3	OCH_3	CH_2=CH-CH_2	S	0.86	8.64	.51	8.43
12	OCH_3	H	OCH_3	OH	C_2H_5	S	0.0	6.69	6.72	6.72
13	OCH_3	Cl	OCH_3	OH	C_2H_5	S	0.71	7.15	7.17	7.16
14	OCH_3	Br	OCH_3	OH	C_2H_5	S	0.86	7.17	7.25	7.25
15	OCH_3	C_2H_5	OCH_3	OH	C_2H_5	S	1.02	7.72	7.35	7.35
16	OCH_3	Br	OCH_3	OH	C_2H_5	R	0.86	5.21	5.53	5.57

Cont.....

Table 3.7 Contd....

No.	R^2	R^3	R^5	R^6	R	Stereochem.	π_3[a]	$-\log EC$ Obsd[b]	$-\log EC$ Eq. [illegible]	$-\log EC$ Eq. [illegible]
17	OCH_3	Br	OCH_3	OH	$CH_2=CH-CH_2$	S	0.86	7.36	7.[illegible]	7.[illegible]
18	NH_2	Br	OCH_3	OCH_3	C_2H_5	S	0.86	7.68	7.01	7.43
19	OH	Br	NH_2	OCH_3	C_2H_5	S	0.86	7.40[c]	7.51	7.43
20	OH	H	OH	OCH_3	C_2H_5	S	0.0	7.25	7.01	7.09
21	OH	Br	OH	OCH_3	C_2H_5	S	0.86	7.72	7.01	7.43
22	OH	Br	H	OCH_3	C_2H_5	S	0.86	7.00	-	7.43
23	OH	Cl	Cl	OCH_3	C_2H_5	S	0.71	7.49	-	7.33
24	OH	C_2H_5	Cl	OCH_3	C_2H_5	S	1.02	8.04	-	7.53

[a] Taken from Ref.2 of Chapter II; [b] Taken from Ref. 38 ; [c] Not included in the derivation of Eqs. 3.26 and 3.27

the formation of a coplanar six-membered pseudoring, involving the amide moiety and the methoxy group (Fig. 3.1), was presumed to be the essential structural requirement for in vitro antidopamine activity.[36,37] A crystal structure study of substituted benzamides has shown the existence of a strong hydrogen bonding between the oxygen atom of 6-methoxy group and the amide hydrogen,[39] thus forming a rigid bicyclic ring system(ring B, Fig. 3.1) and the presence of a hydroxyl group adjacent to the amide carbonyl in salicylamide is supposed to enhance this planar arrangement by forming another six-membered pseudoring (ring A) through another hydrogen bond between the phenol hydrogen and the amide carbonyl oxygen.

The nature of the substituent at 5-position in the phenyl ring (X^2) was not found to influence the activity in vivo or in vitro and nor was found the change in N-substituent of pyrrolidine ring (R^2) to have any effect on the activity.[36,37] The electronic character of any substituent was not found by de Paulis et al. to have any effect on the activity. All these observations were made by de Paulis et al.[36,37] only qualitatively. A quantitative analysis of the correlation of all these characteristics of molecules with their activity will

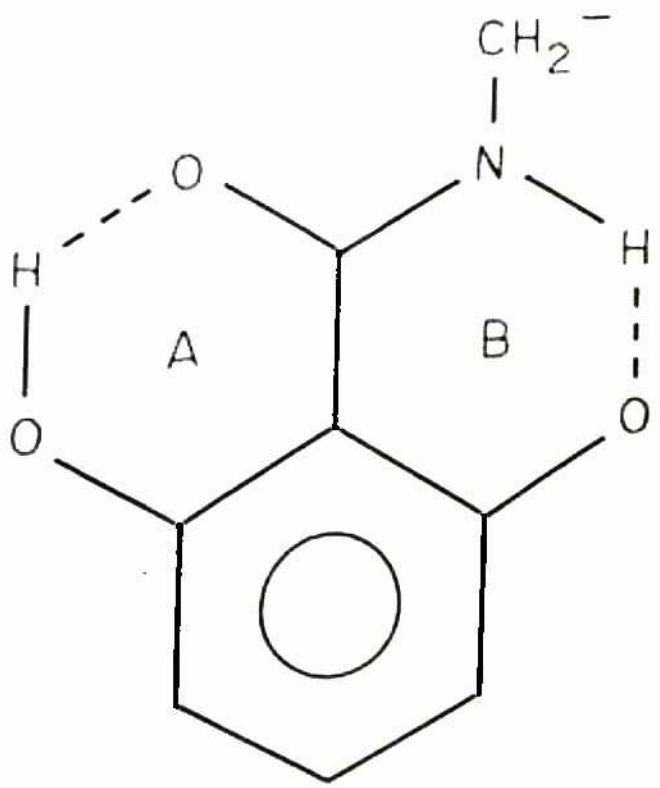


Fig. 3.1. Formation of two pseudorings, A and B, by hydrogen bonding in 6-methoxysalicylamides.

precisely determine the extent of the role played by each characteristic in the drug action of the molecule. Further, the correlation equations may be exploited to design a new better compound or predict the activity of prospective compounds.

The in vitro and in vivo activity data of benzamide neuroleptics reported by de Paulis et al. in their two consecutive studies[36,37] compiled (Table 3.6). In their first study,[36] de Paulis et al. varied all 4 substituents, X^1, X^2, R^1, and R^2 in structure VII (compds 1-24) and in the next study they concentrated only on the variation of X^1 and X^2 substituents, keeping R^1 and R^2 fixed (R^1=H, R^2=C_2H_5, compds 25-46). Compound 47 where R^1 is also changed was included in the next study.[37] We analysed the activity data of these compounds together in relation to hydrophobic and electronic parameters of the substituents. Since R^1 and R^2, except in compound 15 where R^1=CH_3CO, were changed from hydrogen to an alkyl group only, two dummy parameters, D_1 and D_2, were respectively used for them, each with a value of unity for an alkyl group and zero for hydrogen.

With the use of data of Table 3.6, a multiple regression analysis was found to reveal Eq. 3.19 correlating the in vitro activity of all the 47 compounds with π and σ of X^1 and X^2 substituents and the two dummy parameters of R^1 and R^2 substituents. Now

$$-\log IC_{50} = 1.105(\pm 0.372)\pi_{X}1 + 0.062(\pm 0.400)\pi_{X}2 - 1.025(\pm 0.743)\sigma_{X}1 + 0.070(\pm 0.826)\sigma_{X}2 - 2.085(\pm 0.465)D_1 + 0.129(\pm 0.652)D_2 + 6.982$$

$$n=47,\ r=0.894,\ s=0.567,\ F(6,40) = 23.75 \quad \ldots(3.19)$$

as expected, the parameters related to X^2 and R^2 substituents are comoletely insignificant statistically at 95% confidence level. Hence, if these parameters are dropped, the correlation suffers no loss in significance at all (Eq.3.20). Instead, the value of s slightly drops down and that of F

$$-\log IC_{50} = 1.085(\pm 0.351)\pi_{X}1 - 1.038(\pm 0.713)\sigma_{X}1 - 2.108(\pm 0.420)D_1 + 7.048$$

$$n=47,\ r=0.893,\ s=0.549,\ F(3,43) = 54.96 \quad \ldots(3.20)$$

significantly improves. The F-value is significant at 99% level [$F(3,43)(0.01) = 4.27$]. Further, the correlation becomes more significant, when the square t rm of $\pi_X 1$

is also added (Eq. 3.21). Thus Eq. 3.21 shows that while

$$-\log IC_{50} = 2.502(\pm 0.560)\pi_X 1 - 1.283(\pm 0.543)\sigma_{..}1 - 2.238(\pm 0.319)D_1$$
$$-1.013(\pm 0.353)(\pi_X 1)^2 + 6.855$$
$$n=47,\ r=0.942,\ s=0.414,\ F(4,42) = 78.59 \quad ...(3.21)$$

hydrophobic nature of X^1 substituent is important for the activity, it also puts a limit to the latter. This optimization of in vitro activity by hydrophobicity can be attributed to a limited steric bulk tolerance at the active site of the receptor, as in in vitro study there are no transport barriers such as intervening membranes or nonsalective binding to extraneous biological material. The optimum $\pi_X 1$ value is 1.24.

Notwithstanding to de Paulis et al.'s observation,[36,37] Eq. 3.21 also suggests that there is a significant role of electronic character of X^1 substituent in the binding of compound with dopamine receptor. An electron-donating substituent will always favour the binding. One can assume that by donating electron, X^1 will affect C=O group which lies meta to it and thus strengthen some hydrogen bond between C=O and the receptor. When R^1 =H, one carbonyl lone pair is held in intramolecular hydrogen bonding, but

the other is still available for binding to the receptor, and electron donors in the benzene ring should strengthen it.

A large negative coefficient of D_1 in Eq. 3.21 reveals that alkylation of OH group at 2-position will drastically reduce the activity of compounds. This leads to confirm de Paulis et al.'s suggestion[36] that such change at 2-position will produce the steric hinderance in the formation of presumed pseudorings in compounds, deemed to be essential for their activity. This steric factor was found to be important in in vivo activity also. In the correlations of both in vivo assays of compounds, antihyperactivity as well as antistereotypy action, tne parameter D_1 was found to be statistically quite significant. The $\pi_X 1$ was the only other parameter which had surfaced to be important in this case. Thus with the available in vivo data, the best correlation equations obtained were as,

$$-\log ED_{50}(\text{antihyper.}) = 0.970(\pm 0.461)\pi_X 1 - 0.712(\pm 0.400)D_1 + 5.732$$
$$n=34,\ r=0.704,\ s=0.507,\ F(2,31) = 14.76 \qquad ..(3.22)$$

$$-\log ED_{50}(\text{antistereo}) = 0.788(\pm 0.357)\pi_X 1 - 0.792(\pm 0.313)D_1 + 5.418$$
$$n=34,\ r=0.770,\ s=0.395,\ F(2,31) = 21.84 \qquad ..(3.23)$$

Since Eqs. 3.22 and 3.23, though all the variables in them are significant at 95% confidence level and F-values are

significant at 99% level $[F(2.31)(0.01) = 4.49]$, do not represent as good correlations as Eq. 3.21 and since no other variables were found to improve these correlations, it may be assumed that in vitro and in vivo actions of benzamide neuroleptics do not involve exactly the same mechanism and that the receptors in the two systems may be slightly different structurally from each other.

The in vitro activity data of another series of highly potent benzamide derivatives (S & R isomers), recently reported by Hogberg et al.[38] (Table 3.7) was also analysed. In their study, Högberg et al.[38] varied all 5 substituents, R^2, R^3, R^5, R^6, and R in structure VIII. Since R^2, R^5, R^6 were either OH or OCH_3 except in compound 18 where R^2 is NH_2 and in compound 19 where R^5 is NH_2, three dummy parameters I^2, I^5, I^6 were respectively used for them, each with a value of zero for OH/NH_2 and 1 for OCH_3. R was mostly C_2H_5 except in compound 11 and 17 where it is CH_2=CH-CH_2. So a dummy parameter IR was included with a value of zero for C_2H_5 and 1 for CH_2=CH-CH_2. Similarly, to include the effect of different isomeric forms (S or R) of compounds another indicator parameter I_s has been included with a value of 1 for S-isomer, -1 for R-isomer

and zero for recemic mixture, though there was no recemic mixture. Now the analysis was carried out using these indicator parameters and hydrophobic and electronic parameter of R^3.)

