QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON SOME BENZODIAZEPINE RECEPTOR LIGANDS

THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

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Under the supervision of

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CERTIFICATE

This is to certify that the thesis entitled **"QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON SOME BENZODIAZEPINE RECEPTOR LIGANDS"** and submitted by **P. ANITHA**, ID.No. **94PHXF006** for the award of the **Ph.D.** degree of the Institute, embodies the original work done by her under my supervision.

Date 13.11.1998

Signature in full of the Supervisor

(Dr. S.P. Gupta)
Professor
Chemistry Group

To

my uncle

Shri. Bhanuprasad

for all that he is to me

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PREFACE

Long ago it was proposed that the biological activity of a compound is a function of its chemical structure. Today, biological activity is considered as a function of physicochemical properties. With this concept, structure-activity relationships (SAR) are developed when a set of physicochemical properties of a group of congeners are found to explain variations in biological responses of those compounds. This resulted in discovery, examination, and interpretation of SAR in a more systematic way, which led to the introduction of quantitative structure-activity relationship (QSAR) studies.

The discovery of benzodiazepines and their receptors have opened a new era in the discovery and characterization of drugs acting on the central nervous system. The BZR, however, no longer signifies a receptor that selectively interacts with ligands belonging only to the BZ class of compounds. A variety of non-benzodiazepine series of compounds have been identified as endogenous ligands for BZRs. Therefore, it became essential to study the actual mechanism of interaction of compounds with benzodiazepine receptors. Quantitative structure-activity relationship studies provide a deeper insight into the mechanism of drug-receptor interactions, hence attempts have been made to make QSAR studies on some non-benzodiazepine series of ligands of varying molecular structures. The study also may be of great help in rationalizing the drug design.

This thesis contains three Chapters. Chapter 1 presents an introduction to benzodiazepine receptor (BZR) ligands, and also a discussion on QSAR, its applications and limitations. Chapter 2 discusses

the significance of different physicochemical parameters used in the correlation study and the methods of their calculation, and Chapter 3 embodies the results and discussion of our QSAR studies made on a variety of non-benzodiazepine series of BZR ligands.

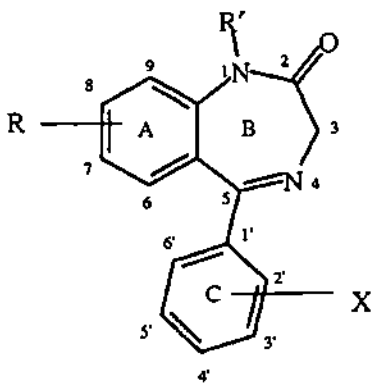
CONTENTS

Chapter 1

Introduction

1.1 Benzodiazepine Receptor (BZR) and Ligands

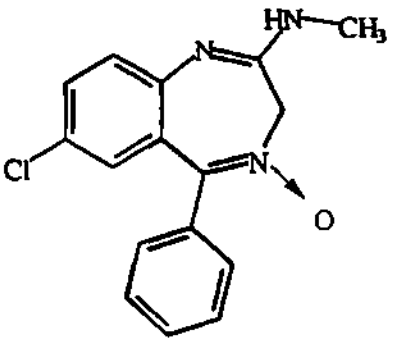
Benzodiazepines (BZs) (1) are widely used as centrally acting therapeutic agents.[1] They can be used in the pharmacotherapy of anxiety and related emotional disorders, in the treatment of sleep disorders, status epileptics and other convulsive states. They also act as centrally acting muscle relaxants and can be the drugs of choice for medication and for inducing agents in anesthesiology. Thus, the discovery of BZs and their receptors opened a new era in the discovery and characterization of drugs acting on central nervous system (CNS).



1

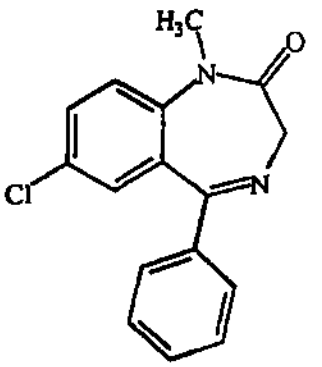
The pioneering and explorative synthetic work by Sternbach in early days of the BZ era led to the development of compound 2 with its unusual pharmacological profile. While its structure was studied by Sternbach and Reeder,[2-5] its sophisticated pharmacological tests were done by Randall and his staff.[6,7] It was given the generic name chlordiazepoxide and was launched under the trade name Librium®. The

synthesis of this compound was rapidly followed by its derivative diazepam (3), which was given the trade name Valium®



2

A number of related compounds were synthesized by Sternbach and his group after the success of these two drugs.



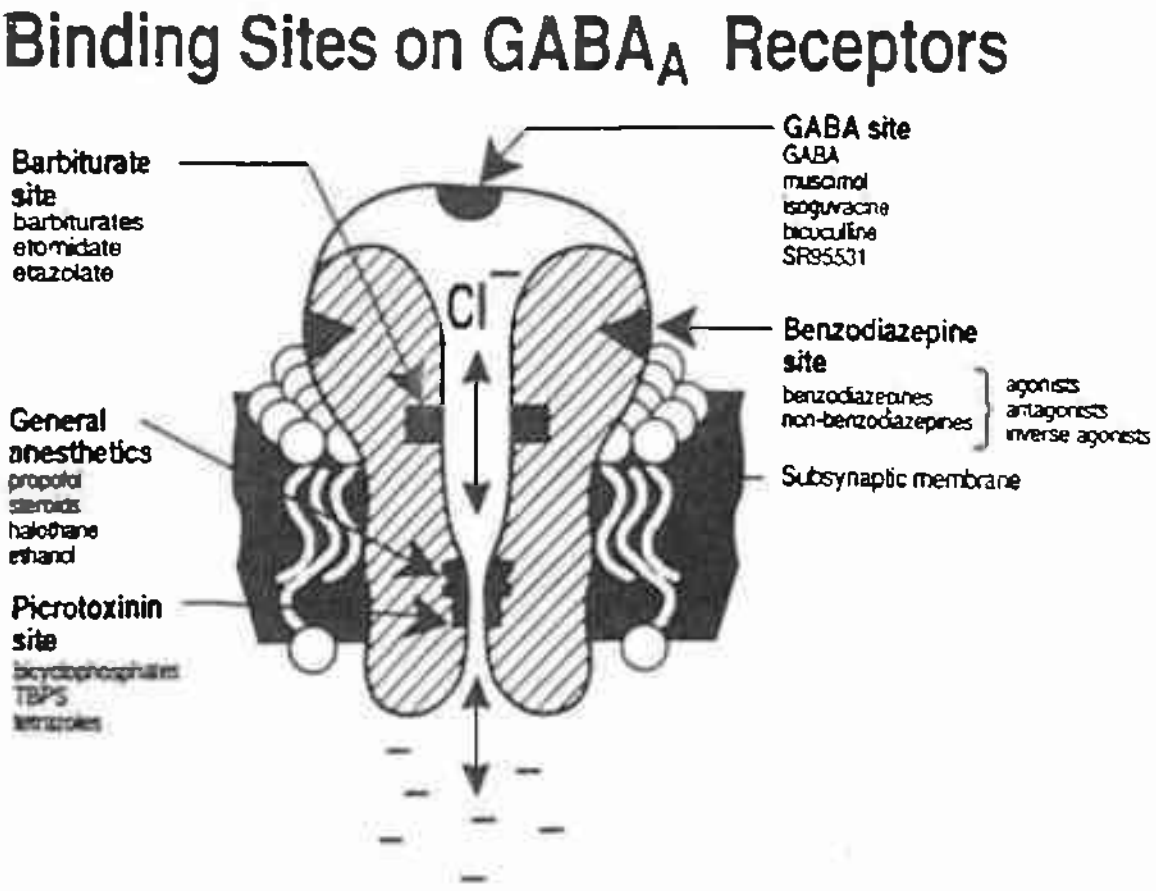
3

By the end of 1983, about 35 benzodiazepine drugs were available for therapy. However, the mechanisms of action of these unusually well tolerated and broadly effective drugs remained obscure for years, as the discovery and characterization of benzodiazepine receptors (BZRs) from the brain tissue came much later than the synthesis of chlordiazepoxide or diazepam.[8,9] The recognition of the BZRs was rapidly followed by the development of the *in vitro* binding assay which was capable of

expeditiously screening a large number of compounds regardless of the structural type. [10,11]

The finding that the BZR is coupled to the γ-aminobutyric acid (GABA) receptor and is part of the $GABA_A$–controlled chloride ion channel ($BZR/GABA_A/Cl^-$) supramolecular complex led to the discovery that BZs are not directly responsible for their observed biological activity but act as modulators of GABA binding to its receptor, which then directly alters the gating of the GABA dependent transmembrane chloride ion channel.[12,13] GABA acts on at least two different receptor types: $GABA_A$ and $GABA_B$.[14-18] $GABA_A$ receptors are part of a supramolecular complex that include the benzodiazepine, the barbiturate, the picrotoxin, and the ethanol receptor sites and also gates a chloride *ionophore as shown in* Figure (1.1), whereas $GABA_B$ receptors are found in both CNS locations and peripheral tissues. It was shown that BZs bind with high affinity and specificity to $GABA_A$ receptors, activation of which increases chloride conductance and inhibits neuronal activity by hyperpolarization and depolarization block.

The benzodiazepine receptors may be, like other pharmacological receptors, proteins, glycoproteins, or nucleic acids. However, little is known about the chemical structure of BZ binding sites. It has been proposed that tyrosinal residue in or very close to the binding site plays a crucial role in the recognition and binding of ligands.[19] It has also been concluded, however, that histidine might be involved as an essential contact residue.[20] Additionally, BZRs have also been found to possess glycoprotein qualities, where carbohydrate moieties are supposed to affect



Figure(1.1): Schematic diagram of the $GABA_A$ receptor channel with the various known binding sites

the biological response of BZ ligands, but it has not been decided yet whether the carbohydrate moieties are involved in the binding of BZs or whether they influence the conformation of the binding site.[21] The BZR, however, no longer signifies a receptor that selectively interacts with ligands belonging only to the BZ class of compounds.

Certain purines, cyclopyrrolones, triazolopyridazines, phenylquinolinones, β-carbolines, and some other structurally different non-BZ molecules have been identified as endogenous ligands for BZR.[22] Also, there are BZs that interact highly specifically with a completely different receptor, such as tifluadom, with the opiate κ receptor. [23-25]

1.1.1 *Types of BZR Ligands*

Three different types of ligands have been identified for BZR; they have been termed by Haefely as full agonists, full antagonists and full inverse agonists.[26] The first and third types act as allosteric modulators of the $GABA_A$ receptor complex, producing mirror biological effects, and the second type acts as a true antagonist at the receptor for the former, but shows no effect on the binding of GABA (Figure 1.2).[27] Therefore, the ligands that exert a positive cooperative effect on GABA binding to its receptor complex, resulting in a full biological response over the complete, agonist profile, i.e., positive intrinsic efficacy, are known as full agonists, and the ligands that exert a negative cooperative effect on GABA binding to its receptor and, when compared to agonists, show a

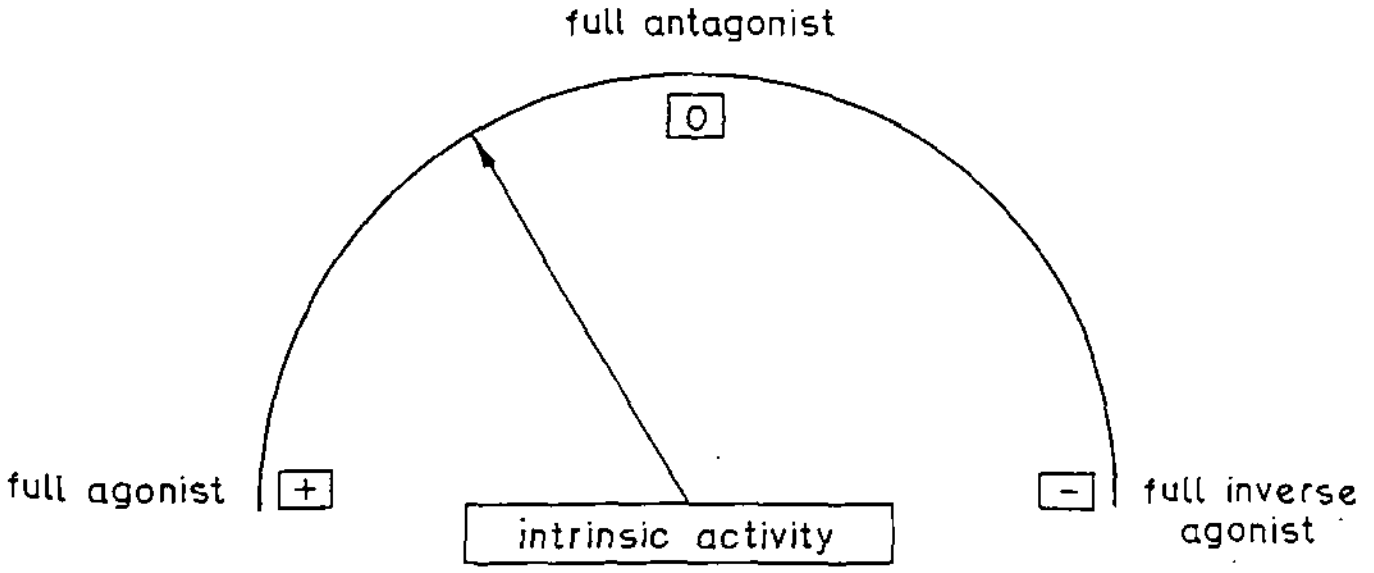


Figure (1.2):A schematic representation of the three different types of ligands

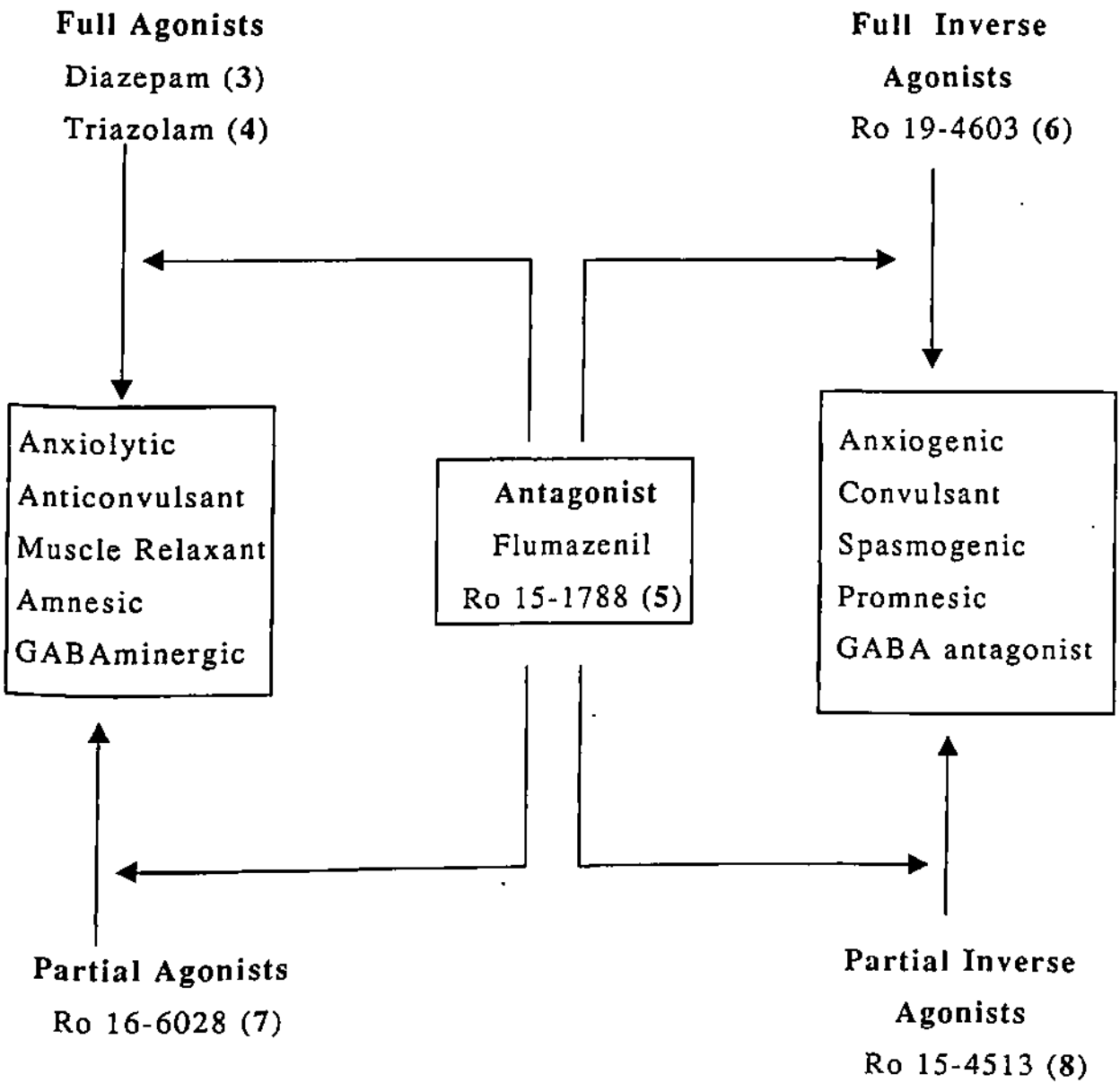
mirror image biological response over the full inverse profile of activity, i.e., negative intrinsic efficacy, are known as full inverse agonists.

Finally, full antagonists are the ligands with high affinity for BZR, having no modulatory effect on GABA binding to its receptor complex, and showing no relevant biological effects of their own, but blocking the effects of both agonist and inverse agonist ligands, i.e., no intrinsic efficacy. While some compounds are known to be full agonists, such as **3** and **4**, full antagonists, such as **5**, or full inverse agonists, such as **6**, the majority of compounds that have been synthesized and found to bind to the BZR, regardless of structure, show properties combining agonistic with antagonistic or inverse agonistic with antagonistic features. Such compounds are known as partial agonists or partial inverse agonists.[22] The example for the former may be **7** and for the latter **8**.

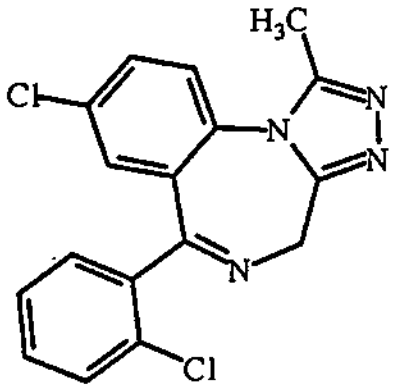
Biological properties of full agonists, antagonists, and inverse agonists acting through BZR are shown in Figure (1.3). However, this classification is best considered as purely based on phenomenology and thus there seems no general rule that would help to decide which of the BZR ligands is an agonist, antagonist, and inverse agonist.

1.1.2 *Interrelationship of Ligands*

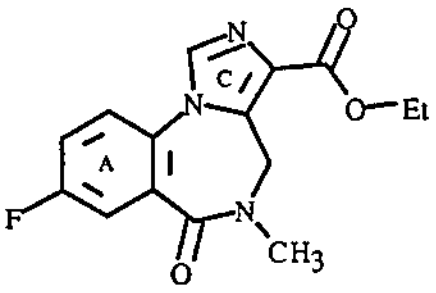
The three types of ligands, discussed above, exhibit an interrelationship, which can be explained on the basis of changes in the conformation of the receptor from its unoccupied resting state.[28,29]



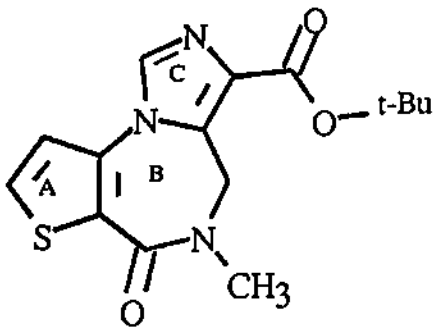
Figure(1.3): Pharmacological properties of BZs



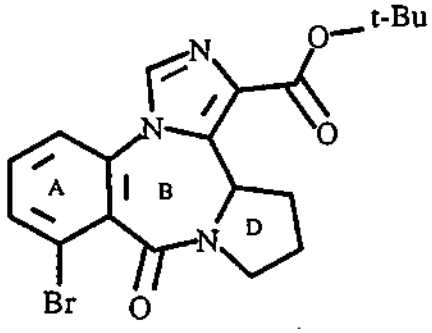
Triazolam (4)



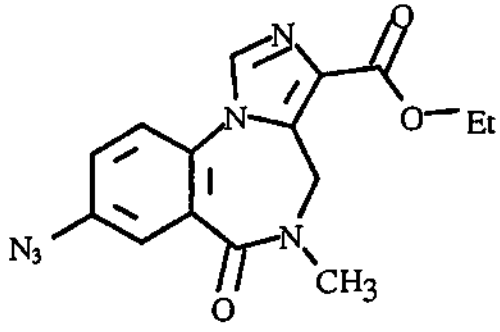
Ro15-1788 (5)



Ro 19-4603 (6)



Ro 16-6028 (7)



Ro15-4513(8)

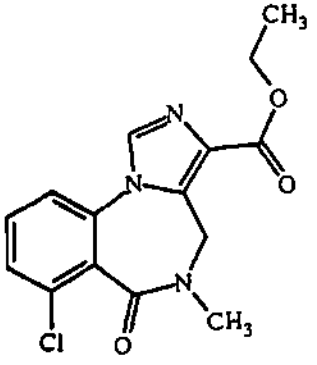
After primary recognition of the essential binding pharmacophores, the receptor will undergo a shift to either an agonist or an inverse agonist conformation, based on the geometry and hydrophobic nature of the ligand. This conformational change, probably energy driven, then allosterically modulates the binding of GABA to $GABA_A$ receptor. It is proposed that no conformational shift takes place when a full antagonist binds to the resting state of the BZR, and it would therefore be expected that such a ligand-receptor complex would show no efficacy at the GABA receptor. [27]

This model for explaining the interrelationship of ligands is known as the three-state receptor model. An alternative two-state receptor model has also been proposed, in which the unoccupied receptor is in equilibrium with agonist and inverse agonist conformations. [30]

1.1.3 *Homogeneity of BZR*

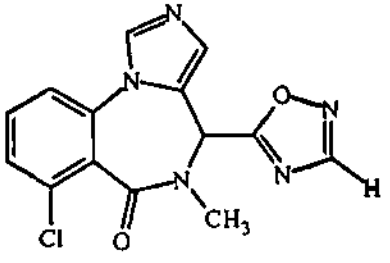
It has been observed that there exists a homogeneity in BZR binding sites for all the three types of ligands. This homogeneity is defined purely in the chemical sense, i.e., it is the amino acid sequence for the receptor protein that should be identical for all three sites. The homogeneity of BZR binding sites has been demonstrated for compounds having only minor structural modifications, which showed similar binding interactions but which displaced activities across the full spectrum from agonist to antagonist to inverse agonist.

While compounds 5, 9 and **10** present an example for the imidazo-benzodiazepin-6-one series,[31] analogous observations have been made for β-carbolines (**11**), pyrazoloquinolines (**12,13,14**), etc. Several structure-activity relationship studies have also supported the homogeneity of BZR.[32]



Ro-15-3505 (**9**)

Inverse Agonist



Ro-19-0528 (**10**)

Agonist

1.1.4 *Compounds Studied for BZR Binding*

The benzodiazepine receptors, as such, exhibit a very high specificity for pharmacologically and clinically active BZs and the minimum structural requirements for bindings in BZs are only the aromatic ring A and the carbonyl group in position 2 (Figure 1.4). The substituents at ring A, the 4,5-(methylene imino) group, the substituted or unsubstituted N1, and the 5-phenyl ring have been shown to produce little effect on *in vitro* binding.[27]

The aromatic ring is believed to undergo π/π stacking, probably with amino acid residues, within the receptor, and the carbonyl oxygen acts as a proton acceptor and is arbitrarily labelled as π_1.[29,33,34]

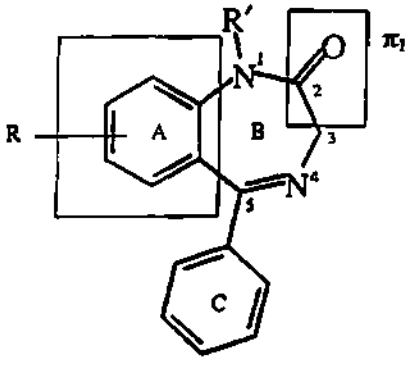
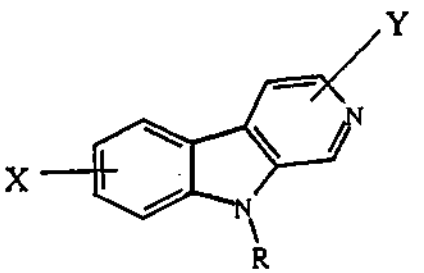


Figure (1.4): *The minimum structural requirements (encircled) in BZs for binding with BZR.*

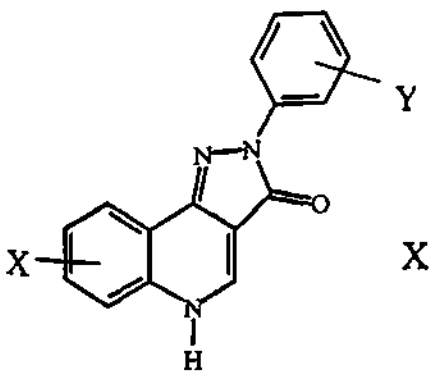
There are now, however, a variety of non-benzodiazepine molecules that bind to BZRs in the same molar range as BZs and exhibit *in vivo* biological response either as agonists, antagonists,or inverse agonists.[35-38] It becomes, therefore, essential to find the common features of ligands that allow for recognition by the receptor regardless of the type of *in vivo* activity and gross structural features.

For this purpose, families of non-benzodiazepines (non-BZs) such as β-carbolines (**11**), pyrazoloquinolines (**12-14**), pyridodiindoles (**15**), [1]benzopyranopyrazolines (**16,17**), [1]benzopyranopyrroles (**18**), [1]benzopyranotriazoles (**19**), pyrazolonaphthyridines (**20**), pyrazoloisoquinolines (**21**), [1,2,4]triazoloquinazolines (**22**), [1,2,4]-triazolophthalazines (**24**),

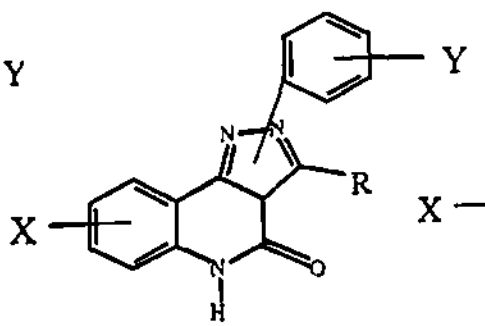
imidazophthalazines (**25**), dipyrazolopyridines (**26**), [1,2,4]triazolopyrimidines (**27**), 9-benzyl-9*H*-purines (**28**), indolyl glyoxylyl amino acid derivative (**29**), and indolylglyoxylylamine derivatives (**30**), in addition to those of BZs, were studied for their *in vitro* binding with BZRs.



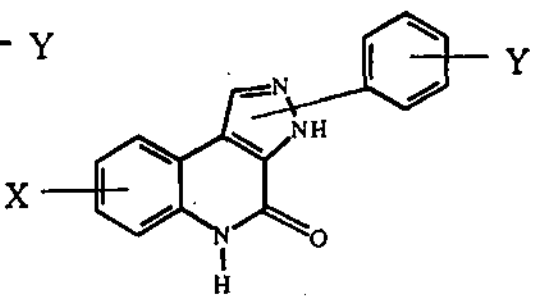
β - carbolines (**11**)



(**12**)

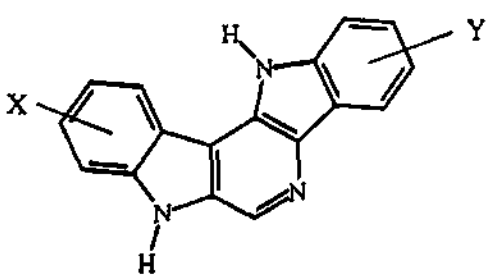


(**13**)

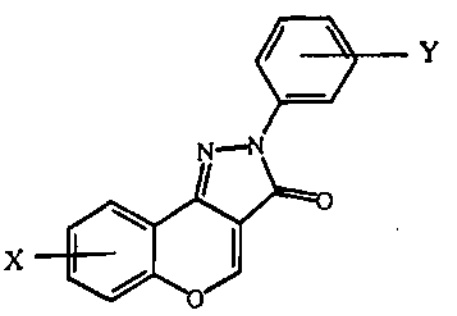


(**14**)

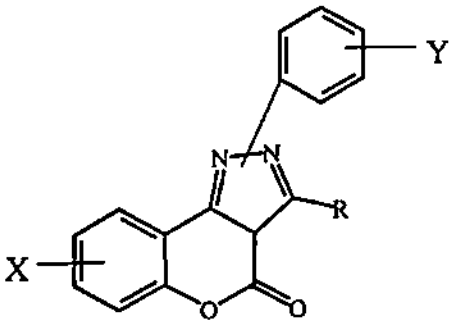
pyrazoloquinolines



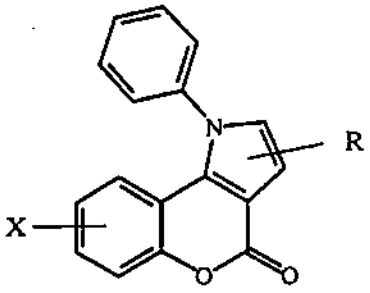
pyridodiindoles (**15**)



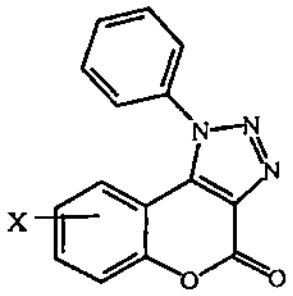
[1]benzopyranopyrazolines (**16**)