For the first 21 compounds of Table 3.7 which were reported[38] by Hogberg et al., the regression analysis revealed the correlation as expressed by Eq. 3.24. Eq.3.24 shows that IR^5, IR and $\sigma_R 3$ are not significant, consequently

$$\begin{aligned}-\log IC_{50} = {} & 7.866 - 2.720(\pm 0.613)IR^2 + 0.866(\pm 0.390)I_s \\ & -1.298(\pm 0.675)IR^6 + 2.314(\pm 1.907)\pi_R 3 \\ & -1.368(\pm 1.314)(\pi_R 3)^2 + 0.591(\pm 0.682)IR^5 \\ & +0.112(\pm 0.780)IR - 1.039(\pm 1.594)\sigma_R 3\end{aligned}$$

$$n=21,\ r=0.956,\ s=0.436,\ F(8,12)=10.72 \qquad \ldots(3.24)$$

the elimination of these parameters produced little effect on the significance of the correlation (Eq. 3.25). In Eq. 3.25, the square term of $\pi_R 3$ has also become insignificant,

$$\begin{aligned}-\log IC_{50} = {} & 8.190 - 2.548(\pm 0.633)IR^2 + 0.883(\pm 0.400)I_s \\ & -1.336(\pm 0.717)IR^6 + 1.535(\pm 1.457)\pi_R 3 \\ & -0.717(\pm 0.961)(\pi_R 3)^2\end{aligned}$$

$$n=21,\ r=0.931,\ s=0.487,\ F(5,15)=19.64 \qquad \ldots(3.25)$$

therefore dropping this too and the compound 19 whose calculated value from Eq. 3.25 largely differs from the observed one, a new correlation (Eq. 3.26) is obtained,

$$-\log IC_{50} = 8.566 - 2.677(\pm 0.609)IR^2 + 0.864(\pm 0.375)I_s - 1.418(\pm 0.675)IR^6 + 0.582(\pm 0.574)\pi_R 3$$

$$n=20,\ r=0.939,\ s=0.461,\ F(4,15) = 22.20 \quad ..(3.26)$$

where all the parameters are statistically quite significant [$F(4,15)(0.01) = 4.89$] . Inclusion of reference compounds 22-24 in Table 3.7 had led to Eq. 3.27.

$$-\log IC_{50} = 8.475 - 2.599(\pm 0.624)IR^2 + 0.842(\pm 0.398)I_s - 1.423(\pm 0.721)IR^6 + 0.620(\pm 0.605)\pi_R 3$$

$$n=23,\ r=0.921,\ s=0.500,\ F(4,18)= 22.26 \quad ...(3.27)$$

Now Eqs. 3.26 and 3.27 re-established that OH group (R^2) is essential for the activity of compounds through the formation of pseudoring A (Fig .1) by hydrogen bonding. Negative value of coefficient of IR^6 indicates that OCH_3 group at 6-position will be inferior to OH group. This may be due to the latter's ability to form stronger

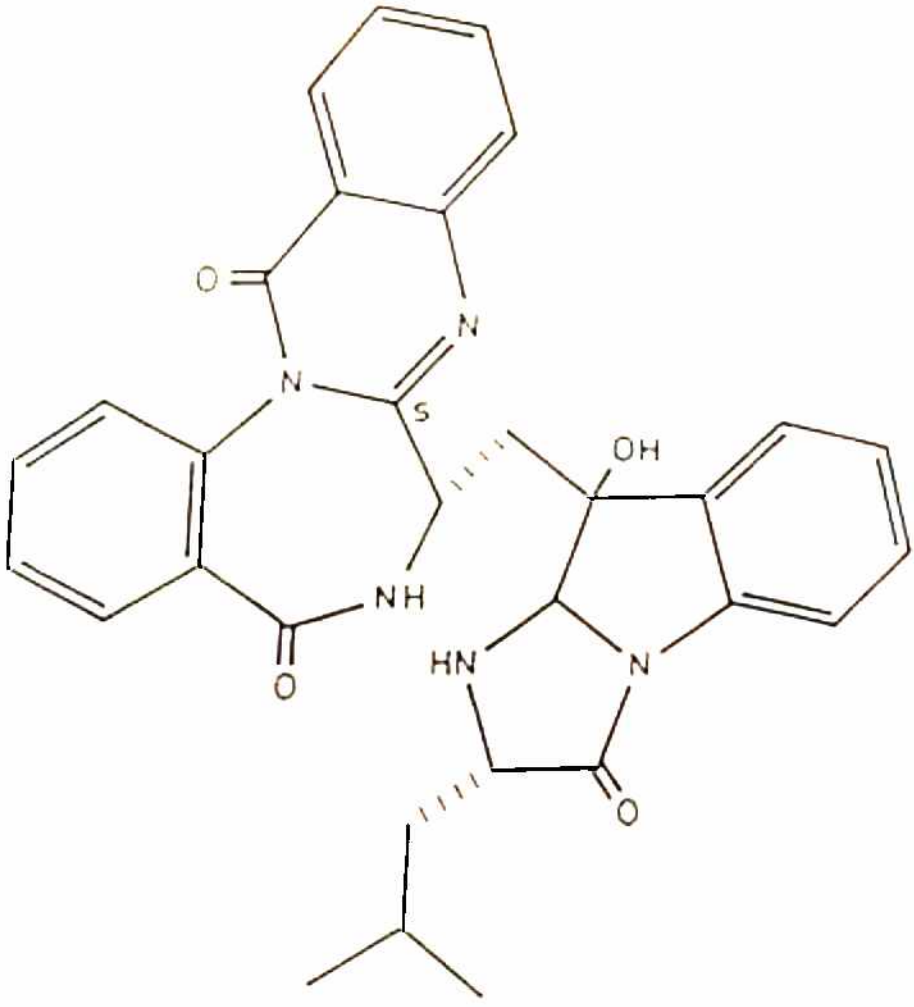
hydrogen bond in ring B than the former. The effect of hydrophobic character of R^3 substituent is not very encouraging. Further the complete absence of σ term for R^3 group denotes that neither the hydrophobic nor the electronic characteristic of R^3 group has any effect on the activity of the compound. This somewhat adverse to the observation that we have made in case of compounds of Table 3.6. This may be due to insufficient number of R^3 substituents in congeners of Table 3.7. The positive value of coefficient of I_s indicates that S-isomers will have better binding affinity than R-isomer or recemic mixture, establishing that stereoselectivity is also an important factor and interfering in the receptor binding.

C. Cholecystokinin Antagonists :

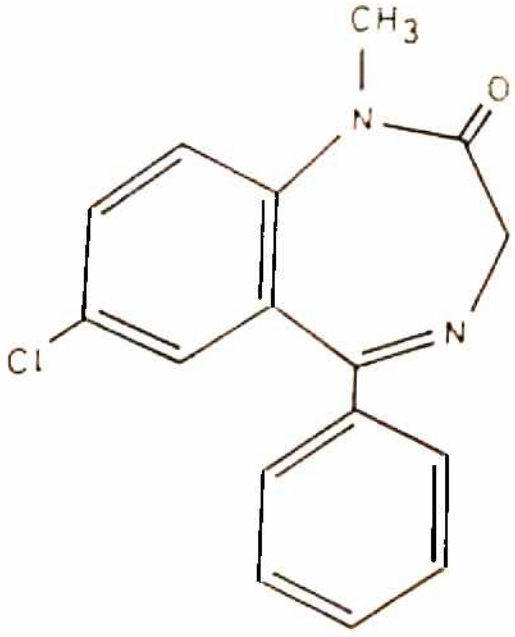
Cholecystokinin [CCK, H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2][40] is a gastrointestinal peptide hormone and putative central neurotransmitter. It is one of a growing list of peptides that play key roles in normal physiology as neurotransmitters and neurohormones. It has been implicated in the modulation of central dopaminergic transmission.[41,42] Activation of CCK receptors in peripheral tissues plays an import role in the control of pancreatic and biliary secretion, gallbladder contraction, and gut motility.[43,44] Whatsoever, the functions of CCK

and CCK-like other peptides are not clearly understood and little information is available for their involvement in disease states. The studies on their functions and pharmacological actions are hampered by the shortage of appropriate pharmacological tools, notably a selection of potent, stable, and selective antagonists.

For CCK, until recently, there have been no known agents ideally suited to elucidate its physiological role. The most widely used amino acid derived antagnoists, proglumide and benzotript, were of marginal potency and poor specificity.[45,47] A significant factor in the shortage of useful peptide antagonists was the peptide structure itself. However, recently a natural product asperlicin (IX) was found to be an effective antagonist of CCK in vitro and in vivo.[48,49] But asperlicin had liabilities as pharmacological or potential therapeutic agent, including lack of oral bioavailability, modest potency, and poor water solubility.[49,50] Whatsoever, this nonpeptidal antagonist provided a basis for the design of improved CCK antagonists.[51,52] Asperlicin contains elements of the 1,4-benzodiazepine ring system found in such anti-anxiety agent as diazepam (X). Since diazepam-like anti-anxiety agents are supposed



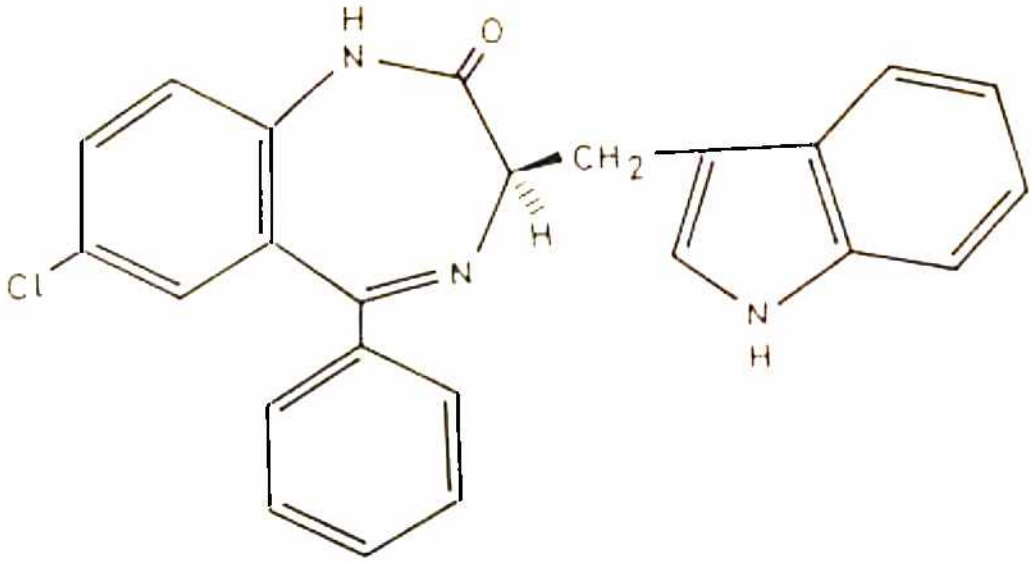
(IX)