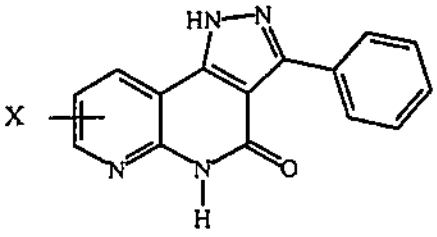
[1]benzopyranopyrazolines (17)



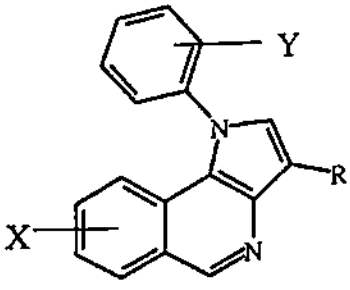
[1]benzopyranopyrroles (18)



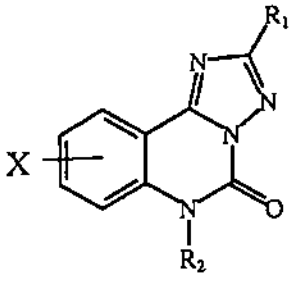
[1]benzopyranotriazoles (19)



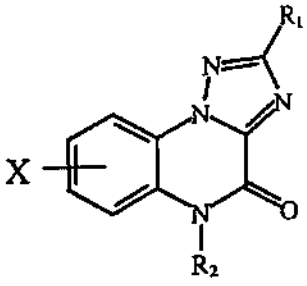
pyrazolonaphthyridines (20)



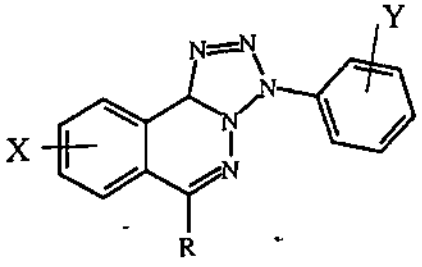
Pyrazoloisoquinolines (21)



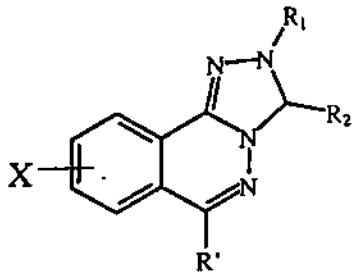
[1,2,4]Triazoloquinazolines (22)



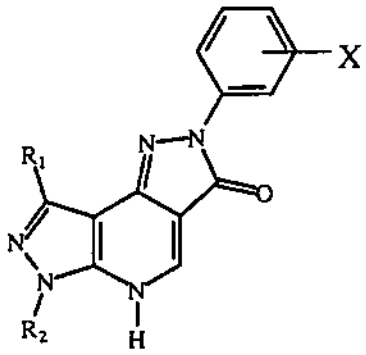
[1,2,4]Triazoloquinoxalines (23)



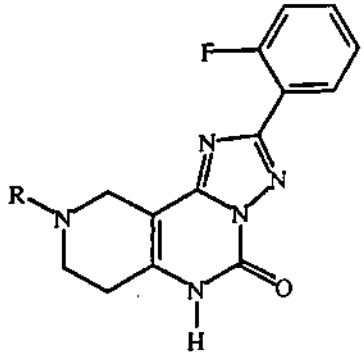
[1,2,4]Triazolophthalazines (24)



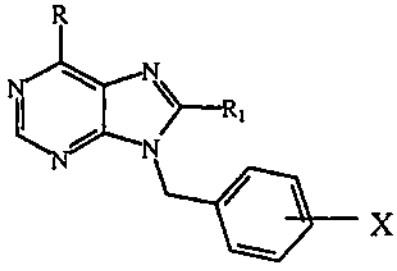
Imidazophthalazines (**25**)



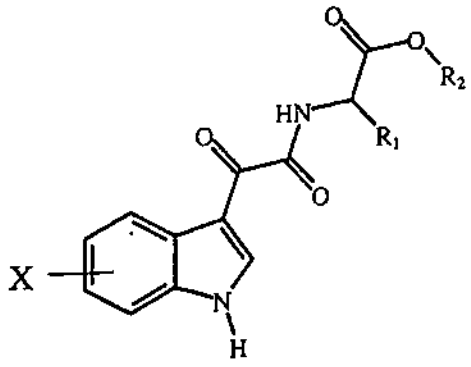
Dipyrazolopyridines (**26**)



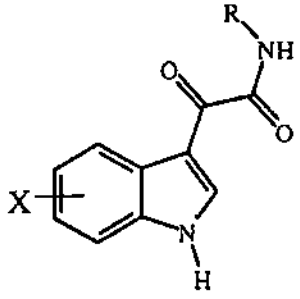
[1,2,4]Triazolopyrimidines (**27**)



9-Benzyl-9*H*-purines (**28**)



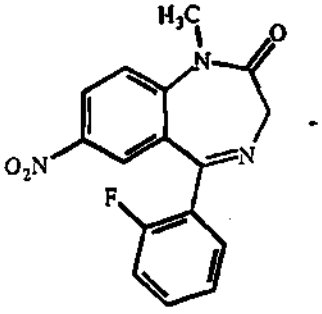
Indolylglyoxylylamino acid derivatives (**29**)



Indolylglyoxylylamine derivatives (**30**)

1.1.5 *Structure-Activity Relationship (SAR) Studies*

There have been many structure-activity relationship (SAR) studies on ligands binding to BZRs.[22,27] Though the early studies were concerned with the compounds of BZ series, more recently all of the known different structural classes of compounds capable of binding to BZRs in the nanomolar range have been included. The simple *in vitro* binding test is relatively well suited for the screening of a large series of compounds and proves useful in detecting compounds acting directly on the receptor, distinguishing them from compounds requiring *in vivo* metabolic transformation in order to become active (prodrugs). Because of this metabolic transformation of many compounds before they become active, the *in vivo* data are not found to be useful for SAR studies. The *in vitro* data usually refer to the molar concentration of drugs leading to a 50% inhibition of [^{3}H]diazepam (3) or [^{3}H]flunitrazepam (31) binding to BZR and are generally expressed by IC_{50} or I_{50}.



31

While it is essential for a meaningful SAR study to distinguish between the agonistic, antagonistic, and inverse agonistic activities of the

ligands, usual conditions of the binding test do not allow such a differentiation. Hence, it could be assumed that different ligands exert their effects by interacting at the same site, each influencing differently the conformation of the receptor glycoprotein environment, which modulates allosterically the BZR, the supramolecular GABA receptor, and the chloride ionophore. The present thesis reports QSAR of some of the non-BZ compounds, underlying the common structural features necessary for their BZR binding .

1.2 Quantitative Structure-Activity Relationship Studies (QSAR)

Earlier, the drug development process involved only random molecular modifications to make qualitative differences in a lead molecule. These random screening methods, which used to be considered as the normal procedure, are extremely expensive and, at the same time, less efficient. In the early 1960's, one could expect to discover a marketable compound out of 2000-3000 tested molecules, whereas this ratio is now close to 1 in 10,000. With the dramatically increasing costs of biological tests, fast access to accurate and reliable information on the drug candidate or similar molecules and the possibility of designing and testing accurate models of chemical structures and interactions, in order to simulate the behaviour and physicochemical properties of a drug molecule and the interactions between a drug and a receptor, have become mandatory.

Crum-Brown and Fraser were the first to suggest that physiological activity depends on "constitution", framing it in the mathematical terms of the following equation.[39]

$$\phi = f\,(c) \qquad (1.1)$$

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 relationship (QSAR) studies.

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 few decades, 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 Hansch and co-workers.[40-42]

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, i.e., 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 the change of the substituents. QSAR studies generally have two important aspects: predictive aspect and diagnostic aspect. The predictive aspect, as the name implies, deals with the extrapolation and interpolation of a correlation study to identify synthesis of more active derivatives and to avoid the synthesis and testing of derivatives of same or equivalent activity, minimizing the time needed to find a better derivative. The diagnostic aspect, on the other hand, answers mechanistic aspects of the reaction, i.e., it helps to obtain the information about the type of binding forces involved and about the mode of actions of drugs. Results of both these aspects can lead to a tailor-made design of new drugs of better activity with lesser or no side effects.

Some important approaches used in QSAR studies are: the nonparametric methods like Free-Wilson approach[43] or Fujita-Ban approach,[44] the parametric method developed by Hansch,[40,42] discriminant analysis,[45] and the pattern recognition technique.[46,47] 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 popular and widely used approach continues to be the so called Hansch approach,[40-42] where the variance in biological effect (ΔBE) is explained by the variance in certain linear free-energy related substituent constants which describe the changes in lipophilic/ hydrophilic (ΔL/ΔH), electronic (ΔE), steric (ΔS), and other properties of the parent molecule induced by the substituents. This model can be expressed as follows:

$$\Delta BE = f(\Delta L/\Delta H, \Delta E, \Delta S, ...) \qquad (1.2)$$

The lipophilicity of a molecule can be described by the logarithm of partition coefficient P, measured in octanol-water system.[48] The change in lipophilicity or hydrophobicity due to a substituent is described by the lipophilic or hydrophobic constant π of the substituent defined as $\pi = \log P_X - \log P_H$, where, X refers to the substituted derivative and H to the parent compound.[49] Lipophilicity can also be described by R_m values obtained from reverse-phase chromatography and by log k obtained from High Pressure Liquid Chromatography. The change in electronic properties can be expressed by Hammett constant (σ),[50] charge densities, spectroscopic properties like chemical shift from IR or UV spectra, field constant ($\mathfrak{F}$), and resonance constant ($\mathfrak{R}$). The steric influence of the substituents can be described by the Taft steric constant (E_s),[51] molar volume (MV), and molar refractivity (MR).[52,53]

Besides, many a drug activities have been found to depend exclusively upon the molecular size,[54-62] which can be described by the van der Waals volume (V_w), and upon the molecular graph which is delineated by molecular connectivity index (χ).[63] In addition to these, Verloop's[64] width parameters B and length parameter L, evaluated by measuring the dimensions of substituents in a restricted number of directions with the aid of a computer program called STERIMOL, were used. These parameters in their dimensional nature are indicative of the deviations of a substituent from spherical shape and their use might provide a better understanding of steric requirements in ligand-receptor interactions. In the present thesis, an extensive use has been made of these parameters.

In a stepwise linear multiple regression analysis, the biological activity (BA) can be related to various physicochemical, electronic, and steric parameters as:

$$BA = a\,\pi \text{ (or } \log P) + b\,\pi^2 \text{ (or } [\log P]^2) + c\sigma + dE_s + k \qquad (1.3)$$

where a, b, c and d are the regression coefficients and k the intercept obtained by least square method. Biological activity can be expressed by negative logarithm of the concentration of drug leading to a desired response. This reciprocal of the concentration used reflects the fact that greater potency is associated with a lower dose. Equation (1.3) shows a nonlinear, i.e., a parabolic dependence of activity on the hydrophobic character of molecules. Actually, Hansch had assumed a "random walk" of

the molecules, where hydrophilic molecules tend to remain in aqueous phase, while hydrophobic molecules tend to go into lipid phase.

Only those molecules that have a optimal hydrophilic/hydrophobic balance tend to reach their goal in reasonable time and concentration. The nonlinear dependence of activity on π or log P value in an *in vivo* system is due to the nonlinear dependence of the rate constant of drug transport through aqueous and bio-organic phases on lipophilicity, whereas in *in vitro* systems, such as enzyme inhibition, such nonlinear relationships result from equilibrium distribution of the drug toward different areas at the enzyme 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[40-42] 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[65-71] were proposed, out of which Kubinyi's bilinear model was found, after Hansch's parabolic model, to be the most useful model[72-78] to describe the nonlinear relationships.

1.2.1 *Applications of QSAR in Drug Design*

After formulation of a statistically significant as well as physicochemically meaningful correlation equation for a given set of compounds, the informations contained in the equation can be used to design new compounds. According to the method of utilization of the informations, examples could be classified into at least three categories:

1. Extrapolation of certain parameters toward directions enhancing the potency. As the correlation may or may not be linear, the best way this can be done is to gradually extend the extrapolation until the maximum potency is generated.
2. Identification of optimum structures with respect to certain parameters. If a parabolic dependence of the activity on certain parameters is revealed, the structure can be optimized by being modified so that the value of the parabolic parameter term is close to the maximum. This way, the best compound in the series can be identified and depending on this one could make a decision to continue or discontinue the synthetic program.
3. Transposition of QSAR informations to other series of compounds. The QSAR informations derived from a set of compounds $A\text{-}X_{1-n}$, prepared mainly on the basis of introduction or replacement of substituents, can be utilized to design new structures, $A\text{-}X_m$, where A is the basal skeletal structure that is kept unchanged and X means variable substituents or substructures.

A number of examples can be quoted where various combinations of the above points have been utilized to design compounds actually exhibiting the predicted activity.

1.2.2 *Limitations of QSAR*

Though QSAR studies can be successfully utilized to predict the activity of new analogues and discuss the mechanisms of drug-receptor interactions, they have some drawbacks and limitations as described below.[79]

The substituent effect on hydrophobicity is characterized by logP 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 logP also depends on the electronic characters and the hydrogen bonding properties of the substituents.[80,81] Thus, if one gets a correlation with logP 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 logP values is steric effect that can prevent the access of water to a hydrophilic group.[82] Steric interactions are extremely difficult to extrapolate from system to system. The use of parameters like MR, MW, Vw, etc., do not give any idea in what way steric effects would effect the drug-receptor interaction. 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 can provide some help in this, but they are time consuming and expensive.

Although molecules are represented as rigid structures on paper, they may, in fact, be quite different in solution and their dynamic nature should be recognized. There is considerable evidence that macromolecules, even in crystalline state, exhibit a wide spectrum of motions.[83-91] These motions may be involved in some molecular conformational changes on substrate or group binding. Both drugs and biomolecules are three dimensional objects whose chemical features are related to their three dimensional structures. The interaction between them involves a complementarity or fit between the two objects. Even a successful QSAR study will provide only indirect information about the three-dimensional aspects of drug-biomolecule interaction.

Many structural features that affect the activity but can not be parametized by the usual variables like 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 feature and 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).[92] 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.[93] 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 interaction does not always represent the true *in vivo* model.

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Chapter 2

Parameters Used

And

Their Calculations

This chapter discusses the methodology of calculation of various distinct parameters, on which most of the biological activities are found to depend and which are very useful in QSAR studies.

2.1 Hydrophobic Parameter [log P]

The fragment method suggested by Hansch and Leo[1] 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.

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

Carefully conducted partitioning experiment and statistical survey of the then available partition data have been used in assigning values to the fragments and factors. The working principle is summarized in the following paragraphs.

In this approach carbon atoms are divided into two categories: isolating carbons (IC) and nonisolating carbons (NIC). ICs are those having either four single bonds (at least two of which are to non heteroatoms) or else are multiply bonded to other carbon atoms.

NIC atoms are carbon atoms multiply bonded to hetero atoms. For example -C= *in* $CH_2=CH_2$ *is an IC but not in* $H_2C=O$. Fragments are of two types: (i) fundamental fragments defined as fragments whose free valency will lead to isolating carbons; (ii) derived fragments, a derivative of fundamental fragments (e.g. CF_3). A fundamental fragment can be either a single atom or a group of multiple atoms (e.g. -C=O, -C=N, etc.). 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:

(i) **Non-polar fragments:** These are simple ICs and hydrogens attached to Ics.

(ii) **H-polar fragments:** A fragment that can be expected to form H-bonds either by accepting or donating an electron pair (e.g. -OH, -COOH, $-NH_2$ etc.).

(iii) **S-polar fragments:** A fragment that is strongly electron withdrawing with little tendency to form H-bonds (e.g. 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 Fs are identified with the help of different subscripts and superscripts. The subscripts are mentioned in Table (2.2). The superscripts are applicable also to fragments.

They are listed as:

1. None = aliphatic structural attachment
2. ϕ = attachment to aromatic ring; if bivalent the attachment is from left as written
3. $1/\phi$ = as 2 but attachment from right as written
4. $\phi\phi$ = two aromatic attachments
5. X = aromatic attachment, value enhanced by second, electron- withdrawing substituent ($\sigma_I \geq \pm 0.35$) and
6. IR = benzyl attachment.

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

In calculating the logP 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 logP of that compound. It is always safe to break any compound, especially compound containing hetero atoms, into fundamental fragment rather than into derived fragment. Some important fragment values and factor values are listed in Tables (2.1) and (2.2), respectively. A simple example for logP calculation is shown below:

Example, Toluene ($C_6H_5CH_3$): This can be treated as a compound comprising six aromatic carbons, one aliphatic carbon and eight hydrogens.

The fragments can be expressed as:

$$6\, f^{\varphi}_{C} + f_C + 8\, f_H = \log P \text{ (Toluene)}$$

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

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

$$f^{\varphi}_{\underline{C_6H_5}} + f\ CH_3 = \log P \text{ (toluene)}$$

$$1.9 + 0.89 = 2.79 \text{ (calcd.)}$$

Sometimes calculated log P values of compounds deviate very much from the experimentally determined values. For example, observed log P of 1,2-methylenedioxybenzene 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 literature.[1]

Table (2.1): Some common fragment constants*

Without Carbon	f	f^{ϕ}	$f^{\phi\phi}$	With Carbon	f	f^{ϕ}	$f^{\phi\phi}$
- 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	-1.27	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^{ϕ}	Without Carbon	f^{ϕ}	With Carbon	f^{ϕ}	With Carbon	f^{ϕ}
- N=	-1.12	-N=N-	-2.14	C	0.13	-CH-	0.35
- N	-1.60	-O-	-0.08	C	0.25[c]	-C(O)-	-0.59
- N^{ϕ}	-0.56	-NH-	-0.65	C*	0.44[d]	-OC(O)-	-1.40

*Taken from ref. 1, [a]Derived fragment, [b]For methyl ethers and ethylene oxide, use -1.54, [c]For ring fusion carbon, [d]For ring fusion –hetero

Table (2.2) : *List of some factors**

	Involving Bonds			
	Unsaturation			Geometric
	Double	Triple	Proportional to Length: x(n-1)	Short Chains: 1-time
Normal	F(=) = -0.55	F(≡) = -1.42	Chain: F_b = -0.12	Alkane Chain: F_{cBr} = 0.13
Conjugate to φ	F^{φ} (=) = -0.42		Ring[a] : F_b = -0.09	H-polar Fragment: F_{gBr} = -0.22
Conjugate To 2 φ	$F^{\varphi\varphi}$ (=) = -0.0	$F^{\varphi\varphi}$ (≡) = -0.0	Branching: F_{bYN} = -0.20[b] F_{bYP} = -0.31[c]	Ring Cluster : F_{rCl} = -0.45

Involving Multiple Halogenation[d]		
On same Carbon (geminal) F_{mhCm}	(n=2) = 0.30 (n=3) = 0.53 (n=4) = 0.72	On adjacent Carbon (vicinal) F_{mhVn} : 0.28(n-1)

Involving H-polar Proximity					
				Aromatic :	
Chain	$F_p^1 = -0.42\,\Sigma f_1 + f_2$ $F_p^2 = -0.26\,\Sigma f_1 + f_2$ $F_p^3 = -0.10\,\Sigma f_1 + f$	Aliphatic: Ring:	$F_p^1 = -0.32\,\Sigma f_1 + f_2$ $F_p^2 = -0.20\,\Sigma f_1 + f_2$		$F^{\varphi}{}_p^1 = -0.16\,\Sigma f_1 + f_2$ $F^{\varphi}{}_p^2 = -0.08\,\Sigma f_1 + f_2$

Involving Intramolecular H-bond	
F_{HBN} = 0.60 for Nitrogen	F_{HBO} = 1.0 for Oxygen

*Taken from Ref.1

[a]Aromatic rings are excluded

[b]For amine

[c]For Phosphorus esters

[d]Value per halogen atom

2.2 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.[2,3] 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

$$\pi_X = \log P_X - \log P_H \qquad (2.2)$$

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

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

$$0.71 = 2.84 - 2.13$$

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 value of π varies somewhat from system to system. Certain π values are given in Table (2.3).

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2.3 Electronic Parameter (σ)

The development of electronic parameter is one of the most important breakthrough for mechanistic organic chemistry which came in 1935 when L.P. Hammett[4] proposed the following equation to define an electronic parameter σ.

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

In equation (2.4), K_H is the ionization constant of benzoic acid in water at 25°C and K_X is the ionization constant for the meta or para derivative under the same experimental conditions. Positive values of σ represent the electron-withdrawing and the negative ones electron-donating character of substituents in the aromatic ring. For certain substituents, σ values are given in Table (2.3).

2.4 Molar Refractivity (MR)

In various organic reactions, dispersion forces play an important role and these could be modeled by the molar refractivity (MR) of substituents. Experimentally, MR is usually obtained via the Lorentz-Lorenz equation.

$$MR = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{MW}{d} \qquad (2.5)$$

where n is the index of refraction, d is the density, and MW is the molecular weight of a compound. Since MR is an additive constituent property of molecules, fragment values have been calculated for many

common groups of atoms. It has generally been assumed that a positive coefficient with an MR term in a correlation equation suggests a binding action via dispersion forces. Such binding could produce a concomitant conformational change in a macromolecular binding site. If the conformational change favoured the process under study, one would certainly expect a positive coefficient with the MR term, however, if the conformational change were detrimental, a negative coefficient could result for the MR term. Negative coefficient with MR have also been assumed to reflect steric hindrance of one kind or another. Some MR value used are tabulated in Table (2.3).

2.5 van der Waals Volume(V_w)

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 interactions.

To calculate V_w of molecules, spherical shapes are assumed for all atoms according to Bondi[5] because of the absence of generally accepted pear shapes.

Table (2.3): Data on physicochemical parameters for some important substituents*

No.	Substituent	π	σ_m	σ_p	MR
1	H	0.00	0.00	0.00	1.03
2	CH_3	0.56	-0.07	-0.17	5.65
3	C_2H_5	1.02	-0.07	-0.15	10.30
4	C_3H_7	1.05	-0.07	-0.13	14.96
5	*i*- C_3H_7	1.53	-0.07	-0.15	14.96
6	*n*- C_4H_9	2.13	-0.08	-0.16	19.61
7	F	0.14	0.34	0.06	0.92
8	Cl	0.71	0.37	0.23	6.03
9	Br	0.86	0.39	0.23	8.88
10	I	1.12	0.35	0.18	13.94
11	OCH_3	-0.02	0.12	-0.27	7.87
12	NH_2	-1.23	-0.16	-0.66	5.42
13	OH	-0.67	0.12	-0.37	2.85
14	COOH	0.32	0.37	0.45	6.93
15	$COOCH_3$	-0.01	0.37	0.45	12.87
16	CF_3	0.88	0.43	0.54	5.02
17	NO_2	-0.28	0.71	0.78	7.36
18	CHO	-0.65	0.35	0.42	6.88
19	C_5H_5	1.96	0.06	-0.01	25.36
20	CN	-0.57	0.56	0.66	6.33
21	N_3	0.46	0.27	0.15	10.20
22	NHOH	-1.34	-0.04	-0.34	7.22
23	$CH=CH_2$	0.82	0.05	-0.02	10.99
24	$COCH_3$	-0.55	0.38	0.50	11.18

Contd..

No.	Substituent	π	σ_m	σ_p	MR
25	$COOC_2H_5$	0.51	0.37	0.45	17.47
26	$COOC_3H_7$	1.07	0.37	0.45	22.17
27	CH_2OH	-1.03	0.00	0.00	7.19
28	$CHOHCH_3$	-0.86	0.00	-0.07	11.82
29	CH_2OCH_3	-0.78	0.02	0.03	12.07
30	SCH_3	0.61	0.15	0.00	13.82
31	NHCHO	-0.98	0.19	0.00	10.31
32	$OCOCH_3$	-0.64	0.39	0.31	12.47
33	$OCH(CH_3)_2$	0.85	0.10	-0.45	17.06
34	OC_3H_7	1.05	0.10	-0.25	17.06
35	$N(CH_3)_2$	0.18	-0.15	-0.83	15.55

* Taken from ref. 1

The values of the van der Waals radii used and calculated volume of atoms are listed in Table (2.4).Since van der Waals radii are greater than covalent radii, a correction 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 values are tabulated in Table (2.5). A correction for branching in the molecule is also included in the V_W calculation. Such correction is also mentioned in the Table (2.5). All these values have been taken from the literature.[6]

2.6 Molecular Connectivity Index (χ)

Kier and Hall[7] introduced this additive topological parameter to drug design. Here the molecular connectivity index, χ, signifies the degree of branching or connectivity in a molecule.

Different versions of χ are calculated from the hydrogen-supressed graph of the molecule. For this purpose the hydrogen-suppressed graph will be decomposed, depending on the χ considered, into uniform parts called as subgraph(s). Here two types of connectivity indices, simple molecular connectivity index ($^m\chi$) and valance molecular connectivity index ($^m\chi^v$) are discussed. The superscript m is known as order of the connectivity index and is numerically equal to the number of non hydrogenic sigma bonds present in the subgraph of the particular χ.

Table (2.4): van der Waals radius and volume of atoms*

Atom		Radius (A°)	Sphere Volume ($10^2 A^{\circ 3}$)
C		1.7	0.206
H		1.1	0.056
N		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	2.1	0.388
B		2.1	0.388
He		1.2	0.072
Ne		1.6	0.171
Ar		1.9	0.287
Kr		2.0	0.335
Xe		2.2	0.446

* Taken from ref. 6

Table (2.5): Correction values of van der Waals volume, for sphere overlapping due to covalent bonding and branching*

Bond	Bond length (A°)	Correction value (10^2 A^{o3})
C-C	1.5	-0.078
C-H	1.1	-0.043
C-N	1.4	-0.060
C-O	1.4	-0.056
C-S	1.8	-0.066
C-F	1.4	-0.056
C-Cl (aliphatic)	1.8	-0.058
C-Cl (aromatic)	1.8	-0.066
C-Br (aliphatic)	1.9	-0.060
C-Br (aromatic)	1.9	-0.068
C-I (aliphatic)	2.1	-0.063
C-I (aromatic)	2.1	-0.072
C-B	1.6	-0.113
H-H	0.7	-0.030
N-H	1.0	-0.038
N-N	1.4	-0.050
N-O	1.4	-0.042
N-S	1.6	-0.061
O-H	1.0	-0.034
O-B	1.5	-0.079
S-H	1.3	-0.040
S-S	2.0	-0.062

Contd...

Bond	Bond length (A°)	Correction value (10^2 $A^{\circ 3}$)
S-F	1.6	-0.052
C=C	1.3	-0.094
C=N	1.3	-0.072
C=O	1.2	-0.068
C=S	1.6	-0.081
N=N	1.2	-0.061
N=O	1.2	-0.053
S=O	1.5	-0.057
C≡C	1.2	-0.101
C≡N	1.2	-0.079
C≡C (aromatic)	1.4	-0.086
Branching for saturated bond except bonding with H		-0.050

* Taken from ref. 6

A simple version of simple molecular connectivity index is first-order simple molecular connectivity index, $^1\chi$, and it is computed by

$$^1\chi = \Sigma C_{ij} = \Sigma(\delta_i\ \delta_j)^{-1/2} \qquad (2.6)$$

where the summation extends to all connections or edges (C_{ij}) of the hydrogen-suppressed graph and δ_i and δ_j are integers assigned to each atom indicating the number of atoms adjacent or connected to atoms i and j which are formally bonded. Here, in deriving this index, only the number of non-hydrogenic adjacent atoms are considered but not the nature of the atoms and the unsaturation in the molecule.

The valence molecular connectivity index, in contrast to the simple molecular connectivity index, takes into account the nature of the atoms as well as the unsaturation present in the molecules. Here the connectivity term, δ^v is defined as:

$$\delta^v = Z^v_i - N_H \qquad (2.7)$$

in which Z^v_i is the number of valence electrons present in atom i and N_H is the number of hydrogens attached to it. A simple version of valence molecular connectivity index is first-order valence molecular connectivity index, $^1\chi^v$, and is formulated as:

$$^1\chi^v = \Sigma C_{ij} = \Sigma(\delta^v_i\ \delta^v_j)^{-1/2} \qquad (2.8)$$

The application of equation (2.7) for atoms beyond the second row in the periodic table leads to the same δ^v value for each family member,

for example, seven for each halogen and six for each chalcogen. Consideration of valence electrons (Z^v_i) of atom i together with its atomic number (Z_i) and the number of hydrogen atoms (h_i) attached to that atom will give appropriate δ^v value for atoms beyond second row in the periodic table.[8] The mathematical expression for this is:

$$\delta^v = (Z^v_i - h_i) / (Z_i - Z^v_i - 1) \qquad (2.9)$$

According to this equation $\delta_{Cl} = 0.78$ and $\delta_{Br} = 0.26$. The δ^v value for some heteroatoms including halogens are listed in Table (2.6).

Only the above discussed connectivity indices are used in our studies. Higher order connectivity indices are discussed by Kier and Hall in their monograph.[7]

2.7 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.[9,10] Following a suggestion of Ingold, Taft defined the steric constant E_s as:

$$E_s = \log (k_x/k_H) \qquad (2.10)$$

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 an essential step for acid hydrolysis by water.

Table (2.6): Valance delta (δ^v) values for heteroatoms*

Group	δ^v	Group	δ^v
NH_2	3	OH	5
NH	4	O	6
N	5	C=O	6
C≡N	5	Furan O	6
C=NH	4	O=NO	6
Pyridine N	5	H_2O	4
Nitro N	6	H_3O^+	3
NH_3	2	F	7
NH_4^+	1	Cl	0.78[a]
N^+	6	Br	0.26[a]
$=NH_2^+$	3	I	0.16[a]
		S	0.67[a]

*Taken from ref. 7, [a]Obtained from equation (2.9)

2.8 Verloop's Steric Parameters (L and B)

Verloop's steric parameters[11] L and B referring to length and breadth of the substituents are calculated by a computer program called STERIMOL. There is only one length parameter L but there are five width parameters B_1-B_5. All are calculated from standard bond angles, bond lengths, van der Waals radii, and user-determined reasonable confirmations. The width parameters are measured perpendicular to the bond axis and describe the positions, relative to the point of attachment and the bond axis, of five planes which closely surround the group. In contrast to Es values which, because of the reaction on which they are based, cannot be determined for many substituents, the Verloop's parameters are available for any substituent. Table (2.7) lists the Verloop's parameters for some important substituents.