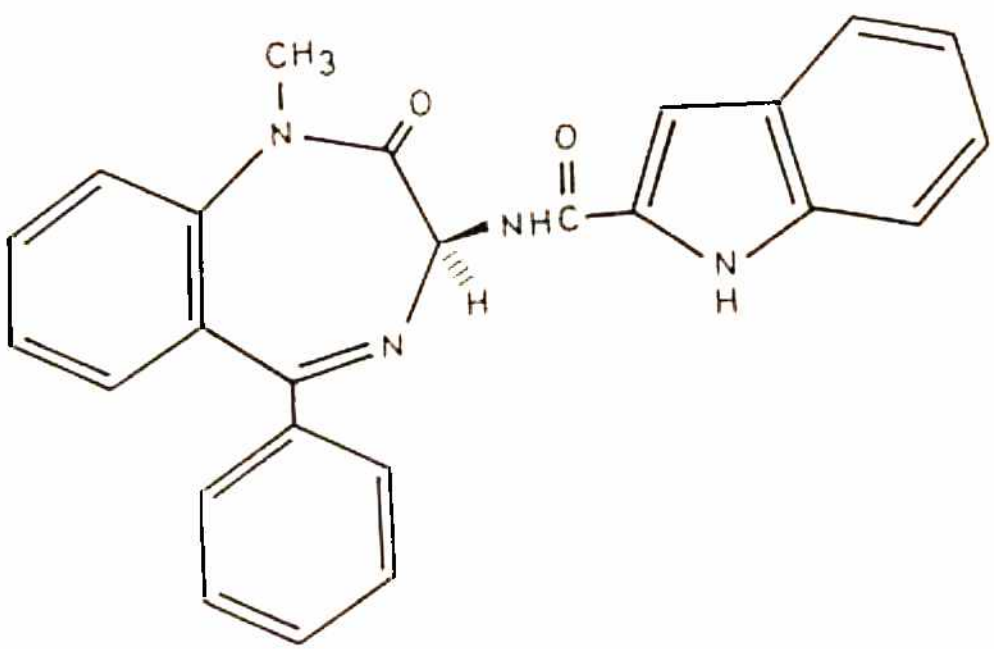
(X)

to be effective ligands for peptide receptors,[53,54] 5-phenyl-1, 4-benzodiazepine ring (e.g. X) was adopted as the basis for the design of improved CCK antagonists,[51,52] In selecting a pathway for elaboration of this basic ring, attention was focused on 3-substitution, and the form which 3-substituent might take was suggested by two factors-the presence in asperlicin of an indolinylmethyl group derived biosynthetically from L-tryptophan (L-Tryp) and the occurence of L-Tryp as a key amino acid in the sequence of CCK.[55] This rationale led to the synthesis of compounds XI and XII and their various analogs.[51,52,56] To provide a rationale to the selection of substituents at various rings of XI and XII in order to optimize the activity, a quantitative structure-activity relationship (QSAR) study has been undertaken. A part of such study is presented here. Further work is in progress.

The two initial series that were subjected to QSAR analysis consists of 3-amidobenzodiazepines(Tables 3.8 and 3.9). These compounds were synthesized and screened for their CCK antagonist activities in vitro by Evans et al.[56] The activities were measured in terms of IC_{50}, the molar concentration of compound required

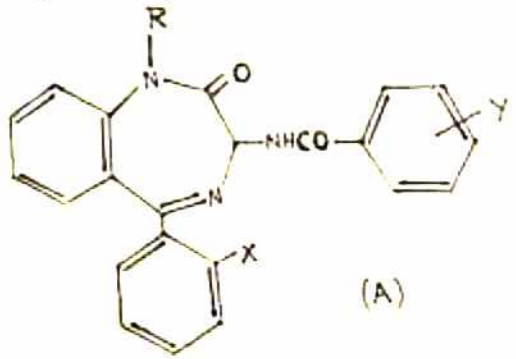


(XI)



(XII)

TABLE 3.8 : 3-(Benzoylamino)benzodiazepines (A) and Their Receptor Binding Affinities.



(A)

Compd	X	Y	R	3-stereo	-log IC_{50} CCK(pancreas)		CCK (brain)		Gastrin	
					Obsd*	Cald[a]	Obsd*	Cald[b]	Obsd*	Cald[c]
1	F	p-NC_2	H	RS	6.96	6.49	d	4.34	d	4.78
2	F	p-Cl	H	RS	8.22	7.21	4.40[e]	4.95	4.85	5.19
3	F	p-Cl	CH_3	RS	8.64	7.59	5.47	5.36	5.54	5.59
4	F	p-Cl	CH_2COOEt	RS	6.96	7.29	5.72	5.04	6.16	5.28
5	H	p-Cl	CH_3	RS	8.08	7.59	4.40	5.36	5.17	5.59
6	H	p-Cl	H	RS	7.39	7.21	d	4.95	5.09	5.19
7	F	p-Cl	CH_2COOH	RS	7.49	6.72	4.68	4.43	5.09	4.67
8	F	p-CF_3	H	RS	7.82	7.19	4.40	4.99	5.02	5.17
9	F	p-CH_3	H	RS	7.68	7.19	4.85	4.90	5.30	5.18
10	F	p-OCH_3	H	RS	7.02	6.81	d	4.57	5.27	4.97

Cont...

Table 3.8 cont..

Compd	X	Y	R	3-stereo	-log IC_{50} CCK(pancreas) Obsd*	CCK(pancreas) Cald[a]	CCK(brain) Obsd*	CCK(brain) Cald[a]	Gastrin Obsd*	Gastrin Cald[a]
11	F	p-$N(CH_3)_2$	H	RS	6.57	7.00	d	4.71	d	5.02
12	H	p-Br	CH_3	R	6.15	6.35	5.54	5.39	5.62	5.22
13	H	p-Br	CH_3	S	6.54	6.79	5.80	5.89	6.19	5.94
14	F	p-Cl	CH_3	RS	7.18	7.58	4.74	5.36	5.62	5.59
15	F	p-Cl	CH_3	S	8.60	8.81	5.54	5.36	6.19	5.95
16	H	p-Cl	CH_3	R	7.31	6.37	4.96	5.36	5.29	5.23
17	H	p-SCH_3	H	RS	6.66	7.20	4.64	4.92	5.10	5.19
18	H	p-F	H	RS	6.32	6.97	4.37	4.68	5.03	5.06
19	F	p-Cl	p-$ClC_6H_4(CO)$	RS	7.74	8.56	5.45[e]	6.39	6.26	6.62
20	H	p-CF_3	H	RS	6.62	7.19	4.19[e]	4.98	4.44	5.17
21	F	p-Br	CH_3	S	9.10	8.79	5.40	5.39	6.17	5.94
22	F	p-t-Bu	CH_3	S	7.72	7.62	5.10	5.13	5.62	5.20
23	F	p-I	CH_3	S	9.10	8.68	5.52	5.41	6.20	5.86
24	F	p-CN	CH_3	S	7.37	7.60	4.51	4.44	5.05	5.26
25	H	p-n-Pr	H	RS	5.44	6.69	4.92	4.93	4.10	4.75
26	H	p-Ph	H	RS	4.00	6.06	4.75	4.74	d	4.46

cont...

Table 3.8 contd..

Compd	X	Y	R	3-stereo	-log IC_{50}					
					CCK(Pancreas)		CCK(rabbit)		Gastrin	
					Obsd[a]	Calc[a]	Obsd[b]	Calc[b]	Obsd[c]	Calc[c]
27	H	p-n-C_5H_{11}	H	RS	4.57	4.95	5.10	4.91	4	4.71
28	H	p-t-Bu	H	RS	5.85	6.02	4.70	4.79	4.40	4.44
29	H	p-OH	H	RS	5.74	5.81	4.00	5.01	4.61	4.56
30	H	p-OH	H	RS	6.14	6.00	d	4.33	4.56	4.59
31	H	p-n-C_5H_{11}	CH_3	S	6.60	6.46	d	4.72	6	4.47
32	H	p-CF_3	CH_3	S	6.89	6.79	5.74	5.30	5.21	5.03
33	H	n-n-C_5H_{11}	CH_3	R	4.92	4.91	d	4.72	4	4.76
34	H	p-CF_3	CH_3	R	5.94	6.36	5.48	5.59	5.39	5.22
35	H	p-Br	CH_3	RS	9.00	7.57	5.62	5.39	5.77	5.58
36	F	p-CF_3	CH_3	RS	7.74	7.57	5.74	5.30	5.74	5.57
37	F	p-CF_3	CH_3	S	7.96	8.79	5.85	5.30	6.36	5.93
38	F	p-n-C_5H_{11}	CH_3	RS	5.96	5.23	d	4.72	d	4.12
39	F	p-n-C_5H_{11}	CH_3	S	6.16	6.46	4.00	4.72	4.32	4.45
40	F	p-t-Bu	CH_3	RS	7.00	6.40	5.64	5.13	5.19	4.84
41	F	3,4-di-OCH_3	H	RS	5.77	6.81(6.08)	d	4.57(4.77)	d	4.97(4.9

contd...

Table 3.8 contd..

Compd	X	Y	R	3-Stereo	-log IC_{50} CCK(pancreas)		CCK(brain)		Others	
					Obsd*	Cald[a]	Obsd*	Cald[a]	Obsd	Cald[a]
42	H	3,4-di-Cl	H	RS	7.54	7.30(7.54)	n	4.35(4.12)	4.00	7.12(6.72)
43	H	o-NH_2,p-Cl	CH_3	RS	5.05	7.50(5.64)	5.62	5.30(5.73)	5.72	5.19(5.61)
44	F	H	H	RS	5.82	6.84(6.15)	i	4.51(4.76)	4.53	4.97(4.93)
45	F	o-Cl	H	RS	5.26	4.94	d	4.29	4.74	4.67
46	H	o-Cl	H	RS	5.12	4.94	d	4.54	4.20	3.85
47	H	o-Cl	CH_3	S	5.77	6.23	4.08	4.21	4.19	4.62
48	H	o-Cl	CH_3	R	4.96	5.23	4.66	4.64	5.12	4.62
49	H	o-Cl	CH_3	RS	5.47	5.73	d	4.42	4.52	4.62
50	H	m-Cl	H	RS	7.09	7.54	4.12	4.12	4.72	4.72
51	F	m-Br	CH_3	S	8.46	8.78	5.45	5.33	6.12	6.19
52	H	m-SCF_3	H	RS	5.82	6.24	d	4.12	d	3.81
53	F	o-Br	CH_3	S	5.21	5.98	4.15	4.75	4.72	5.29
54	H	3,5-di-Cl	H	RS	6.30	6.31	d	4.12	3.74	3.85
55	F	m-I	CH_3	S	9.12	8.44	5.77	5.33	6.41	5.92
56	F	M-I	CH_3	R	7.82	7.44	5.62	5.76	5.76	5.92
57	F	o-I	CH_3	R	4.24	4.53	5.42	5.01	5.36	5.15

Contd...

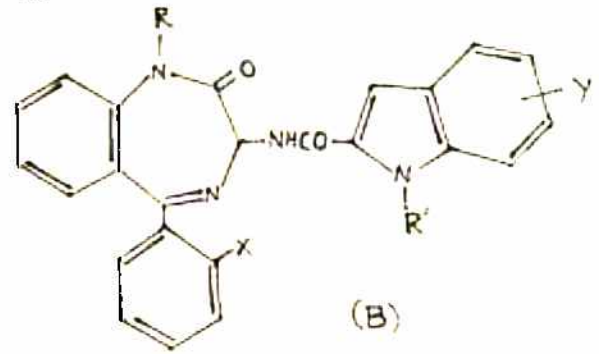
Table 3.8 contd..

Compd	X	Y	R	3-stereo	-log IC_{50} CCK (pancreas)		CCK (Brain)		Gastrin	
					Obsd*	Cald[a]	Obsd*	Cald[b]	Obsd*	Cald[c]
58	F	o-I	CH_3	S	6.10	5.53	4.35	4.98	4.46	4.16
59	F	o-Br	CH_3	R	5.05	4.98	5.23	5.14	5.24	5.29
60	F	o-Cl	CH_3	R	5.47	5.23	4.80	5.39	5.43	5.36
61	F	o-Cl	CH_2OCOEt	RS	d	5.70	5.49	5.07	5.89	5.56
62	H	o-I	CH_3	S	5.96	5.53	4.15	3.93	4.42	4.41

* Taken from Ref. 56;

[a] For compounds 1-44 from eq. 3.29 (data within parentheses are those calculated from 3.33). Data reproduced for compounds 45-62 are also from eq. 3.33. [b] For compounds 1-44 from eq. 3.32 (data within parentheses are those calculated from eq. 3.35). Data reproduced for compounds 45-62 are also from eq. 3.35. [c] For compounds 1-44 from eq. 3.31 (data within parentheses are those calculated from eq. 3.34). Data reproduced for compounds 45-62 are also from eq. 3.34. [d] Uncertain data, [e] Not used in the derivation of eq. 3.32 [f] This compound contains N-oxide on the endocyclic nitrogen at the 4-position in the seven-membered ring. However, it was very well accommodated in the correlation.