For QSAR studies in this thesis, standard values for different parameters for various substituents have been taken from literature.[1]

Table (2.7): Verloop's parameters for some important substituents*

No.	Substituent	L (Å)	B_1 (Å)	B_2 (Å)	B_3 (Å)	B_4 (Å)
1	H	2.06	1.00	1.00	1.00	1.00
2	F	2.65	1.35	1.35	1.35	1.35
3	Cl	3.52	1.80	1.80	1.80	1.80
4	Br	3.83	1.95	1.95	1.95	1.95
5	I	4.23	2.15	2.15	2.15	2.15
6	CH_3	3.00	1.52	1.90	1.90	2.04
7	C_2H_5	4.11	1.52	1.90	1.90	2.97
8	n-C_3H_7	5.05	1.52	1.90	1.90	3.49
9	i-C_3H_7	4.11	2.04	2.76	3.16	3.16
10	c-C_3H_7	4.14	1.98	2.24	2.29	2.88
11	$CH_2C_6H_5$	3.63	1.52	3.11	3.11	6.02
12	CF_3	3.30	1.98	2.44	2.44	2.61
13	COOH	3.91	1.60	1.60	2.36	2.66
14	$COOCH_3$	4.85	1.90	1.90	2.36	3.36
15	$CONH_2$	4.06	1.60	1.60	2.42	3.07
16	CN	4.23	1.60	1.60	1.60	1.60
17	C_6H_5	6.28	1.70	1.70	3.11	3.11
18	p-ClC_6H_4	7.74	1.80	1.80	3.11	3.11
19	OH	2.74	1.35	1.35	1.35	1.93
20	OCH_3	3.98	1.35	1.90	1.90	2.87
21	OC_2H_5	4.92	1.35	1.90	1.90	3.36
22	$OCH_2C_6H_5$	8.20	1.35	3.03	3.11	3.11
23	NH_2	2.93	1.50	1.50	1.84	1.84
24	$NHCH_3$	3.53	1.50	1.90	1.90	3.08
25	NO_2	3.44	1.70	1.70	2.44	2.44

*Taken from ref.11

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Chapter 3

QSAR Studies :

Results And Discussion

As discussed earlier, benzodiazepine receptor ligands, because of their wide range of therapeutic activities, are the most frequently prescribed CNS drugs. Because of their safety and better effectiveness, benzodiazepines have even replaced barbiturates in the treatment of both anxiety and insomnia. Apart from the classic BZs, a number of structurally different compounds have affinity for this receptor.

More over, the heterogeneity and the functional and structural complexity of the GABA receptor /Cl^- ionophore complexes have strongly hampered up to now a clear detection and definition of the stereoselectivity requirements necessary for eliciting a specific intrinsic activity. A total of at least 14 subunits[1,2] of the $GABA_A$ receptor have been identified by molecular cloning, and these subunits are taught to assemble into a pentameric structure to form a Cl^-channel.

Most functional subtypes of $GABA_A$ receptors contain α, β and γ subunits, with different subtypes showing high sensitivity to different benzodiazepine receptor ligands.[3-5] Unfortunately, the key structural and dynamic properties of the BZR cannot be directly measured, or modeled, because of the limited current data and methodologies, and therefore several indirect studies have been carried out to detect the common structural features of diverse classes of BZR ligands most likely responsible for high affinity and possibly for specific intrinsic activity and definite pharmacological profile.

Despite the recent significant advances in the structural and functional studies of BZR[2] and in the molecular pharmacology and physiopathology of anxiety disorders[6], much remains to be achieved for the design and development of truly innovative drugs, lacking the numerous side effects of BZs.[7]

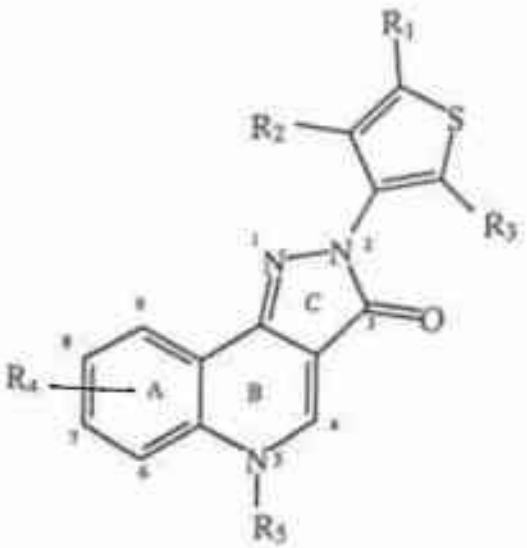
With this objective, a number of non-BZ class of compounds were synthesized in the recent years. A quantitative analysis of the biological activity and the physicochemical properties of these compounds will precisely determine the extent of the role played by different physicochemical properties of the compounds in the drug receptor binding. Further, the correlation equations thus obtained may be exploited to propose possible model for the receptor, allowing the binding of the corresponding ligands. Hence, a detailed QSAR study has been carried out on some non-BZ compounds, in order to understand their mode of interaction with the receptor and to rationalize the selection of substituents. The values of the various parameters were taken from the literature[8] or calculated as per the methods discussed in the preceding Chapter. A least square method has been applied for the regression analysis.[9] The activity parameters used are the binding constant K_i or the molar concentration IC_{50} leading to 50% inhibition of the substrate binding to the receptor. The K_i is related to IC_{50} as

$$K_i = IC_{50} / (1 + C/K_d) \qquad (3.1)$$

where C is the concentration of the radioligand used and K_d is the apparent dissociation constant of the radioligand-receptor complex.

3.1 Thienylpyrazoloquinolines

Two different series of thienylpyrazoloquinolines (1 and 2) were studied for their BZR binding affinity by Takada et al.,[10,11] which are listed in Tables (3.1) and (3.2). We performed a multiple regression analysis on these series of compounds and obtained equation (3.2) for the compounds of Table (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 within the parenthesis are 95% confidence intervals. The value for F given in the parenthesis is at 99% level.



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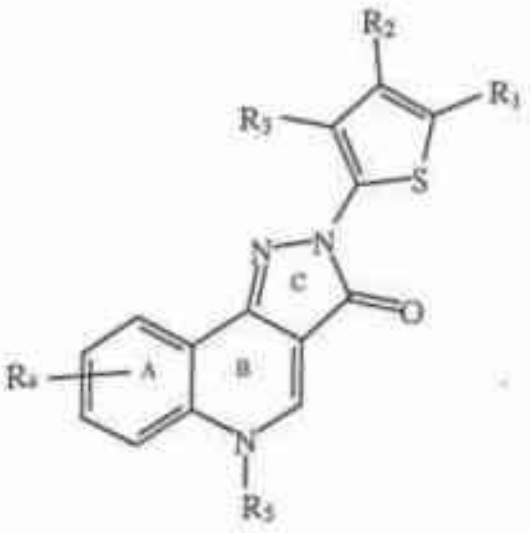


2

$$\log (1/K_i) = 2.90\ (\pm 0.76)\pi_{R3} - 5.73(\pm 1.02)\ (\pi_{R3})^2 - 2.74\ (\pm 0.26)I_5 - 0.12\ (\pm 0.05)\ (\pi_{R1})^2 - 1.13(\pm 0.35)I_2 + 9.297 \qquad (3.2)$$

$$n = 20,\ r = 0.989,\ s = 0.15,\ F_{5,14} = 126.78(4.69)$$

Table (3.1):Thienlypyrazoloquinolines (1) and their BZR-binding constants and related physicochemical parameters

No.	R_1	R_2	R_3	R_4	R_5	π_{R1}	π_{R3}	log $(1/K_i)^a$ Obsd[b]	Calcd, Eq.(3.2)
1	CH_3	H	H	H	H	0.56	0.00	9.49	9.25
2	H	H	H	H	H	0.00	0.00	9.14	9.29
3	H	CH_3	H	H	H	0.00	0.00	8.16	8.16
4[c]	H	H	$COOCH_3$	H	H	0.00	-0.01	6.84	9.26
5	H	H	COOH	H	H	0.00	-0.32	7.75	7.78
6	C_2H_5	H	H	H	H	1.02	0.00	9.39	9.16
7	Cl	H	H	H	H	0.71	0.00	9.14	9.23
8	CH_3	H	Cl	H	H	0.56	0.71	8.33	8.42
9	CH_3	H	Br	H	H	0.56	0.86	7.57	7.50
10	CH_3	H	H	7-Cl	H	0.56	0.00	9.11	9.25
11	CH_3	H	H	8-Cl	H	0.56	0.00	9.20	9.25
12	CH_3	H	H	7-F	H	0.56	0.00	9.19	9.25
13	CH_3	H	H	8-F	H	0.56	0.00	9.43	9.25
14	CH_3	H	H	7-CH_3	H	0.56	0.00	9.02	9.25
15	CH_3	H	H	8-CH_3	H	0.56	0.00	9.42	9.25
16	CH_3	H	H	H	CH_3	0.56	0.00	6.66	6.51
17	CH_3	H	H	H	C_2H_5	0.56	0.00	6.36	6.51
18	*n*-C_3H_7	H	H	H	H	1.55	0.00	8.98	9.00
19	*i*-C_3H_7	H	H	H	H	1.53	0.00	9.02	9.01
20	*n*-C_4H_9	H	H	H	H	2.13	0.00	8.73	8.74
21	*i*-C_4H_9	H	H	H	H	2.28	0.00	8.64	8.67

[a] K_i: binding constant against [^{3}H] diazepam. [b] Compiled from refs. 10 and 11. [c] Not included in the regression

Table (3.2): Thienlypyrazoloquinolines (2) and their BZR-binding constants and related physicochemical parameters

No.	R_1	R_2	R_3	R_4	R_5	π_{R1}	π_{R3}	log (1/K_i)[a] Obsd[b]	Calcd, Eq.(3.3)
1	CH_3	H	H	H	H	0.56	0.00	9.49	9.03
2	C_2H_5	H	H	H	H	1.02	0.00	8.99	9.03
3	*n*-C_4H_9	H	H	H	H	2.13	0.00	8.82	9.03
4	CH_3	CH_3	H	H	H	0.56	0.00	9.13	9.03
5	H	H	H	H	H	0.00	0.00	9.38	9.03
6	H	CH_3	H	H	H	0.00	0.00	9.32	9.03
7	Cl	H	H	H	H	0.71	0.00	8.60	9.03
8	CH_3	H	$COOC_2H_5$	H	H	0.56	0.51	8.00	8.00
9	CH_3	H	COOH	H	H	0.56	-0.32	7.66	7.66
10	CH_3	H	H	7-Cl	H	0.56	0.00	8.97	9.03
11	CH_3	H	H	8-Cl	H	0.56	0.00	9.12	9.03
12	CH_3	H	H	7-F	H	0.56	0.00	9.19	9.03
13	CH_3	H	H	8-F	H	0.56	0.00	9.03	9.03
14	CH_3	H	H	7-CH_3	H	0.56	0.00	8.63	9.03
15	CH_3	H	H	8-CH_3	H	0.56	0.00	9.08	9.03
16	CH_3	H	H	H	CH_3	0.56	0.00	5.99	5.99
17	*n*-C_3H_7	H	H	H	H	1.55	0.00	9.06	9.03
18	*i*-C_3H_7	H	H	H	H	1.53	0.00	8.99	9.03
19	*i*-C_4H_9	H	H	H	H	2.28	0.00	8.69	9.03

[a] K_i: binding constant against [^{3}H]diazepam. [b] Compiled from refs. 10 and 11.

Equation (3.2) correlates the binding affinity with hydrophobic property of R_1 and R_3 substituents and two indicator variables I_2 and I_5 which have been used for R_2 and R_5 substituents, respectively, with a value of 1 each for an alkyl group and zero for H. This correlation seems to be highly significant and accounts for about 98% of the variance in the activity (r^2=0.98). It should be noted that we have used no physicochemical or dummy parameter to account for the effect of R_4 substituent at ring A. A close observation of Table (3.1) shows that, of the R_4 substituents, the 7-substituent has some lowering effect, but when we tried to account for the effect in the correlation, using a dummy parameter I_7 with a value of unity, it produced little effect on the significance of the correlation and the parameter itself was insignificant at 95% confidence level (equation 3.3). It has been discussed in fact that in pyrazoloquinolines the substituents at ring A are of little importance.[12] Equations (3.2) and (3.3) suggest that in thienyl derivatives the important substituents are those present at the thienyl ring.

$$\log (1/K_i) = 3.00\ (\pm 0.69)\pi_{R3} - 5.95(\pm 0.95)\ (\pi_{R3})^2 - 2.80\ (\pm 0.24)I_5 - 0.14(\pm 0.05)\ (\pi_{R1})^2 - 1.19\ (\pm 0.32)I_2 - 0.20(\pm 0.21)\ I_7 + 9.35 \qquad (3.3)$$

$$n = 20,\quad r = 0.992,\quad s = 0.14,\quad F_{6,13} = 132.71(4.62)$$

A negative coefficient of $(\pi_{R1})^2$ suggests that neither a highly hydrophobic nor a highly hydrophilic R_1 substituent at the 5-position of this ring will be conducive to the binding affinity. Thus, either no substituent or a substituent with a hydrophilic-lipophilic balance (π=0)

will be most favourable at this position. A negative coefficient of I_2 suggests that a methyl group at the 4-position of the ring will produce steric hindrance. Only the R_3 substituent at position 2 is exhibited to produce some positive effect provided it is not highly hydrophobic as both equations (3.2) and (3.3) represent a parabolic correlation in π_{R3}, giving an optimum value of $\pi_{R3} = 0.25$.

The negative coefficient of I_5 indicates that the substitution at N5 will lead to a decrease in the activity. This corroborates to the finding for pyrazoloquinolin-3-ones (but not for -4-ones) that unsubstituted N5-H is conducive to the activity as it participates in hydrogen bonding.[12]

In case of thienyl derivatives of Table (3.2), which differ from those of Table (3.1) only in respect of the positional attachment of the thienyl group (the former are thien-2-yl derivatives and the latter thien-3-yl derivatives), the best correlation obtained was

$$\log (1/K_i) = 1.86\ (\pm 1.14)\pi_{R3} - 7.60(\pm 2.56)\ (\pi_{R3})^2 - 3.04\ (\pm 0.56)I_5 + 9.03 \qquad (3.4)$$

$$n = 19, \quad r = 0.957, \quad s = 0.25, \quad F_{3,15} = 54.41(5.42)$$

which incorporates only two parameters π_{R3} and I_5, exhibiting that for this series of compounds only the R_3 substituent at the 3-position of the thienyl ring and R_5 of N5 will be important. The latter, as usual, would be detrimental to the activity and the former will increase the activity

until it becomes highly hydrophobic. The optimum value of π_{R3} in this case is around 0.12, which is just half of that obtained for the compounds of Table (3.1). This value of $(\pi_{R3})_{opt}$ or even that for the compounds of Table (3.1) is not very high. Therefore, it appears that any bulky R_3 group which is closer to the axis of rotation of the thienyl ring in both the series would not be favourable to the activity. This probably may tilt the plane of the thienyl ring whose position may be essential for the binding. Unlike in equation (3.3), the absence of π_{R1} and I_2 in equation (3.4) exhibited that R_1 and R_2 substituents are not very effective in thien-2-yl derivatives.

In the derivation of equations (3.2) and (3.3) for Table (3.1), the compound 4 was not included, as it exhibited aberrant behaviour. Its observed activity is much lower than the one predicted by equation (3.2), and it is difficult to exlplain this difference. Its analogues 5 of Table (3.1) (R_3 = COOH) and 8 of Table (3.2) (R_3 = $COOC_2H_5$), having very low and high π_{R3} values equal to –0.32 and 0.51, respectively, as compared to its own (-0.01), are found to possess the observed activity practically in full agreement to the corresponding predicted ones.

In equations (3.2) and (3.4) discussed above, the independent variables used were found to be almost orthogonal to each other (Tables 3.3 and 3.4).

Table (3.3): Mutual correlations (r-value) between independent variables used in equation (3.2)

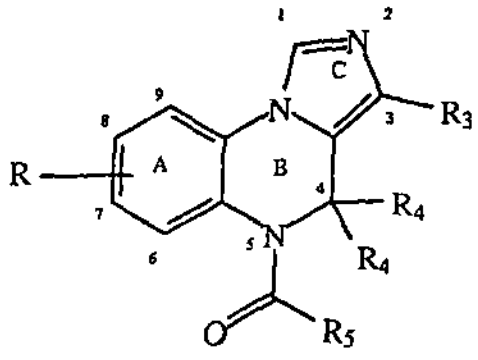
	π_{R3}	$(\pi_{R3})^2$	$(\pi_{R1})^2$	I_2	I_5
π_{R3}	1.0	0.467	0.099	0.057	0.083
$(\pi_{R3})^2$		1.0	0.131	0.075	0.109
$(\pi_{R1})^2$			1.0	0.154	0.088
I_2				1.0	0.00
I_5					1.0

Table (3.4): Mutual correlations (r-value) between independent variables used in equation (3.4)

	π_{R3}	$(\pi_{R3})^2$	I_5
π_{R3}	1.0	0.602	0.017
$(\pi_{R3})^2$		1.0	0.073
I_5			1.0

3.2 Imidazoquinoxaline Amides and Carbamates

For the series of imidazoquinoxaline amides and carbamates (3) (Table 3.5) studied by TenBrink et al.,[13] an interesting result was obtained



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in which the binding constant was found to be correlated only with some dummy parameters as given in equation (3.5). These parameters are defined as follows:

$I_3 = 0$ for R_3 = N, N-ox and 1 for other R_3 substituents; $I_4 = 0$ for R_4 = H and 1 for $R_4 = CH_3$; $I_{6,7} = 0$ for R = H and 1 for R = 6- or 7-F.

$$\log (1/K_i) = 8.69 - 0.34\ (\pm 0.23)\ I_3 - 0.47\ (\pm 0.25)\ I_4 + 0.48\ (\pm 0.23)\ I_{6,7} \qquad (3.5)$$

$$n = 15,\quad r = 0.943,\quad s = 0.15,\quad F_{3,11} = 29.47\ (5.67)$$

Equation (3.5) indicates that for quinoxaline series an N,N-ox group would be preferred than any other group at the 3-position and that methyl

Table (3.5): Imidazoquinoxaline amides and carbamates (3) and their BZR-binding constants

No.	R_3	R_4	R_5	R	log $(1/K_i)^a$ Obsd[b]	Calcd, Eq.(3.5)
1	N,O-ox[c]	H	CH_3	H	8.18	8.36
2	N,N-ox[d]	H	CH_3	6-F	9.15	9.13
3	N,N-ox	H	CH_3	7-F	9.16	9.20
4	N,N-ox	H	CH_3	H	8.82	8.69
5	N,N-ox	CH_3	CH_3	H	8.32	8.22
6	CO_2-*t*-Bu	H	CH_3	H	8.55	8.36
7	N,N-ox	H	Ph	H	8.37	8.69
8	N,N-ox	H	Ph	7-F	9.24	9.20
9	N,N-ox	H	Ph	6-F	9.11	9.13
10	N,N-ox	H	2-ClPh	H	8.85	8.69
11	CO_2-*t*-Bu	H	2-ClPh	H	8.40	8.36
12	N,N-ox	CH_3	OCH_3	H	8.17	8.22
13	N,N-ox	CH_3	O-*i*-Pr	H	8.17	8.22
14	N,N-ox	H	O-*t*-Bu	H	8.72	8.69
15	CO_2-*t*-Bu	H	O-*t*-Bu	H	8.29	8.36

[a] K_i : binding constant against [^{3}H]flunitrazepam. [b] Taken from ref.13. [c] N,O-ox:3-Cyclopropyl-1,2,4-oxadiazolyl-5. [d] N,N-ox:5-Cyclopropyl-1,2,4-oxadiazol-3-yl.

Table (3.6): Mutual correlations (r-value) between independent variable used in equation (3.5)

	I_3	I_4	$I_{6,7}$
I_3	1.0	0.302	0.364
I_4		1.0	0.302
$I_{6,7}$			1.0

groups at the 4-position will be detrimental to the activity. However, the presence of fluorine at 6- or 7-position is shown to be advantageous for the binding. No parameter for R_5 substituent was found to be correlated with the activity. Hence it can be said that this group is of little value. A comparison of the observed activity values with those calculated using equation (3.5) shows that the dummy parameters are well qualified for the prediction. All the parameters used in equation (3.5) were found to be orthogonal to each other (Table 3.6).

3.3 6-Arylpyrrolo[2,1-d][1,5]benzothiazepines

A series of 6-arylpyrrolo[2,1-d][1,5]benzothiazepines (**4**) were studied by Fiorini et al.[14] Since in this series of compounds (Table 3.7) the variation in the substituents at each substituted position is small, a Fujita-Ban approach[15] has been adopted to estimate the *de novo* contribution of substituents to the activity of the molecules.

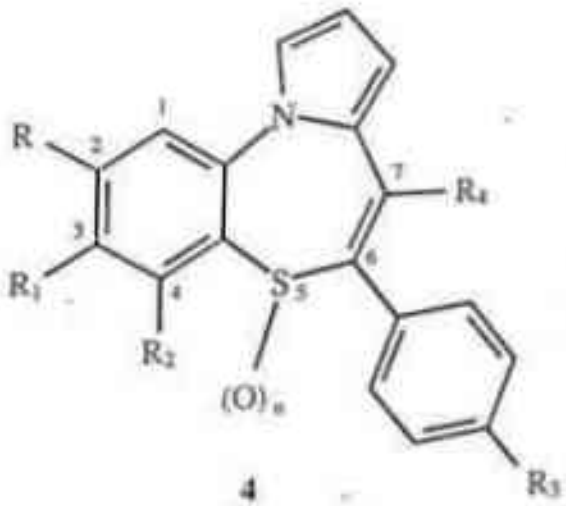

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Table (3.7): Benzothiazepines (4) and their BZR-binding affinity and physicochemical parameters

No.	R	R_1	R_2	R_3	R_4	n	log (1/IC_{50}) Obsd[a]	Calcd,[b]	Calcd,[c]
1	H	H	H	H	OSO_2CH_3	0	7.22	7.16	7.36
2	H	H	H	H	$OCOCH_3$	0	7.70	7.44	7.36
3	H	H	H	H	$OCOC_2H_5$	0	7.17	7.44	7.36
4	H	H	H	H	$OCOC_3H_7$	0	7.66	7.44	7.36
5	H	H	H	H	$OCOC_4H_9$	0	7.32	7.44	7.36
6	H	H	H	H	$OCOC_5H_{11}$	0	7.64	7.44	7.36
7	H	H	H	H	$OCON(CH_3)_2$	0	8.05	8.16	8.10
8	H	H	H	OCH_3	$OCOC_3H_7$	0	7.55	7.36	7.36
9	CF_3	H	H	OCH_3	$OCOCH_3$	0	5.34	5.56	5.56
10	CF_3	H	H	OCH_3	$OCOC_2H_5$	0	5.33	5.56	5.56
11	CF_3	H	H	OCH_3	$OCON(CH_3)_2$	0	6.54	6.29	6.30
12	Cl	H	H	H	$OCOCH_3$	0	6.25	6.22	6.17
13	Cl	H	H	H	$OCOC_2H_5$	0	6.09	6.22	6.17
14	Cl	H	H	OCH_3	$OCOCH_3$	0	6.19	6.15	6.17
15	Cl	H	H	OCH_3	$OCOC_2H_5$	0	6.31	6.15	6.17
16	Cl	H	H	OCH_3	$OCON(CH_3)_2$	0	6.77	6.87	6.92
17	H	Cl	H	H	$OCOCH_3$	0	6.64	6.67	6.63
18	H	Cl	H	OCH_3	$OCOCH_3$	0	6.62	6.59	6.63
19	H	H	Cl	H	$OCOCH_3$	0	8.10	7.94	7.90
20	H	Cl	H	OCH_3	$OCOCH_3$	0	7.70	7.86	7.90
21	H	H	H	H	$OCOCH_3$	1	6.16	6.61	6.55
22	H	H	H	H	$OCOCH_3$	2	5.94	5.79	5.75
23	H	H	H	OCH_3	$OCOCH_3$	1	5.68	6.53	6.55
24	H	H	H	OCH_3	$OCOCH_3$	2	6.19	5.71	5.75

Contd...

No.	R	R_1	R_2	R_3	R_4	n	log (1/IC_{50}) Obsd[a]	Calcd[b]	Calcd[c]
25	H	H	H	OCH_3	$OCOC_3H_7$	1	6.57	6.53	6.55
26	H	H	H	OCH_3	$OCON(CH_3)_2$	0	8.05	8.09	8.10
27	H	H	H	OCH_3	$OCOCH_3$	0	7.47	7.36	7.36
28	H	H	H	OCH_3	OSO_2CH_3	0	7.02	7.08	7.36
29	H	H	H	OCH_3	$OCOC_2H_5$	0	7.52	7.36	7.36

[a]Taken from ref.14. [b]Using the activity contributions of substituents as given in Table (3.8). [c]Using the activity contributions of substituents as given in Table (3.9).

In Fujita-Ban approach, the total activity of a molecule is given by

$$BA = \sum_i G_i X_i + \mu \qquad (3.6)$$

where G_i is the activity contribution of the ith substituent relative to H, and X_i is a parameter which takes the value of 1 or 0 according to the presence or absence of the ith substituent in the molecule. The constant μ is the activity of the unsubstituted molecule. Thus, equation (3.6) yields simultaneous equations equal in number to that of the compounds in the set. If the number of compounds in a set is sufficiently large as compared to the number of total substitutents, a least-square method is used to find out the values of G_i and μ.

For the 29 compounds of Table (3.7), we had only 9 variables including μ. The substituent R_4 was divided into 2 subsets: one that included only $OCON(CH_3)_2$ substituent and other that had the remaining substituents, all being of the type OCO-alkyl or OSO_2–alkyl. For the variable 'n' its real values as given in the table were taken for X_i. A regression analysis was then performed which revealed the activity contribution as shown in Table (3.8). The data within parentheses are 95% confidence intervals. The correlation coefficient (r), standard deviation (s), and the F-statistics (F) have also been calculated for this analysis and are given in the table. The values of these parameters are quite significant, but judged from 95% confidence level the activity contributions of OCH_3 of R_3–substituent and OCOX or OSO_2X type of R_4–substituent were not found to be very significant. Otherwise also they are

very small. Hence they were neglected and fresh regression analysis was performed with only 7 variables and the results that were obtained are listed in Table (3.9). A comparison of these results with Table (3.8) shows that the neglect of these substituents hardly made any difference. Not only the activity contributions of the remaining susbtituents remained almost same, even the values of r and s remained unaffected and the F-value became rather more significant [significant at 99% level, $F_{7,21}(0.01)=3.65$].

The results of Tables (3.8) and (3.9), however, exhibit that R- and R_1-substituents make the negative contributions to the activity, hence they should not be preferred. Similarly, the value of 'π' other than zero would be detrimental to the activity. A positive contribution is made only by R_2- and R_4-substituents and the highest contribution is of R_4= $OCON(CH_3)_2$. In this substituent the lone pair of electrons at the nitrogen seems to play some role in the drug-receptor interaction. It is quite likely that a charge-transfer phenomenon takes place in which the nitrogen acts as a donor.

Table (3.8) : Activity contributions of substituents in Table (3.8) obtained by Fujita-Ban approach

R	R_1	R_2	R_3	R_4	n	μ
CF_3= −1.80(±0.43) Cl= −1.21(±0.34)	Cl= −0.77(±0.48)	Cl= 0.50(±0.48)	OCH_3 = −0.08(±0.25)	$OCON(CH_3)_2$=1.00(±0.56) OCO-alkyl or } =0.28(±0.48) OSO_2-alkyl	−0.82(±0.23)	7.16

n=29, r=0.945, s=0.30, $F_{6,20}$=23.20

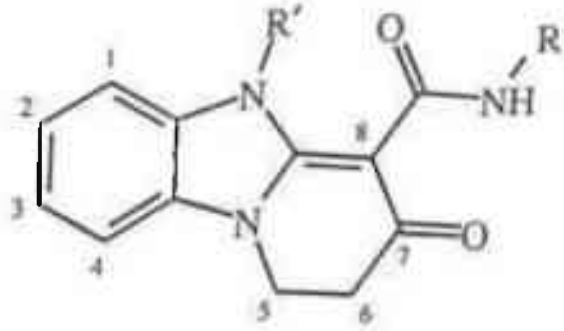
Table (3.9) : Activity contributions of substituents when R_3, and R_4 = OCO-alkyl/OSO_2 –alkyl substituents neglected

R	R_1	R_2	R_4	n	μ
CF_3=−1.80(±0.40)	Cl=−0.73(±0.47)	Cl=0.55(±0.47)	$OCON(CH_3)_2$=0.74(±0.35)	−0.80(±0.22)	7.36
Cl=−1.18(±0.33)					

n=29, r=0.945, s=0.30, $F_{7,21}$=30.69

3.4 *Pyridobenzimidazoles*

The series of pyrido[1,2-a]benzimidazoles (5) were studied by Maryanoff et al.[16] For this series of benzimidazoles (Table 3.10), the BZR binding affinity was measured in the absence and presence of GABA.