TABLE 3.9 : 3-[(2-Indolylcarbonyl)amino]benzodiazepines (B) and Their Receptor Binding Affinities.



(B)

Compd	X	R	R'	Y	3-stereo	-log IC_{50} CCK(pancreas) Obsd*	CCK(pancreas) Cald[a]	CCK(brain) Obsd*	CCK(brain) Cald[b]	Gastrin Obsd*	Gastrin Cald[c]
1	H	H	H	H	RS	8.33	8.70	5.10	5.64	5.40	6.15
2	F	H	H	H	RS	8.68	8.70	5.22	5.64	5.34	6.52
3	F	CH_3	H	H	RS	8.85	8.70	6.52	6.09	6.72	6.52
4	F	CH_3	CH_3	H	RS	8.89	8.70	6.00	5.47	5.30	5.69
5	F	CH_2COOH	H	H	RS	8.82	8.70	5.25	5.06	6.41	6.52
6	F	H	H	5'-F	RS	8.30	8.65	4.92	4.57	f	6.45
7	F	H	H	5'-Cl	RS	7.15	7.29	4.42	4.47	4.70	4.52
8	H	CH_3	H	H	RS	9.10	8.70	6.10	6.08	6.14	6.15
9	H	CH_3	CH_3	H	RS	8.85	8.70	4.82	5.47	5.70	5.31
10	H	CH_2COOH	H	H	RS	8.85	8.70	5.22	5.06	6.19	6.15
11	F	H	H	5'-Br	RS	6.92	6.63	4.30	4.41	f	3.58

Contd...

Table 3.9 contd...

Compd	X	R	R'	Y	3-stereo	-log IC_{50} CCK(Pancreas)		CCK(Brain)		Gastrin	
						Obsd*	Cald[a]	Obsd*	Cald[b]	Obsd*	Cald[c]
12	F	H	H	5'-OH	RS	7.15	7.45	4.80	5.26	4.54	4.74
13	F	H	H	5'-OCH_3	RS	6.70[d]	6.70	4.54[e]	5.45	f	6.52
14	F	H	CH_3	H	RS	6.33	6.70	5.15	5.02	6.20	5.69
15	H	CH_3	H	H	S	10.10	9.58	6.57	6.51	6.77	6.61
16	H	CH_3	H	H	R	8.08	7.82	5.43	5.66	5.85	5.69
17	F	CH_3	H	H	S	9.22	9.58	6.52	6.51	7.55	6.93
18	F	CH_3	H	H	R	7.72	7.82	5.96	5.66	6.62	6.06
19	F	CH_2COOt	H	H	RS	8.52	8.70	6.70[e]	5.74	6.92	6.52

*Taken from Ref. 56;

[a] From eq. 3.37 [b] From eq. 3.39 [c] From eq. 3.38 [d] Not included in the derivation of eq. 3.37 [e] Not included in the derivation of eqs. 3.39 and 3.40

[f] Uncertain data.

for half-maximal inhibition of binding of [^{125}I] CCK-33 or [^{125}I] CCK-8 (+) to CCK receptors in rat pancreatic or guinea pig brain tissues, or for half-maximal inhibition of binding of [^{125}I] gastrin to guinea pig gastric glands.[56] Efforts were made to correlate these activities by least square method with physicochemical parameters. The physicochemical parameters were mainly the Hansch hydrophobic constant π and the Hammett electronic constant σ (Table 3.10)

A close look at Table 3.8 that contains a large series of 3- (benzoylamino)benzodiazepines shows that a majority of compounds (compds 1-40) have only para substituent in the phenyl ring of their benzoylamino moiety. Compounds 45-62 are ortha or meta substituted, and 41-43 are disubstituted containing substituents at para and ortho or meta positions. Therefore, for QSAR analysis the whole series was divided into two groups- group one containing all the para substituted derivatives and group two containing ortho ro meta substituted derivatives. Compounds 41-43 and the unsubstituted one (compd 44) were included in both the groups.

TABLE 3.10 : Physicochemical Parameters Used in QSAR Study

Substituent	π	σ_p [a]	σ_m [a]
H	0.00	0.00	0.00
NO_2	-0.28	0.78	0.71
F	0.14	0.06	0.34
Cl	0.71	0.23	0.37
Br	0.86	0.23	0.39
I	1.12	0.18	0.35
CH_3	0.56	-0.17	-0.07
CF_3	0.88	0.54	0.43
OCH_3	-0.02	-0.27	0.12
$N(CH_3)_2$	0.18	-0.83	-0.15
SCH_3	0.61	0.00	0.15
SCF_3	1.44	0.50	0.40
CN	-0.57	0.66	0.56
C_6H_5	1.96	-0.01	0.06
$n\text{-}C_3H_7$	1.55	-0.13	-0.07
$t\text{-}C_4H_9$	1.98	-0.20	-0.10
$n\text{-}C_5H_{11}$	2.5	-0.15	-0.08
NH_2	-1.23	-0.66	0.02
OH	-0.67	-0.37	0.12
CH_2COOH	-0.72		
$CH_2COOC_2H_5$	0.13		
$p\text{-}ClC_6H_5CO$	1.99		

[a] Used for only Y-substituent in both the series of congeners (Tables 3.8 and 3.9) . In Table 3.9 the 5'-position of indolyl ring was treated as meta position.

For a complete regression analysis, two dummy parameters were to be defined, one for the configuration of the compound at 3-position and one for the X-substituent in 5-phenyl ring. The parameter I_s, meant for the configuration, was given a value of -1,0, or +1, depending upon whether the compound had R-, RS- or S-configuration. Since the X-substituent was either F or H, the other parameter I_x, meant for it, was given a value of 1 for F and zero for H. Now for compounds of group 1, a multiple regression analysis revealed Eq.3.28, correlating the CCK(pancreas) [CCK-A] receptor binding affinity of compounds with physicochemical and dummy parameters.

$$\begin{aligned} -\log IC_{50}(CCK\text{-}A) = {} & 1.048(\pm 0.716)\pi_{Y,p} - 0.704(\pm 0.306)(\pi_{Y,p})^2 \\ & + 0.589(\pm 0.685)\pi_R + 0.224(\pm 0.898)\sigma_Y \\ & + 1.140(\pm 0.488)I_s + 0.350(\pm 0.545)I_X \\ & + 6.605 \end{aligned}$$

$$n=44,\ r=0.810,\ s=0.83,\ F(6,37) = 11.44,\ \pi_{Y,p}(Opt)=0.74 \qquad \ldots(3.28)$$

From the point of view of confidence intervals, the coefficients of the electronic parameter σ_Y and the dummy

parameter I_v appear to be highly insignificant, hence if these two parameters are dropped, the correlation does not loose much of its significance (Eq.3.29). The coefficient of π_R also does not appear to be statistically

$$-\log IC_{50}(CCK\text{-}A) = 1.044(\pm 0.712)\pi_{Y,p} - 0.735(\pm 0.293)(\pi_{Y,p})^2 + 0.680(\pm 0.720)\pi_R + 1.220(\pm 0.471)I_s + 6.835$$

$$n=44,\ r=0.800,\ s=0.83,\ F(4,39)=15.96,\ \pi_{Y,p}(Opt)=0.71 \quad \ldots(3.29)$$

significant, but if it is also dropped, the correlation becomes slightly inferior (Eq.3.30). Therefore, Eq.3.29 is taken to express the best correlation between the CCK-A receptor binding affinity and the physicochemical parameters. Likewise, Eq.3.31 expresses the best correlation for the inhibition of gastrin binding to

$$-\log IC_{50}(CCK\text{-}A) = 1.204(\pm 0.721)\pi_{Y,p} - 0.779(\pm 0.301)(\pi_{Y,p})^2 + 1.295(\pm 0.485)I_s + 6.960$$

$$n=44,\ r=0.774,\ s=0.87,\ F(3,40)=19.43 \quad \ldots(3.30)$$

$$-\log IC_{50}(gastrin) = 0.604(\pm 0.369)\pi_{Y,p} - 0.445(\pm 0.186)(\pi_{Y,p})^2 + 0.720(\pm 0.330)\pi_R + 0.358(\pm 0.257)I_s + 4.984$$

$$n=36,\ r=0.801,\ s=0.40,\ F(4,31)=12.57,\ \pi_{Y,p}(Opt)=0.68 \quad \ldots(3.31)$$

$$-\log IC_{50}(CCK\text{-}B) = 0.765(\pm 0.493)\pi_{Y,p} - 0.349(\pm 0.208)(\pi_{Y,p})^2 + 0.726(\pm 0.524)\pi_{R} + 4.582$$

$$n=30,\ r=0.692,\ s=0.44,\ F(3,26)=7.35,\ \pi_{Y,p}(Opt)=1.09 \qquad \ldots(3.32)$$

gastric glands. A poor correlation (Eq.3.32) was however obtained for the inhibition of CCK binding to the receptor in brain [CCK-B], even though compds 1,19 and 20, because of their marked deviation, were not included in the regression. Our all attempts had failed to improve this correlation. The parameter I_s had also to be dropped in this correlation because of its high statistical insignificance. It is to be noted however that, unlike in eq. 3.29 , π_R is significant not only in Eq. 3.31 but also in Eq. 3.32.

For group 2, the best correlations obtained were:

$$-\log IC_{50}(CCK\text{-}A) = 6.153 - 1.706(\pm 0.457)\pi_{Y,o} + 3.798(\pm 1.820)\pi_{Y,m} - 2.591(\pm 1.403)(\pi_{Y,m})^2 + 0.790(\pm 0.484)I_R + 0.500(\pm 0.309)I_s$$

$$n=21,\ r=0.955,\ s=0.47,\ F(5,15)=31.33,\ \pi_{Y,m}(Opt)=0.73 \qquad \ldots(3.33)$$

$$-\log IC_{50}(\text{gastrin}) = 4.193 - 0.510(\pm 0.403)\pi_{Y,o} + 1.729(\pm 1.692)\pi_{Y,m} - 1.386(\pm 1.382)(\pi_{Y,m})^2 + 0.792(\pm 0.448)I_R + 0.433(\pm 0.408)I_X$$

$$n=20,\ r=0.883,\ s=0.40,\ F(5,14)=7.06,\ \pi_{Y,m}(\text{Opt})=0.62 \qquad ..(3.34)$$

$$-\log IC_{50}(\text{CCK-B}) = 4.120 - 0.674(\pm 0.379)\pi_{Y,o} + 0.645(\pm 0.529)I_X + 0.782(\pm 0.968)I_R - 0.217(\pm 0.261)I_s$$

$$n=14,\ r=0.877,\ s=0.38,\ F(4,9)=6.65 \qquad ..(3.35)$$

In eq.3.35, I_R and I_s are statistically not significant, but if they are dropped, there occurs a drastic change in the significance of the correlation (eq. 3.36). I_R is a dummy parameter used in place of π_R, as in case of group 2 compounds, the R-substituent is mostly either CH_3 or H. For only one compound, 61, this R is different (CH_2COOEt), but it shows the effect like CH_3. I_R has thus the value of 1 for CH_3(or CH_2COOEt) group and zero for H group.