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In both the situations the affinity was expressed in terms of IC_{50}, the molar concentration of the compound leading to 50% inhibition of [^{3}H]flunitrazepam binding to BZR. These IC_{50} values for both the measurements were found to be well correlated with hydrophobic constant (π) of R moiety in 8-CONHR group and electronic constant (Hammett constant σ) of ortho-substituent, if any, present in this moiety (equations 3.7 and 3.8).

$$\log(1/IC_{50})_{noGABA} = 4.005(\pm 1.375)\pi_R - 1.427(\pm 0.464)\pi_R^2 + 7.179(\pm 3.262)\sigma_o + 5.470 \quad (3.7)$$

$$n=18, \quad r=0.881, \quad s=0.54, \quad F_{3,14}=16.10$$

Table (3.10): Pyrido[1,2-a]benzmidazoles (5) and their BZR-binding affinity and physicochemical parameters

No.	R	R'	π_R	σ_o	log (1/IC$_{50}$) no GABA Obsd[a]	log (1/IC$_{50}$) no GABA Calcd, Eq.(3.9)	log (1/IC$_{50}$) GABA Obsd[a]	log (1/IC$_{50}$) GABA Calcd, Eq.(3.10)
1	Ph	H	1.96	0.00	8.04	7.62	8.23	7.82
2	4-ClPh	H	2.44	0.00	6.21	6.63	6.58	6.87
3	3-ClPh	H	2.44	0.00	6.92	6.63	7.02	6.87
4	2-ClPh	H	2.44	0.23	7.92	8.20	8.28	8.51
5	2-FPh	H	1.87	0.06	8.77	8.14	8.85	8.34
6	4-MeOPh	H	1.71	0.00	7.39	7.90	7.80	8.06
7	3-MeOPh	H	1.71	0.00	7.59	7.90	7.80	8.06
8	2-MeOPh	H	1.71	-0.27	6.00	6.05	6.03	6.14
9	2,6-Cl$_2$Ph	H	2.92	0.23	6.49	6.64	6.77	6.96
10	2,6-F$_2$Ph	H	1.78	0.06	8.55	8.24	8.77	8.43
11	c-C$_6$H$_{11}$	H	2.51	0.00	6.85	6.44	7.14	6.69
12	c-C$_4$H$_7$	H	1.80	0.00	7.52	7.81	7.60	7.99
13	c-C$_3$H$_5$	H	1.14	0.00	7.80	7.94	7.74	7.96
14	H	H	0.00	0.00	5.62	5.56	5.22	5.14
15	Ph	Me	1.96	0.00	8.24	8.42	8.68	8.79
16	2-FPh	Me	1.87	0.06	9.38	8.95	9.59	9.32
17	Ph	Et	1.96	0.00	8.68	8.42	8.85	8.79
18	Ph	PhCH$_2$	1.96	0.00	7.92	8.42	8.57	8.79

[a]Taken from ref. 16

$$\log (1/IC_{50})_{GABA} = 4.592(\pm 1.387)\pi_R - 1.549(\pm 0.468)\pi_R^2 + 7.539(\pm 3.290)\sigma_o + 5.033 \quad (3.8)$$

$n=18,\ r=0.899,\ s=0.54,\ F_{3,14}=19.57$

In both the cases, the correlations were further improved significantly when an indicator parameter I equal to unity was used for R' substituents present in the last 4 compounds (equations 3.9 and 3.10). The positive and reasonably large coefficient of this parameter suggests that a substituent present at N9 would increase the activity, which may be probably due to

$$\log(1/IC_{50})_{noGABA} = 3.530(\pm 1.074)\pi_R - 1.266(\pm 0.363)\pi_R^2 + 6.831(\pm 2.456)\sigma_o + 0.809(\pm 0.513)I + 5.559 \quad (3.9)$$

$n=18,\ r=0.937,\ s=0.41,\ F_{4,13}=23.48$

$$\log (1/IC_{50})_{GABA} = 4.019(\pm 0.865)\pi_R - 1.355(\pm 0.292)\pi_R^2 + 7.120(\pm 1.977)\sigma_o + 0.975(\pm 0.413)I + 5.142 \quad (3.10)$$

$n=18,\ r=0.966,\ s=0.33,\ F_{4,13}=45.69$

some steric effect (the steric fit with the receptor site). A large positive value of σ_o (since the value of σ for ortho-substituents is not defined, its value has been taken equal to that of σ_p) in all equations (3.7-3.10) suggests that an electron-withdrawing ortho-susbstituent in the R moiety will be beneficial to the binding of the compounds with the receptor in both the situations, i.e., presence or absence of GABA. The electron-

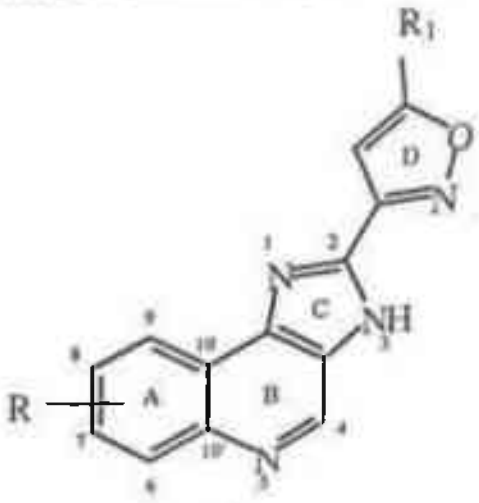
withdrawing effect of the substituent may probably increase the polarity of CONH fraction, which may be expected to be involved in some dipole-dipole interaction with the receptor.

The parabolic correlation with π_R suggests that the R moiety may be involved in some hydrophobic interaction with the receptor but that the effect would be optimized with essentially an equal value of $\pi_{R,opt}$ =1.40 (absence of GABA, equation 3.9) and $\pi_{R,opt}$ =1.48 (presence of GABA, equation 3.10). Both equations (3.9) and (3.10) represent highly significant correlations and account for 87.8 and 93.3% of the variance in the activity, respectively. All the parameters used in these equations were mutually orthogonal [r : (π_R/σ_o) = 0.36, (π_R/I) =0.03, (σ_o/I) = 0.03].

3.5 Fused Imidazopyridines

For the series of fused imidazopyridines (6) and (7) (Table 3.11), studied by Takada et al.,[17] first the Fujita-Ban analysis was performed and the contributions of the substituents were assessed. Table (3.12) lists the activity contribution of each substituent along with that of parent structure.The statistical parameters of this analysis were highly significant. However, judged from 95% confidence interval the activity contributions of some substituents (marked by an asterisk) seemed to be insignificant. Therefore, they were neglected and the activity contributions of the remaining substituents were re-assessed (Table 3.13) and found that they were essentially same as obtained previously. The

alues of statistical parameters (r and s) also remained essentially same.



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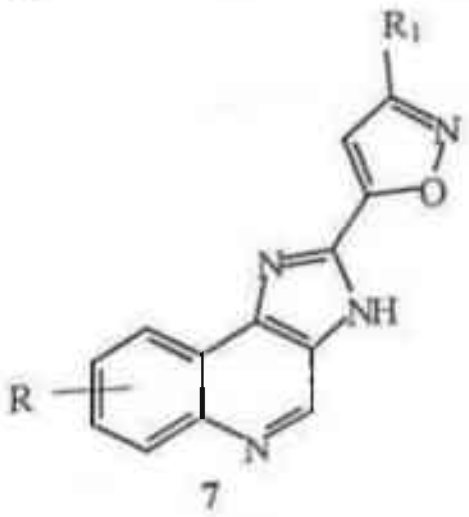

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From this analysis, it is observed that all the substituents whose activity contribution is statistically significant produce negative effect. They, therefore, can be assumed to have some steric effects that can be accounted for by some steric parameters.

Using various steric parameters in Hansch analysis, the best correlation that we could find was

$$\log (1/K_i) = 9.258 - 1.724(\pm 0.151)B_{1,6} - 1.449(\pm 0.346)MR_7 - 0.591\ (\pm 0.151)B_{1,9} - 0.293(\pm 0.167)I \qquad (3.11)$$

$$n=26,\quad r=0.982,\quad s=0.20,\quad F_{4,21}=145.84$$

In this equation, B_1 is Verloop's width parameter[18] and MR is the molar refractivity scaled by 0.1. The use of B_1 for 6- and 9- substituents and MR for 7- substituents significantly accounts for the steric effects of these substituents.

Table (3.11): Imidazopyridines (6) and (7) and their BZR-binding affinity and physicochemical parameters

No.	R	R_1	$B_{1,6}$	MR	$B_{1,9}$	log (1/K_i) Obsd[a]	Calcd,[b]	Calcd,[c]	Calcd,Eq.(3.11)
1	H	H	0	0	0	9.22	9.23	9.17	9.25
2	H	Me	0	0	0	9.05	8.94	8.92	8.96
3	H	Et	0	0	0	8.96	8.94	9.17	8.96
4	6-F	H	1.35	0	0	7.40	7.11	7.09	6.93
5	6-Cl	H	1.80	0	0	6.28	6.02	6.00	6.15
6	7-F	H	0	0.10	0	9.05	9.10	9.17	9.11
7	7-Cl	H	0	0.60	0	8.46	8.24	8.22	8.39
8	7-MeO	H	0	0.79	0	8.14	8.23	8.21	8.11
9	8-F	H	0	0	0	9.10	9.18	9.17	9.26
10	8-Cl	H	0	0	0	9.10	9.35	9.17	9.26
11	8-MeO	H	0	0	0	9.30	9.32	9.17	9.26
12	9-F	H	0	0	1.35	8.47	8.54	8.52	8.46
13	9-Cl	H	0	0	1.80	8.12	8.14	8.11	8.19
14	H	H	0	0	0	9.00	9.23	9.17	9.26
15	H	Me	0	0	0	9.05	8.94	8.92	8.96
16	H	Et	0	0	0	8.92	8.94	9.17	8.96
17	6-F	Me	1.35	0	0	6.52	6.82	6.84	6.64
18	6-Cl	Me	1.80	0	0	5.47	5.73	5.75	5.86
19	7-F	Me	0	0.10	0	8.85	8.81	8.92	8.82
20	7-Cl	Me	0	0.60	0	7.73	7.95	7.97	8.10
21	7-MeO	Me	0	0.79	0	8.02	7.94	7.96	7.82
22	8-F	Me	0	0	0	8.96	8.89	8.92	8.96
23	8-Cl	Me	0	0	0	9.30	9.06	8.92	8.96
24	8-MeO	Me	0	0	0	9.05	9.03	8.92	8.96
25	9-F	Me	0	0	1.35	8.31	8.25	8.27	8.17
26	9-Cl	Me	0	0	1.80	7.86	7.85	7.87	7.90

[a]Taken from ref. 17. [b]Using the activity contributions of substituents as given in Table (3.12). [c]Using the activity contributions of substituents as given in Table (3.13).

The parameter I in equation (3.11) is an indicator variable used with a value of unity for the substituent R_1= Me/Et. Although in Fujita-Ban analysis, the activity contribution of Et was statistically not significant, in magnitude it was essentially same as that of Me. Therefore, I was used for both and it is seen in equation (3.11) that its coefficient exactly matches with activity contributions of Me/Et obtained by Fujita-Ban approach.

The series of Table (3.11) contains, however, two types of derivatives with respect to 2-isoxazolyl ring. The first 13 compounds are 2-isoxazol-3-yl derivatives and the remaining 13 compounds (14-26) are 2-isoxazol-5-yl derivatives. An attempt was made to account for the effect of this difference, using a dummy variable D with a value of zero for all 2-isoxazol-3-yl derivatives and one for all 2-isoxazol-5-yl derivatives, but the resulting correlation (equation 3.12) did not exhibit any significance of this parameter rather marginalized the effect of R_1 substituent, suggesting that the point of attachment in 2-isoxazolyl ring hardly matters, instead its R_1–substituent shows a detrimental effect.

$$\log (1/K_i) = 9.265 - 1.720(\pm 0.152)B_{1,6} - 1.438(\pm 0.348)MR_7 - 0.586(\pm 0.152)B_{1,9} - 0.192(\pm 0.264)I - 0.130(\pm 0.263)D \qquad \textbf{(3.12)}$$

$$n=26,\quad r=0.983,\quad s=0.20,\quad F_{5,20}=117.24$$

Table (3.12): Activity contributions of substituents in Table (3.11) by Fujita-Ban approach

R	R_1	μ	
6-F = -2.12(±0.41)	Me = -0.29(±0.19)	9.23	
6-Cl = -3.21(±0.41)	Et* = -0.28(±0.42)		
7-F* = -0.13 (±0.41)			n = 26
7-Cl = -0.99 (±0.41)			r = 0.987
7-OMe = -1.00(±0.41)			s = 0.22
8-F* = -0.05(±0.41)			$F_{12,13}$ = 41.11
8-Cl* = 0.12(±0.41)			
8-OMe* = 0.10(±0.41)			
9-F = -0.69(±0.41)			
9-Cl = -1.09(±0.41)			

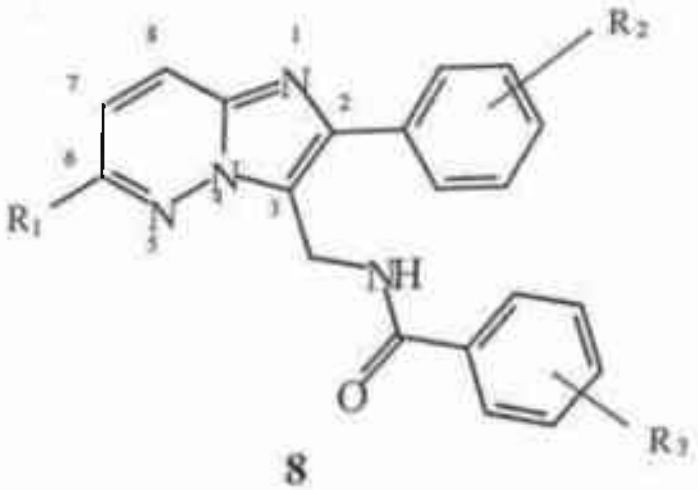
Table (3.13): Activity contributions of substituents when insignificant substituents (marked with asterisk in Table 3.12) neglected

R	R_1	μ	
6-F = -2.09(±0.35)	Me = -0.25(±0.18)	9.17	
6-Cl = -3.17(±0.35)			n = 26
7-Cl = -0.95(±0.35)			r = 0.983
7-OMe = -0.97(±0.35)			s = 0.22
9-Cl = -1.06 (±0.35)			$F_{5,20}$ = 72.06
9-F = -0.66(±0.35)			

3.6 6-Alkoxyimidazo[1,2-b]pyridazines

A series of 6-alkoxyimidazo[1,2-b] pyridazines (8) (Table 3.14) were also found to bind with benzodiazepine receptors by Harrison et al.[19] A Fujita-Ban analysis for this series of compounds was performed. The activity contributions of only those substituents for which they were statistically significant are reported.

From the results as listed in Table (3.15), we find that, of R_1 substituents, the OCH_3 group and, of R_2 substituents, the 3,4-OCH_2O group make the largest contributions.



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At R_1 the OCH_3 group might be having the optimum shape and size required for the interaction with the receptor and hence the less favourable effect of OC_2H_5 may be attributed to some steric role played by it. This proposition is based on the observation that of all the alkoxy

Table (3.14):3-substituted imidazo[1,2-b]pyridazines (8) and their BZR binding affinities against [^{3} H]diazepam binding.