$$-\log IC_{50}(\text{CCK-B}) = 4.694 - 0.641(\pm 0.433)\pi_{Y,o} + 0.834(0.570)I_X$$

$$n=14,\ r=0.777,\ s=0.45,\ F(2,11)=7.61 \qquad ..(3.36)$$

For compounds of Table 3.9 which are 3- (2-indolyl-carbonyl) amino benzodiazepines, the best correlation obtained were as follows

$$-\log IC_{50}(CCK\text{-}A) = 8.703 - 2.798(\pm 0.703)(\pi_{Y,5'})^2 + 0.880(\pm 0.322)I_s$$

$$n=18,\ r=0.936,\ s=0.30,\ F(2,15) = 49.40 \quad ..(3.37)$$

$$-\log IC_{50}(gastrin) = 6.147 - 3.979(\pm 2.050)(\pi_{Y,5'})^2 - 0.834(0.794)I_{R'} + 0.462(\pm 0.606)I_s + 0.378(\pm 0.658)I_X$$

$$n=16,\ r=0.824,\ s=0.55,\ F(4,11) = 4.23 \quad ...(3.38)$$

$$-\log IC_{50}(CCK\text{-}B) = 5.637 + 0.803(\pm 0.502)\pi_R - 3.141(1.520)\sigma_{Y,5'} + 0.425(\pm 0.418)I_s - 0.614(\pm 0.556)I_{R'}$$

$$n=17,\ r=0.887,\ s=0.38,\ F(4,12) = 11.11 \quad ..(3.39)$$

In Eq. 3.38 , I_s and I_X are not statistically significant at 95% confidence interval, but if they are dropped, the significance of the correlations is considerably reduced (Eq. 3.40). In the derivation of Eq. 3.37, compd

$$-\log IC_{50}(\text{gastrin}) = 6.354 - 3.620(\pm 2.086)(\pi_{Y,5'})^2 - 0.786(\pm 0.844)I_{R'}$$

$$n=16,\ r=0.742,\ s=0.60,\ F(2,13)=7.34 \qquad ..(3.40)$$

13 was not included, and in the derivation of Eq. 3.39 compds 13 and 19 were not included, as all these compounds proved outliers in respective correlations. The too low observed activities than expected for compound 13 can be attributed to the steric effect produced by the bulky OCH_3 group at 5'-position but how $R=CH_2COOEt$ in compound 19 with a low π value gives higher observed activity is hard to explain.

Now all these correlations for both series lead to establish primarily the importance of hydrophobic character of substituents particularly at benzoyl or indolyl ring. In case of para substituted benzoyl analogs, Eqs. 3.29 and 3.31 exhibit that π is a dominant factor to account for the activity. However, since these equations are quadratic in π, the π puts a limit on the activity, and from both the equations it is obtained that $\pi \approx 0.7$ will lead to the optimum activity. The occurrence of π_R in both the equations

further reveals that a hydrophobic R-group at benzodiazepine ring will also increase the activity. Equations 3.29 and 3.31 have also I_s parameter denoting that an S-configuration of the group at 3-position will lead to an increase in the activity, while an R-configuration will lead to the decrease in the activity. The optimum activity is suggested to be possessed by the racemic mixture of the isomers. Thus Eqs. 3.29 and 3.31 which are related to the inhibition of peripheral CCK and gastrin bindings, respectively, lead to identical conclusions, suggesting that peripheral CCK and gastrin receptors behave in a similar way with their substrates or ligands. Equation 3.32 which is related to CCK binding in brain neither represents as good correlations as Eqs. 3.29 and 3.31 nor does it contain all the parameters that the latter have. Hence, the CCK receptor in brain does not appear to behave in the same manner as the peripheral CCK receptor. This difference in peripheral and brain CCK receptors is more prominantly exhibited by Eqs. 3.33 and 3.35 which were derived for ortho and/or meta substituted benzoyl benzodiazepines. In case of CCK-A binding inhibition, the hydrophobic characters of both ortho and meta substituents appear to play a role, while in case of CCK-B binding inhibition is exhibited only the role of hydrophobic

character of ortho substituent. It leads to suggest that in case of the latter, the meta substituents of the ligands do not find the opportunity to interact with the receptor, thus brain CCK receptor is structurally quite different from peripheral one. Equations 3.33 and 3.34 which are almost similar, except that in the former appears the I_s parameter and in the latter the I_x instead, demonstrate however again that peripheral CCK and gastrin receptors may be structurally similar.

In Eqs. 3.33-3.35 the negative coefficient of $\pi_{Y,o}$ suggests that increase in the lipophilic character of ortho substituent will lead to a decrease in the activity. This indicates the size effect of ortho substituent. It can be assumed that probably the active site of receptors is not able to accommodate the bigger substituent at this position of the ligand.

Equation 3.33 shows the configurational effect on inhibition of CCK-A binding, while Eq. 3.34 shows, instead, the positive effect of a methyl group at 5-phenyl ring in the inhibition of gastrin binding. This latter effect is absent in the former case but is present in case of

inhibition of CCK-B binding (Eq.3.35) The configurational effect in inhibition of CCK-B binding is also shown to be the reverse of that in CCK-A binding inhibition.

Although, the lipophilic character of all ortho, meta, and para substituents have been shown to account for the activity, an analysis of the differences in the predicted and observed activities of compds 41-43, which are disubstituted presents an intersting picture. In case of CCK-A binding inhibition, there is a large difference between the observed data and the data predicted by Eq. 3.29(related to para analogs), while there is comparatively a much smaller difference between the observed data and those predicted by Eq. 3.33 (related to ortho/meta derivatives). It shows that in disubstituted analogs, the physicochemical properties of ortho or meta substituent account better for the activity than those of para substituent. But this appears to be true particularly for the inhibition of CCK-A binding, as the differences in predictions by para and ortho/meta related equations are not so high in gastrin or CCK-B binding inhibition (Table 3.8), It can be therefore assumed that in case of interaction of disubstituted ligands with CCK-A receptor, ortho or meta

substituent would have better orientation towards the active site than para substituent. Such an orientation difference of disubstituted analogs does not surface in case of their interactions with the gastrin or CCK-B receptor.

The QSAR analysis of 3-[(2-indolylcarbonyl)amino] benzodiazepines (Table 3.9) which has revealed Eqs. 3.37-3.40 presents a more interesting picture. The occurrence of only squared term of π and not the unsquared one for the substituent in indolyl ring at 5'-position with negative sign in Eqs. 3.37 and 3.38 suggests that no substituent at this position, whether hydrophilic or lipophilic will be tolerated for the interaction of the ligand with CCK-A or gastrin receptor. These equations exhibit that the optimum activity of any compound would be associated with only H substituent for which π is zero. The complete absence of $\pi_{Y,5'}$ in Eq. 3.39 indicates that 5'-substituent is totally irrelevant for the interaction of the compound with CCK-B receptor. The I_R, parameter used for the methyl group at N_1 -position of indolyl group does not appear in Eq. 3.37 and in Eqs. 3.38 and 3.39 is this with negative sign, suggesting that no substituent will be tolerated at this

position too of the indolyl group. Also the lipophilic character of R-group at N_1-position of benzodiazepine ring was found to be significant only in case of inhibition of CCK-B binding (Eq. 3.39) and not in other two cases. Similarly, the involvement of X-substituent of 5-phenyl ring was expressed (Eq.3.38) only in the binding with gastrin receptor and not with peripheral or brain CCK receptor. Surprizingly enough, an electronic effect of 5'-substituent had surfaced (Eq. 3.39) in case of CCK-B binding inhibition. Since in no other correlations, any electronic effect was observed, the appearance of σ^- in Eq. 3.39 might be only a chance appearance.

From what has been discussed so far, we draw the following conclusions regarding the interactions of CCK antagonists with peripheral CCK, gastrin, and brain CCK receptors and propose three different interaction models as represented by Figs. 3.2 and 3.3.

1. The peripheral CCK receptor and the gastrin receptor are not much different structurally and behave almost in the same manner with their ligands. The brain CCK receptor is however quite different from these two and thus its mode of interaction with its ligands also differs.

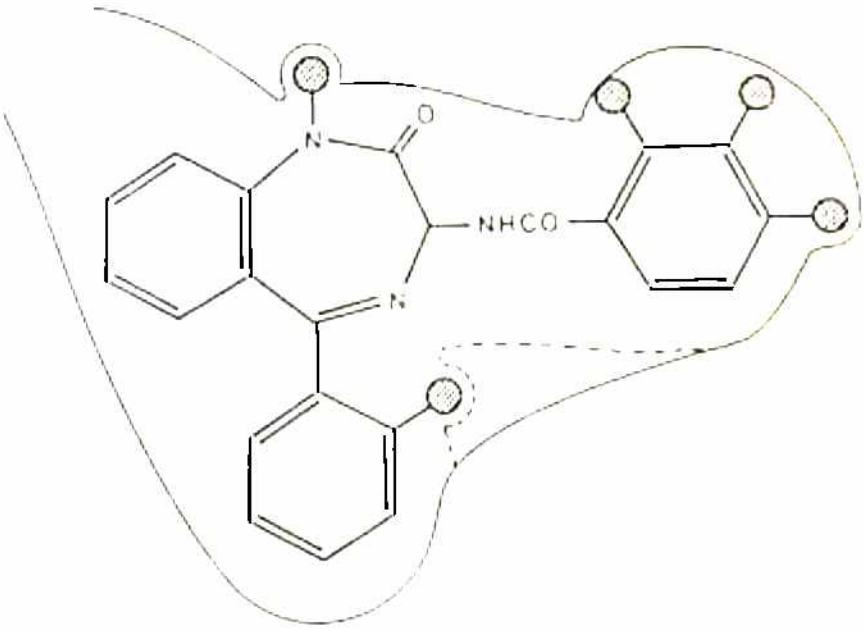


Fig.3.2. Hypothetical model of interactions of 3-(benzoylamino)benzodiazepines with peripheral CCK and gastrin receptors. The dotted portion belongs to the gastrin receptor.

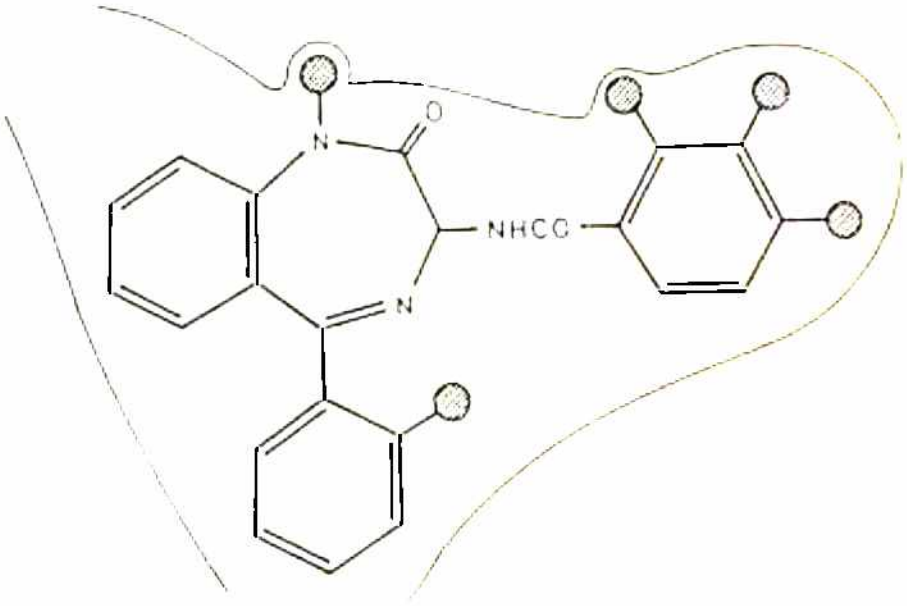


Fig.3.3. Hypothetical model of interaction of 3-(benzoylamino)benzodiazepines with brain CCK receptor.

2. The peripheral CCK and gastrin receptors seem to possess a very large hydrophobic area which can be easily approached and fully covered by unsubstituted indolyl group of indolyl antagonists, but which is approachable by benzoyl group of benzoylantagonists only when it is substituted. Further, when the benzoyl group is disubstituted, i.e., at para and ortho/meta positions, the substituent at the latter seems to get better opportunity to come in contact with the active site than that at the former. No such large hydrophobic site appears to be present in brain CCK receptor.

3. There seems to be present another small hydrophobic region, a little away from the larger one, in peripheral CCK and gastrin receptors to accommodate the substituent at N_1-position of benzodiazepine ring of antagonists. Such a small hydrophobic region to accommode N_1-substituent appears to be present in brain CCK receptor also. A third hydrophobic region can also be assumed to be present in gastrin receptor, and not in others, to engulf the X-substituent of 5-phenyl ring.

Figures 3.2 and 3.3 represent the models of interactions, based upon the above assumptions, for 3- (benzoylamino) benzodiazepines with the receptors. Identical models can be drawn for 3- (indolylcarbonyl)amino benzodiazepines.

References:

1. Hollister, L.E., Pharmacology of Benzodiazepines; Usdin, E., et al., Eds; Verlag Chemie, Weinheim, p-29 (1983).

2. Squires, R.F., Braestrup, C., Nature (London), 266, 732(1977).

3. Möhler, H., Okada T., Science, 198, 849 (1977).

4. Braestrup, C., Squires, R.F., Eur. J. Pharmacol., 48, 263 (1978).

5. Haefely, W., Kyburz, E., Gerecke, M., Möhler, H., in 'Advances in Drug Research', Testa, B., Ed., Academic Press, London, Vol. 14, p-165 (1985).

6. Olsen, R.W., Annu. Rev. Pharmacol. Toxicol., 22, 245 (1982).

7. Martin, I.L., TIPS, 5, 343 (1984).

8. Braestrup, C., Honore, T., Nielsen, M., Petersen, E.N., Jensen, L.H., Biochem. Pharmacol., 33, 859(1984).

9. Marangos, P.J., Paul, S.M., Goodwin, F.K., Skolnick,P., Life Sci., 25, 1093(1979).

10. Braestrup, C., Nielsen, M., Olsen, C.E., Proc. Natl. Acad. Sci., USA, 77, 2288(1980).

11. Skolnick, P., Marangos, P.J., Goodwin F.K., Edwards, M., Paul, S., Life Sci., 23, 1473(1978).

12. Asano, T., Spector, S., Proc. Natl. Acad. Sci., USA,76 , 977(1979).

13. Cecchi, L., Melani, F., Palazzino, G., Filacchioni, G., Martini, C., Pennacchi, E., Lucacchini, A., Farmaco, Ed. Sci., 40, 509 (1985).

14. Melani, F., Cecchi, L., Palazzino, G., Filacchioni, G., Martini, C., Pennacchi, E., Lucacchini, A., J. Med. Chem., 29, 291 (1986).

15. Melani, F., Cecchi, L., Palazzino, G., Filacchioni,G., Martini, C., Pennacchi, E., Lucacchini, A., J. Pharm. Sci., 75, 1175 (1986).

16. Primofiore, G., Marini, A.M., Settimo, F.D., Martini, C., Bardellini, A., Giannaccini, G., Lucacchini, A., J.Med. Chem., 32, 2514 (1989).

17. Goldstein, A., Lowney, L.I., Pal, B.K., Proc. Natl. Acad. Sci., USA, 68, 1742 (1971).

18. Dax, E.M., Partilla, J.S., Gregerman, R.I., J. Receptor Res., 2, 267 (1981).

19. Cain, M., Weber, R.W., Guzman, F., Cook, J.M., Barker, S.A., Rice, K.C., Crawley, J.N., Paul, S.M., Skolnick, P., J. Med. Chem., 25, 1081 (1982).

20. Lippke, K.P., Müller, W.E., Schunack, W.G., J. Pharm. Sci., 74, 676(1985).

21. Kelley, J.L., McLean, Ed.W., Ferris, R.M., Howard, J.L., J.Med. Chem., 32, 1020(1989).

22. Kelley, J.L., McLean, Ed.W., Linn, J.A., Krochmal, M.P., Ferris R.M., Howard, J.L., J. Med. Chem., 33, 196(1990).

23. Davies, L.P., Cook, A.F., Poonian, M., Taylor, K.M., Life Sci., 26, 1089(1980).

24. Geller, I., Seifter, J., Psychopharmacologia, 1, 482(1960).

25. Pollard, G.T., Howard, J.L., Psychopharmacology, 62, 117 (1979).

26. Langer, S.Z., Arbilla, S., Pharmacol. Biochem., Behav., 29, 763 (1988).

27. Watzen, F., Baker, R., Engelstoff, M., Herbert, R., Macleod, A., Knight, A., Merchant, K., Moseley, J., Saunders, J., Swain, C.J., Wong, E., Springer, J.P., J. Med. Chem., 32, 2282 (1989).

28. Petersen, E.N., Jensen, L.H., Diejer, J., Honore, T., Pharmacopsychiatry, 19, 4 (1986).

29. (i) Mertz, W.A., et al., Abst. From IV World Congress of Biological Psychiatry, Sept. 1985 Philadelphia, Raven Press, New York, p 408; (ii) Haefely, W., Clin. Neuropharmacol, 7, (Supl.1), 670 (1984).

30. Bondo Hansen, J., Engelstoft, M., Honre, T., Soebrog, W.F., Schmiechen, R., IX International Symposium on Medicinal Chemistry, Berlin, Sept. 1986.

31. Möhler, H., Burkard, N.P., Keller, H.H., Richards, J.G., Haefely, W., J. Neurochem., 37, 714 (1981).

32. Seeman, P., Biochem. Pharmacol., 26, 1741 (1977).

33. Florvall, L., Oegren, S.O., J. Med. Chem., 25, 1280(1982).

34. Florvall, L., Persson, M.L., Oegren, S.O., Acta. Pharm. Suec., 20, 365 (1983).

35. Oegren, S.O., Hall, H., Köhler, C., Magnusson, O., Lindbom, L. O., Angeby-Möller, K., Florvall, L., Eur. J. Pharmacol., 102,459 (1984).

36. de Paulis, T., Kumar, Y., Sohansson, L., Ramsby, S., Florvall, L., Hall, L.H., Angeby-Möller, K. Ögren, S.O., J. Med. Chem., 28, 1263 (1985).

37. de Paulis, T., Kumar, Y., Johansson, L., Ramsby, S., Hall, L.H., Salemark, M., Angeby-Möller, K., Ögren, S.O., J. Med. Chem., 9, 61 (1986).

38. Högberg, T., Bengtsson, S., de Paulis, T., Johansson,L., Ström, P., Hall, H., Ögren, S.O., J. Med. Chem., 33, 1155 (1990).

39. van de Waterbeemd, H. Testa, B., J. Med. Chem., 26, 203 (1983).

40. Ondetti, M.A., Pluscec, J., Sabo, E.F., Sheehan, J.T., Williams, N., J.Am. Chem. Soc. 92, 195 (1970).

41. Hokfelt, T., Rehfeld, J.F., Skirboll, L., Ivemark, B., Goldstein, M., Markey, K., Nature (London) 285, 476(1980).

42. Chang, R.S.L., Lotti, V.J., Martin, G.E., Chen, T.B., Life Sci. 32, 871(1983).

43. Kerstens, P.J.S.M., Lamers, C.B.H.W., Jansen, J.B.M.J., De Jong, A.J.L., Hessels, M., Hafkenscheid, J.C.M. Life Sci. 36, 565(1985).

44. Morley, J.E. , Life Sci. 30, 479 (1982).

45. Hahne, W.F., Jensen, R.T., Lemp, G.F., Gardner, J.D., Proc. Natl. Acad. Sci.(U.S.A.) 78, 6304 (1981).

46. Gardner, J.D., Jensen, R.T., Am. J. Physiol. 246 G471(1984).

47. Crawley, J.N., Stivers, J.A., Hommer, D.W., Skirboll,L.R., Paul, S.M., J. Pharmacol.Exp. Ther. 236, 320 (1986).

48. Liesch, J.M., Hensens, O.D., Springer, J.P., Chang, R.S.L., Lotti, V.J., J. Antibiot. 38, 1638 (1985).

49. Chang, R.S.L., Lotti, V.J., Monaghan, R.L., Birnbaum,J., Stapley, E.O., Goetz, M.A., Albers-Schonberg, G., Patchett, A.A., Liesch, J.M., Hensens, O.D., Springer,J.P., Science (Washington, D.C.) 230, 177 (1985).

50. Lotti, V.J., Cerino, D.J., Kling, P.J., Chang, R.S.L., Life Sci. 39, 1631 (1986).

51. Evans, B.E., Bock, M.G., Rittle, K.E., Dipardo, R.M., Whitter, W.L., Veber, D.F., Anderson, P.S., Freidinger, R.M., Proc. Natl. Acad. Sci.(U.S.A.) 83, 4918 (1986).

52. Evans, B.E.,Rittle, K.E., Bock, M.G., Dipardo, R.M., Freidinger, R.M., Whitter, W.L., Gould, N.P., Lundell, G.F., Homnick, C.F., Veber, D.F., Anderson, P.S., Chang, R.S.L., Lotti, V.J., Cerino, D.J., Chen, T.B., King, P.J., Kunkel, K.A., Springer, J.P., Hirshfield, J., J. Med. Chem. 30, 1229 (1987).

53. Guidotti, A., Furchetti, C.M., Corda, M.G., Konkel, D., Bennett, C.D., Costa, E., Proc. Natl. Acad. Sci. (U.S.A.) 80, 3531 (1983).

54. Alho, H., Costa, E., Ferrero, P., Fujimoto, M., Cosenzamurphy. D., Guidotti, A., Science (Washington,D.C.) 229, 179(1985).

55. Rajh, H.M., Mariman, E.C.M., Tesser, G.L., Nivard, R.J.F., Int. J. Pept. Protein Res. 15. 200(1980).

56. Evans, B.E., Rittle, K.E., Bock, M.G., DiPardo, R.M., Freidinger, R.M., Whitter, W.L., Lundell,G.F., Veber, D.F., Anderson, P.S., Chang, R.S.L., Lotti, V.J., Cerino, D.J., Chen, T.B., Kling, P.J., Kunkel, K.A., Springer, J.P., Hirshfield, J., J. Med. Chem. 31 , 2235 (1988).

CHAPTER IV

QSAR Studies on Some Local Anesthetics.

Local Anesthetics :

Local anesthetics are drugs that block nerve conduction when applied locally to nerve tissue in appropriate concentrations. They act on any part of the nervous system and on every type of nerve fiber. For example when they are applied to the motor cortex, impulse transmission from that area stops, and when they are injected into the skin they prevent the initiation and the transmission of sensory impulses. A local anesthetic in contact with a nerve trunk can cause both sensory and motor paralysis in the area innervated. The great practical advantage of the local anesthetics is that their action is reversible.

Although it is widely accepted that local anesthetics exert their pharmacological action by interacting with the cell membranes, the sites of their action in membranes are still not clearly resolved. Some authors suggest that local anesthetics interact with membrane phospholipids[1,2] and some suggest that they interact with proteins associated with the membrane.[3,4] It has also been proposed that local anesthetics act by causing perterbation of the bulk membrane structure.[5,6] However some recent investigations have indicated that local anesthetics interact with specific receptors in the membranes.[7,8] It was long back suggested that local anesthetics increase the surface pressure of the lipid layer that constitutes the nerve membrane and thus close the pores through which ions move.[9]

Most of the theories discussed above however broadly speak of the mecnanism of the blockade of the nerve conduction and thus no satisfactory theory has been established to discuss the mechanism of drug action at the molecular and receptor level. To propose anything on this aspect of local anesthetics would require a thorough knowledge of their respective activity relationship. We have taken here two completely different series of local anesthetics to make a QSAR study. Although some pharmacologists consider that local anesthetics do not exactly fall in the group of CNS agents still some classify them under the categories of drugs acting on CNS,[10] because drugs used to treat diseases of peripheral organs but also affecting tne brain are considered to be CNS agents.

Local anesthetics, in addition to blocking conduction in nerve axons in peripheral nervous system, interefere with the function of all organs in which conduction or transmission of impulses occurs. Thus they have important effects on the CNS. Following absorption local anesthetics may cause stimulation of the CNS, producing restlessness and tremor that may lead to clonic convulsions. In general, the more potent the anesthetic the more readily convulsions may be produced. Alterations of CNS activity are thus predictable

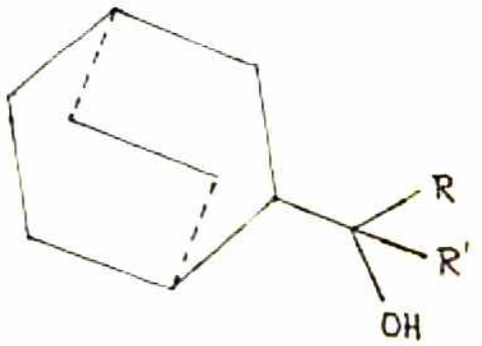
from the local anesthetic agent in question and the blood concentration achieved. Some local anesthetics may produce loss of consciousness that is preceded only by symptoms of sedation, while few are having effect on mood and behavior.[10]

(i) Mono-and Diaryl-2-Quinuclidinylcarbinols:

Irrespective of the site of action, the lipophilicity has been found to be an important factor for the local anesthetic activity. For a variety of local anesthetics, such as paracaines,[11] benzyl alcohols,[12] procaine analogues,[13] ethers of dihydroxyarenes,[14] carbalinates,[15] lidocaine analogues,[16,17] and certain nonspecific compounds,[18,19] the activity has been shown to be a significant function of the hydrophobic parameter, log P.

Table 4.1 lists a series of arylquinuclidinylcarbinols with their local anesthetic activity (LA) studied by Nelson et al.[20] The activity mentioned is relative to that of propranolol, a prominent local anesthetic. For all the nineteen compounds, the activity data were found to be correlated with calculated log P values (calculated as mentioned in Chapter II) as shown by Eq. 4.1

Table 4.1 : Local Anesthetic Activity and Related Physicochemical Parameters of Arylquinuclidinylcarbinols



S.No.	R	R'	log P	V_w[a] (10^{2} Å^3)	LA	
					Obsd[b]	Cald.Eq.4.2
1	H	Ph(threo)	1.78	1.031	0.18	0.33
2	H	Ph(erythro)	1.78	1.031	0.25	0.33
3	H	2-thiazolyl	0.14	0.896	0.80[c]	-0.10
4	H	1-Me-2-imidazolyl	1.41	0.975	0.61	0.23
5	H	1,6-methano [10] annulen-2-yl	3.14	1.589	0.99	0.68
6	H	2-furyl	1.00	0.924	0.09	0.13
7	H	4-pyridyl	0.31	0.980	0.08	-0.05
8	H	p-ClC_6H_4	2.49	1.196	0.43	0.51
9	H	p-MeC_6H_4	2.31	1.185	0.23	0.47
10	H	t-Bu	2.42	0.783	0.13	0.50
11	Ph	Ph	3.36	1.543	0.85	0.74

.....2

Table 4.1 contd...

S.No.	R	R^1	log P	V_w[a] (10^2 Å^3)	LA	
					Obsd[b]	Calcd, Eq. 4.2
12	2-furyl	2-furyl	1.79	1.329	0.27	0.33
13	p-MeC_6H_4	p-MeC_6H_4	4.42	1.851	0.86	1.01
14	p-ClC_6H_4	p-ClC_6H_4	4.78	1.873	1.04	1.11
15	p-$OMeC_6H_4$	p-$OMeC_6H_4$	3.02	2.013	0.54	0.65
16	2-MeO-5-BrC_6H_3	2-MeO-5-BrC_6H_3	4.74	2.425	0.92	1.10
17	p-$OMeC_6H_4$	2-MeO-5-BrC_6H_3	3.88	2.219	1.20	0.86
18	2-MeO-5-BrC_6H_3	p-$OMeC_6H_4$	3.88	2.219	1.23	0.86
19	Ph	2-thiazolyl	1.72	1.466	0.25	0.31

[a] Calculated for only substituents according to Ref. 10 of Chapter II;

[b] Taken from Ref. 20 ; [c] Not included in deriving Eq. 4.2

$$LA = 0.198(\pm 0.103)\log P + 0.072$$
$$n=19,\ r=0.70,\ s=0.29,\ F(1,17) = 16.36 \qquad (4.1)$$

In this equation, compound 3 was found to be misfit, as it has very low log P value but comparatively very high activity. This anomalous behavior of compound 3 can be explained by assuming that its cationic form - all the commonly used local anesthetics are supposed to exist mostly as positively charged tertiary or secondary ammonium cation - must be having very high dissociation constant and thus furnishing, unlike others, a comparatively high concentration of neutral form which diffuses through the cellular membrane. Therefore, if this compound is excluded from the regression analysis, a significant correlation (Eq. 4.2) is obtained, where the

$$LA = 0.260(\pm 0.088)\log P + 0.132$$
$$n=18,\ r=0.84,\ s=0.23,\ F(1,16) = 38.75 \qquad (4.2)$$

lipophilic factor, being highly significant at 95% confidence interval, is found to account for about 71% ($r^2 = 0.71$) of the variance in the activity. Also the F-value in Eq. 4.2 is significant at 99% level [$F(1,16)(0.01) = 8.53$]. Almost similar correlation (Eq. 4.3) were obtained with the van der Waals volume (V_w). However, since the van der Waals volume

$$LA = 0.659(\pm 0.227)V_w - 0.409$$
$$n=18,\ r=0.84,\ s=0.23,\ F(1,16)=37.91 \qquad (4.3)$$

had significant correlation with log P, one can say that it is only the lipophilicity that plays a major role in the anesthetic activity of this new class of local anesthetics. This study therefore provides another example to support the role of lipophilicity in the action of local anesthetics. in this study, the square term of any parameter was not found to be significant.

(ii) Inhibitory Effects of Local Anesthetics on Batrachotoxin-Elicited Sodium Flux and Phosphoinositide Breakdown and Batrachotoxin Binding to Sodium Channels:

It has also been proposed that local anesthetics act by causing perturbations of the bulk membrane structure.[5,6] Their effects on volatage-sensitive sodium channels have appeared to be fundamental to their local anesthetic activity.[21] They inhibit not only stimulus-evoked opening of sodium channels, but also opening of channels by agents such as batrachotoxin (BTX). Such local anesthetic activity appears to be correlated with inhibitory effects of drugs on binding of a radioactive BTX analog to sites on the sodium channel.[8,22] Recently, it

has been shown that agents like BTX, which enhance sodium channel function, can induce phosphoinositide breakdown in brain synaptoneurosomes.[23,24] Nishizawa et al.[25] therefore made a study on the mutual correlations of the effects of certain local anesthetics on BTX-elicited sodium flux, BTX-elicited phosphoinositide breakdown, and binding of [^{3}H] BTX-A 20α-benzoate to sodium channels in guinea pig cerebral cortical synaptoneurosomes. This study aims to investigate physichochemical properties of molecules that govern these effects of local anesthetics and hence to discuss the mechanism of drug action.

The data on the inhibitory effects of local anesthetics as listed in Table 4.2 on BTX-elicited sodium flux, BTX-elicited phosphoinositide breakdown, and binding of [^{3}H] BTX-A 20α-benzoate to sodium channels in guinea pig cerebral cortical synaptoneurosomes were obtained by Nishizawa et al.[25] The IC_{50} in each case refers to the minimum inhibitory concentration of drug leading to 50% effect. The physicochemical parameters, log P and V_w, listed in Table 4.2 were calculated as discussed in Chapter II. Log P characterizes the hydrophobic character of the molecule, while V_w refers to the molecular size of the drug and constitutes an important parameter to describe the dispersion force or steric effects

Table 4.2 : V_w and log P Values and Inhibitory Effects of Local Anesthetics on BTX-elicited Sodium Flux, BTX-elicited Phosphoinositide Breakdown, and Binding of [^{3}H] BTX-A 20α-benzoate to Sodium Channels in Guinea pig Cerebral Cortical Synaptoneurosomes.

S.No.	Compound	V_w (10^2 Å^3)	Log P	-log IC_{50}(Sod. flux)		-log IC_{50}(Phos. Breakdown)		-log IC_{50}(BTX binding)	
				Obsd*	Cald, Eq. 4.8	Obsd*	Cald, Eq. 4.9	Obsd*	Cald, Eq. 4.10
1	Dibucaine	3.233	1.030	6.00	5.47	5.68	5.48	5.85	5.70
2	Tetracaine	2.532	0.690	5.37	4.63	5.35	4.57	5.47	4.79
3	Euprocin	3.676	0.755	5.54	5.95	5.48	6.02	6.13	6.15
4	Bupivacaine	2.809	3.550	4.89	5.01	4.85	4.76	5.27	5.49
5	Dimethisoquin	2.693	2.315	6.15[a]	4.93	5.48	4.80	5.47	5.27
6	Quinacrine	3.476	6.840	5.17	5.03	4.36	4.29	5.48	5.57
7	Phenacaine	2.802	3.130	5.16	5.04	4.64	4.83	5.77	5.44
8	QX-572	2.861	-2.730	4.04	4.05	4.08	3.97	5.41[c]	3.68
9	QX-314	2.631	-1.875	3.92	4.11	4.07	4.06	4.01	3.90
10	Diphenhydramine	2.506	4.200	4.35	4.59	4.00	4.22	5.22	5.05
11	Lidocaine	2.297	0.295	3.85	4.30	3.96	4.22	3.62	4.40
12	Diphenhydramine methiiodide	2.636	-0.580	3.48[a]	4.50	3.14[b]	4.48	4.19	4.50

.....2

Table 4.2 contd..

S.No.	Compound	V_w (10^2 Å^3)	log P	-log IC_{50}(Sod. flux)		-log IC_{50}(Phos. breakdown)		-log IC_{50}(STX binding)	
				Obsd*	Cald. Eq. 4.8	Obsd.*	Cald. Eq. 4.9	Obsd.*	Cald. Eq. 4.10
13	Piperocaine	2.475	2.940	4.50	4.67	4.02	4.45	4.89	5.06
14	Prilocaine	2.085	-0.640	4.07	3.86	3.37	3.77	4.27	3.83
15	Cocaine	2.640	1.240	4.32	4.82	2.85[b]	4.75	4.31	5.05
16	Etidocaine	2.703	2.065	5.09	4.94	4.92	4.82	5.46	5.26

566

* Taken from ref. 25

[a] Not used in deriving Eq. 4.8 [b] Not used in deriving Eq. 4.9 [c] Not used in deriving Eq. 4.10

in drug receptor interaction.

When we first attempted to correlate the data on sodium flux with log P and V_w, we found that V_w was a better descriptor of the activity (Eq.4.4) than log P(Eq.4.5). However, neither of Eqs. 4.4 and 4.5 reflects any satisfactory

$$-\log IC_{50} = 1.047(\pm 0.958)V_w + 1.874$$
$$n=16,\ r=0.53,\ s=0.71,\ F(1,14)=5.48 \qquad (4.4)$$

$$-\log IC_{50} = 0.142(\pm 0.174)\log P + 4.550$$
$$n=16,\ r=0.42,\ s=0.76,\ F(1,14)=3.04 \qquad (4.5)$$

correlation and therefore we used both the parameters together, as they were not mutually correlated ($r = 0.30$), and obtained Eq.4.6 which exhibited a slightly improved correlation.

$$-\log IC_{50} = 0.876(\pm 0.991)V_w + 0.098(\pm 0.168)\log P + 2.202$$
$$n=16,\ r=0.60,\ s=0.69,\ F(2,13) = 3.35 \qquad (4.6)$$

The 95% confidence interval values in Eq.4.6 exhibit that neither of V_w and log P is statistically significant, as they are larger than the corresponding coefficient of the parameters. However, when we made the equation quadratic in log P, a satisfactory correlation was obtained (Eq. 4.7),

where each parameter, though marginally was significant at 95% confidence interval and F-value was also significant at 95% level [$F(3,12)(.05)=3.49$]. A further improvement in the correlation was obtained (Eq. 4.8) when compounds 5 and 12, which were outliers, were excluded. Similar correlations were obtained for the data on phosphoinositide breakdown

$$-\log IC_{50} = 1.138(\pm 0.905)V_w + 0.261(\pm 0.215)\log P - 0.048(\pm 0.046)(\log P)^2 + 1.608$$
$$n=16,\ r=0.74,\ s=0.60,\ F(3,12) = 4.93 \qquad (4.7)$$

$$-\log IC_{50} = 1.146(\pm 0.690)V_w + 0.197(\pm 0.169)\log P - 0.041(\pm 0.036)(\log P)^2 + 1.610$$
$$n=14,\ r=0.82,\ s=0.45,\ F(3,10)=6.20 \qquad (4.8)$$

(eq.4.9) and BTX binding (Eq.4.10) with exclusion of one or two compounds, as indicated in the Table in each case. In Eqs. 4.8 - 4.10 all, r and s values are almost same indicating that

$$-\log IC_{50} = 1.259(\pm 0.674)V_w + 0.182(\pm 0.163)\log P - 0.056(\pm 0.035)(\log P)^2 + 1.283$$
$$n=14,\ r=0.84,\ s=0.44,\ F(3,10)=7.35 \qquad (4.9)$$

$$-\log IC_{50} = 1.172(\pm 0.685)V_w + 0.334(\pm 0.228)\log P - 0.051(\pm 0.042)(\log P)^2 + 1.617 \qquad (4.10)$$

$$n=15,\ r=0.85,\ s=0.45,\ F(3,11)= 7.97$$

all the three inhibitory activities of local anesthetics are equally well affected by the molecular size and the hydrophobic character of the molecules. This was as expected, because the inhibitory effect on sodium flux was shown to be linearly related with other two effects.[25] Since the inhibition of BTX binding to sodium channels appears to be the main cause of the local anesthetic action of drugs, one may concentrate on finding the nature of interaction of drugs with BTX receptor. The dependence of inhibitory effect on V_w suggests that the drug-receptor interaction must involve the dispersion interaction and the dependence on log P indicates that over all effect should be governed by the ability of molecules to reach the receptor site crossing the lipoid membrane of the cell. The existence of an outlier (compound 8) in the correlation of BTX binding inhibitory effect may be explained assuming that compound required a conformational change in the receptor for its effect which could not be accounted for by V_w or log P. However, the existence of different outliers in other two cases, both related to the inhibition of BTX binding, is hard to explain.

So far as the role of dispersion interaction in the action of local anesthetics is concerned, Agin et al.[26] had assumed that all molecules acting as local anesthetics interacted with the receptor in a similar way and that the controlling factor in the interaction could be expressed in terms of molecular properties. This assumption of Agin et al. was verified by Handa et al.[27] by correlating the local anesthetic action of a series of nonspecific local anesthetics, including some of the compounds mentioned in Table 4.2, with molecular polarizability (α) and V_w, as shown by Eqs. 4.11 and 4.12, respectively. MBC in the equations refers to the minimum concentration in an external solution necessary

$$\log (\text{MBC}) = 3.66 - 0.08(0.002)\alpha$$
$$n=39,\ r=0.986,\ s=0.34 \qquad (4.11)$$

$$\log (\text{MBC}) = 3.765 - 2.650(0.123)V_w$$
$$n=39,\ r=0.962,\ s=0.568 \qquad (4.12)$$

to completely block excitibality. Thus the role of dispersion interaction in the action of local anesthetic appears to be an important factor and for such interaction a receptor is needed which may be either BTX-receptor or some other kind of the receptor. According to a hypothesis, the local anesthetic molecules combine with an acetylcholine

10. Ritchie, J.M., Green, N.M., in 'The Pharmacological Basis of Therapentics", Gilman, A.G., Goodman, L.S., Rall, T.W., Murad, F. , Eds., MackMillan, New York, p-322 (1985).

11. Hansch,C., Fujita, T., J. Am. Chem.Soc., 86, 1616(1964).

12. Hansch, C., Kerley, R., J. Med.Chem., 13, 957 (1970).

13. Johnson, C.L., Goldstein, M.A., Schwartz, A., Mol. Pharmacol., 9, 360(1973).

14. Zaagsma, J., Nauta, W.Th., J.Med.Chem., 17, 507(1974).

15. Pešàk, M., Kopecký, E., Borovanský, A., in "QSAR in Design of Bioactive Compounds; Kucher, M., Ed., J.R. Proms. Science, Spain, p-209 (1984).

16. Courrière, Ph., Paubel, J.P., Nivière, P., Foussard-Blanpin, O., Eur. J. Med. Chem., 13, 121 (1978).

17. Ehrhardt, J.D., Rouot, B., Schwartz, J., Eur. J. Med. Chem., 13. 235(1978).

18. Casanovas, A.M., Labat, C., Courrière, Ph., Oustrin, J., Eur. J. Med. Chem., 17, 333(1982).

19. Casanovas, A.M., Malmary-Bebot, M.F., Courrière, Ph., Oustrin, J., Biochem. Pharmacol., 32, 2715 (1983).

20. Nelson, P.H., Strosberg, A.M., Untch, K.G., J. Med. Chem., 23, 180(1980).

21. Catterall, W.A., Trends Pharmacol.Sci., 8, 57(1987).

22. Creveling, C.R., McNeal, E.T., Daly, J.W., Brown, G.B., Mol. Pharmacol., 176, 475 (1983).

23. Gusovsky, F., Daly, J.W., FEBS Lett., 199, 107(1986).

24. Gusovsky, F., Hollingsworth, E.B., Daly, J.W., Proc. Natl. Acad. Sci., USA, 83, 3003 (1986).

25. Nishizawa, Y., Gusovsky, F., Daly, J.W., Mol.Pharmacol., 34, 707 (1988).

26. Agin, D., Hersh, L., Holtzman, D., Proc. Natl. Acad. Sci., USA., 53, 952 (1965).

27. Handa, A., Bindal, M.C., Prabhakar, Y.C., Gupta, S.P., Indian J. Biochem. Biophys., 20, 318(1983).

28. Bartels, E., Nachmansohn, D., Biochem. Z., 342, 359(1965).

29. Casanovas, A.M., Labat, C., Courrière, Ph., Oustrin, J., Biochem. Pharmacol., 34, 3187 (1985).

receptor in the neuronal membrane,[28] while another theory suggests that local anesthetics elicit their effect through the inhibition of cytochrome c oxidase.[19,29]

References :

1. Kelusky, E.C., Smith, I.C.P., Mol.Pharmacol., 26, 314(1984).

2. Eftink, M.R., Puri, R.K., Ghahramani, M.D., Biochim. Biophys. Acta. 813, 137(1985).

3. Weber, M., Changeux, J.P., Mol. Pharmacol., 10, 35(1974).

4. Trudell, J.R., Anesthesiology, 46, 5(1977).

5. Seeman, P., Pharmacol. Rev., 24, 583(1972).

6. Lee, A.G., Nature, 262, 545 (1976).

7. Hille, B., in, 'Theories of Anesthesia; General Purturbations Versus Specific Receptors, Progress in Anesthesiology', Fink, B.R., Ed., Raven, New York,Vol. 2, p.1(1980).

8. Postma, S.W., Catteral, W.A., Mol. Pharmacol. 25, 219(1984).

9. Shanes, A.M., Pharmacol. Rev., 8, 57(1987).

10. Ritchie, J.M., Green, N.M., in 'The Pharmacological Basis of Therapentics", Gilman, A.G., Goodman, L.S., Rall, T.W., Murad, F. , Eds., MackMillan, New York, p-322 (1985).

11. Hansch,C., Fujita, T., J. Am. Chem.Soc., 86, 1616(1964).

12. Hansch, C., Kerley, R., J. Med.Chem., 13, 957 (1970).

13. Johnson, C.L., Goldstein, M.A., Schwartz, A., Mol. Pharmacol., 9, 360(1973).

14. Zaagsma, J., Nauta, W.Th., J.Med.Chem., 17, 507(1974).

15. Pešák, M., Kopecký, E., Borovanský, A., in "QSAR in Design of Bioactive Compounds; Kucher, M., Ed., J.R. Proms. Science, Spain, p-209 (1984).

16. Courrière, Ph., Paubel, J.P., Nivière, P., Foussard-Blanpin, O., Eur. J. Med. Chem., 13, 121 (1978).

17. Ehrhardt, J.D., Rouot, B., Schwartz, J., Eur. J. Med. Chem., 13, 235(1978).

18. Casanovas, A.M., Labat, C., Courrière, Ph., Oustrin, J., Eur. J. Med. Chem., 17, 333(1982).

19. Casanovas, A.M., Malmary-Bebot, M.F., Courrière, Ph., Oustrin, J., Biochem. Pharmacol., 32, 2715 (1983).

20. Nelson, P.H., Strosberg, A.M., Untch, K.G., J. Med. Chem., 23, 180(1980).

21. Catterall, W.A., Trends Pharmacol.Sci., 8, 57(1987).

22. Creveling, C.R., McNeal, E.T., Daly, J.W., Brown, G.B., Mol. Pharmacol., 176, 475 (1983).

23. Gusovsky, F., Daly, J.W., FEBS Lett., 199, 107(1986).

24. Gusovsky, F., Hollingsworth, E.B., Daly, J.W., Proc. Natl. Acad. Sci., USA, 83, 3003 (1986).

25. Nishizawa, Y., Gusovsky, F., Daly, J.W., Mol.Pharmacol., 34, 707 (1988).

26. Agin, D., Hersh, L., Holtzman, D., Proc. Natl. Acad. Sci., USA., 53, 952 (1965).

27. Handa, A., Bindal, M.C., Prabhakar, Y.C., Gupta, S.P., Indian J. Biochem. Biophys., 20, 318(1983).

28. Bartels, E., Nachmansohn, D., Biochem. Z., 342, 359(1965).

29. Casanovas, A.M., Labat, C., Courrière, Ph., Oustrin, J., Biochem. Pharmacol., 34, 3187 (1985).