No.	R_1	R_2	R_3	log $(1/IC_{50})$ Obsd[a]	log $(1/IC_{50})$ Calcd,[b]
1	OCH_3	3,4-OCH_2O	H	8.15	7.90
2	OCH_3	4-Cl	H	7.54	7.53
3	OCH_3	H	2-F	6.86	7.09
4	OCH_3	4-CH_3	2-F	7.68	7.61
5	OCH_3	3,4-OCH_2O	2-F	7.85	7.90
6	OC_2H_5	H	H	6.73	6.77
7	OC_2H_5	4-CH_3	H	7.46	7.29
8	OC_2H_5	3,4-OCH_2O	H	7.60	7.57
9	OC_2H_5	4-Cl	H	7.19	7.20
10	OC_2H_5	H	2-F	6.68	6.77
11	OC_2H_5	4-CH_3	2-F	7.29	7.29
12	OC_2H_5	3,4-OCH_2O	2-F	7.51	7.57
13	OC_3H_7	4-CH_3	H	6.74	6.78
14	OC_3H_7	3,4-OCH_2O	H	6.94	7.06
15	OC_3H_7	H	2-F	6.62	6.26
16	OC_3H_7	4-CH_3	2-F	6.84	6.78
17	OCH_2OCH_3	4-CH_3	H	6.50	6.78
18	OCH_3	3,4-OCH_2O	3-NO_2	8.10	7.90
19	OCH_3	3,4-OCH_2O	4-NO_2	7.64	7.90

[a] Taken from ref.19, [b] Calculated using data of Table (3.15)

Table (3.15): Activity contributions of substituents in Table (3.14) obtained by Fujita-Ban approach.

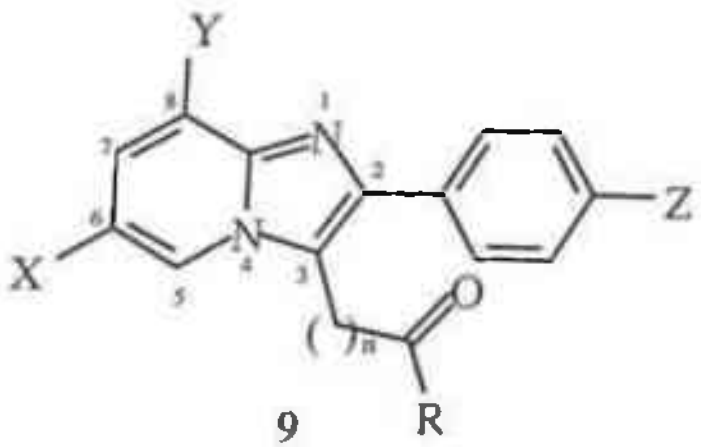
R_1	R_2	μ
OCH_3 =0.837 (± 0.270)	3,4-OCH_2O=0.803(±0.269)	6.257
OC_2H_5 = 0.513 (± 0.255)	4-Cl = 0.433 (± 0.366)	
	4-CH_3 = 0.518 (± 0.274)	
n = 19 r = 0.946	s = 0.197 $F_{5,13}$ = 22.05 (4.86)	

substituents used, or that can be used, for R_1 , the OCH_3 group is the smallest in size and has the activity contribution larger than the next smallest group, OC_2H_5. Groups bigger than OC_2H_5 have not been found to make any significant contributions. This essentially indicates the shape and size effects and points out that the receptor site may not be able to accommodate a substituent bigger than OCH_3. Just next in the alkoxy series OC_2H_5 is probably accommodated but with some strain, resulting linto a statistically significant effect but less than that of OCH_3.

The largest contribution of 3,4-OCH_2O at R_2 can be attributed to its ring structure and its planarity, producing the best possible interaction with the receptor through polar interaction or hydrogen bonding.

3.7 2-Phenylimidazo[1,2-α]pyridines

For the series of 2-phenylimidazo[1,2-α]pyridines (9) (Table 3.16) studied by Trapani et al.,[20] we used the Hansch approach and found



9 R

the binding data to be correlated as

$$\log (1/IC_{50}) = 2.051(\pm 0.929)\pi_X - 1.320 (\pm 1.087)(\pi_X)^2 - 1.650 (\pm 0.376)I_Z + 1.054 (\pm 0.290)I_R + 5.028 \quad (3.13)$$

$n = 22, \quad r = 0.936, \quad s = 0.26, \quad F_{4,17} = 29.92 (4.67), \quad (\pi_X)_0 = 0.78$

The π_X in this equation refers to the hydrophobic constant of X-substituent and I_Z and I_R are two indicator parameters used for Z- and R-substituents. I_Z takes a value of 1 for Z=Cl and zero for any other

Table (3.16): 2-Phenylimidazo[1,2-α]pyridine derivatives (9) and their BZR binding affinities against [^{3}H]flunitrazepam binding and physicohemical parameters.

No.	X	Z	R	n	π_X	log (1/IC_{50}) Obsd[a]	log (1/IC_{50}) Calcd, Eq.(3.13)
1	Cl	Cl	$N(C_2H_5)_2$	0	0.71	5.08	5.22
2	Cl	Cl	$N(C_3H_7)_2$	0	0.71	5.06	5.22
3	Cl	Cl	N-$(CH_2)_4$-	0	0.71	5.53	5.22
4	H	H	$N(C_3H_7)_2$	1	0	6.33	6.08
5	Cl	H	$N(C_3H_7)_2$	1	0.71	7.07	6.87
6	Br	H	$N(C_3H_7)_2$	1	0.86	6.94	6.87
7	I	H	$N(C_3H_7)_2$	1	1.12	6.54	6.73
8	CH_3	H	$N(C_3H_7)_2$	1	0.56	6.90	6.82
9	CH_3O	H	$N(C_3H_7)_2$	1	-0.02	5.55	6.04
10	NO_2	H	$N(C_3H_7)_2$	1	-0.28	5.56	5.40
11	Br	H	$N(C_3H_7)_2$	1	0.86	7.24	6.87
12	Cl	H	N-$(CH_2)_4$-	1	0.71	7.04	6.87
13	Cl	H	N-$(CH_2)_5$-	1	0.71	6.62	6.87
14	Cl	H	$N(C_3H_7)_2$	2	0.71	6.51	6.87
15	Cl	H	OC_2H_5	0	0.71	5.67	5.82
16	CH_3	CH_3	OC_2H_5	0	0.56	5.94	5.76
17	CH_3	H	OC_2H_5	1	0.56	5.86	5.76
18	Cl	H	OC_2H_5	1	0.71	5.93	5.82
19[b]	Cl	Cl	OC_2H_5	1	0.71	6.16	4.17
20	Cl	H	OC_2H_5	2	0.71	5.95	5.82
21	CH_3	H	OC_4H_9	1	0.56	5.50	5.76
22	Cl	H	OC_4H_9	1	0.71	5.60	5.82
23[b]	Cl	Cl	OC_4H_9	1	0.71	5.56	4.17
24	Cl	H	OC_4H_9	2	0.71	5.93	5.82

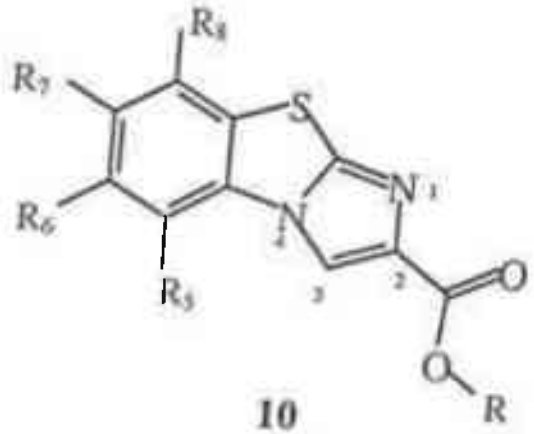
[a]Taken from ref. 20. [b]Not included in the derivation of equation (3.13)

substituent. Similarly, I_R takes a value of 1 for R = an alkylamine group and zero for others. Thus equation (3.13) expresses that an X-substituent will have a hydrophobic effect on the activity, but since there is a parabolic correlation with π_X, the π_X will have an optimum value equal to 0.78, suggesting a limited bulk tolerance at the receptor site. The negative coefficient of I_Z points out that Z = Cl would have a detrimental effect, probably because of having a number of lone pairs of electrons that can cause a repulsive effect with a negatively charged site of the receptor. The positive coefficient of I_R, however, suggests that an amine group at the position of R will have a favourable effect, which can be attributed to the formation of highly polar amide group, [-CON-], that can participate in strong polar interaction with the receptor.

The chain length at the 3-position (the value of n) was not found to matter. However, in the derivation of equation (3.13), compounds 19 and 23 were not included as they exhibited aberrant behaviour. The equation predicts very low activity for them as compared to their corresponding observed activity (Table 3.16). The reasons for these aberrations are not very apparent.

3.8 Imidazo[2,1-b]benzothiazoles

Trapani et al.[21] also reported the synthesis and BZR binding affinity for a series of imidazo[2,1-b]benzothiazoles (**10**) which are listed in Table (3.17).



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For this series of ligands, the best correlation obtained from the Hansch analysis was as

$$\log (1/IC_{50}) = 3.549(\pm 1.089)\ \pi_{R7} - 5.169\ (\pm 1.290)\ (\pi_{R7})^2 + 7.123 \qquad (3.14)$$

$$n = 14,\ r = 0.945,\ s = 0.23,\ F_{3,11} = 46.16\ (6.22),\ (\pi_{R7})_0 = 0.34$$

which suggested the hydrophobic role of R_7-substituent with an optimum value of (π_{R7})=0.34. Such a low optimum value points out that highly hydrophobic R_7-substituents will not be tolerated. In the derivation of this equation, compound 12, whose observed activity (6.47) was found to be much lower than the predicted one (7.49), was not included. Its lower observed activity than expected can be due to the presence, at the two adjacent positions (6 and 7), of CH_3 groups in tetrahedral geometry, producing steric hindrance for each other.

In this series the variation in R group in 2-position substituent was not found to matter, which meant that R moiety had nothing to do in the

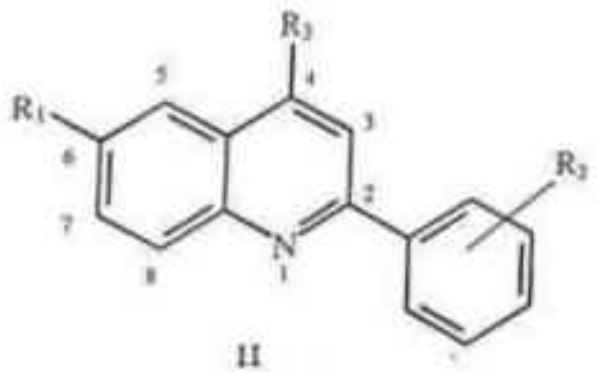
Table (3.17):2-(Alkoxycarbonyl)-4*H*-imidazo[2,1-b]benzothiazoles (10) and their BZR binding affinities against [^{3}H]flunitrazepam and physicochemical parameters.

No.	R_1	R_5	R_6	R_7	π_{R7}	log (1/IC_{50}) Obsd[a]	log (1/IC_{50}) Calcd, Eq.(3.14)
1	OCH_3	H	H	H	0	7.22	7.12
2	OC_2H_5	H	H	H	0	6.92	7.12
3	$OCH_2C_6H_5$	H	H	H	0	7.30	7.12
4	OC_2H_5	H	H	C_2H_5	1.02	5.40	5.37
5	OC_2H_5	H	H	F	0.14	7.29	7.52
6	OC_2H_5	H	H	Cl	0.71	6.83	7.04
7	OC_2H_5	H	H	Br	0.86	6.51	6.35
8	OC_2H_5	H	H	NO_2	-0.28	5.54	5.72
9	OC_2H_5	Cl	H	H	0	7.44	7.12
10	OC_2H_5	OCH_3	H	H	0	7.40	7.12
11	OC_2H_5	CH_3	H	H	0	7.01	7.12
12[b]	OC_2H_5	H	CH_3	CH_3	0.56	6.47	7.49
13	OC_2H_5	H	Cl	Cl	0.71	6.98	7.64
14	OCH_3	Cl	H	H	0	7.21	7.12
15	$OCH_2C_6H_5$	Cl	H	H	0	6.86	7.12

[a]Taken from ref. 21. [b] Not included in the derivation of equation (3.14)

interaction, if any, of COOR group with the receptor. *In this group, it may* be only COO moiety which can have a constant polar interaction or hydrogen bonding with the receptor. As suggested in the case of derivative of **9** (equation 3.13), a -CON- may have better effect than -COO- group, and it may be due to the greater polarity of the former than the latter.

3.9 2-Aryl-4-piperidinoquinolines



11

For the series of 2-aryl-4-piperidinoquinolines (**11**) (Table 3.18), studied by Andersen et al.,[22] we could correlate the activity of the compounds as

$$\log (1/IC_{50}) = 2.574(\pm 0.477)\, D - 1.850\,(\pm 0.985)\,\pi_{R2} + 3.362\,(\pm 1.914)\sigma_{R2} + 1.784\,(\pm 1.573)\,\sigma_{R1} - 1.148\,(\pm 0.469)\,V_{w,R3} + 6.220 \qquad (3.15)$$

$$n = 34,\quad r = 0.923,\quad s = 0.43,\quad F_{5,28} = 32.32\ (3.76)$$

which suggested that the hydrophobic nature of R_2-substituent will not be

Table (3.18):2-Arylquinolines (**11**) and their BZR binding affinities against [^{3}H]flunitrazepam binding and physicochemical parameters.

No.	R_1	R_2	R_3	π_{R2}	σ_{R1}	σ_{R2}	V_{vdw} (10^2A$^{\circ 3}$)	log (1/IC_{50}) Obsd.	log (1/IC_{50}) Calcd. Eq.(3.15)
1	H	H	OH	0	0	0	0.137	5.19	6.06
2	CH_3	H	OH	0	-0.17	0	0.137	6.23	5.76
3	Cl	H	OH	0	-0.23	0	0.137	6.37	6.47
4	H	H	A	0	0	0	1.410	7.07	7.18
5	H	H	B	0	0	0	1.372	7.30	7.22
6	H	H	C	0	-0.17	0	1.133	7.95	7.49
7	CH_3	H	C	0	0.23	0	1.133	6.98	7.19
8	Cl	H	C	-0.02	0	-0.27	1.133	7.69	7.90
9	H	2-OCH_3	C	-0.67	0	-0.37	1.133	5.97	6.62
10	H	2-OH	C	-0.02	0.06	-0.27	1.133	7.71	7.49
11	F	2-OCH_3	C	-0.67	0.06	-0.37	1.133	7.29	6.73
12	F	2-OH	C	0.14	0.06	0.06	1.133	8.15	7.60
13	F	4-F	C	0.71	0.06	0.23	1.133	8.06	7.54
14	F	4-Cl	C	0	0	0	1.423	7.44	7.06
15	H	H	D	-0.67	0.06	-0.37	1.423	6.74	7.16
16	F	2-OH	D	0	0	0	1.437	6.86	7.26
17	CH_3	H	E	0	-0.17	0	1.437	7.20	7.14
18	Cl	H	E	0	0.23	0	1.437	6.60	6.84
19	H	H	E	-0.02	0	-0.27	1.437	7.09	7.55
20	H	2-OCH_3	E	-0.67	0	-0.37	1.437	5.76	6.27
21	H	2-OH	E	-0.02	0.06	-0.27	1.437	7.50	7.14
22	F	2-OCH_3	E	-0.67	0.06	-0.37	1.437	6.72	6.38
23	F	2-OH	E	0.14	0.06	0.06	1.437	7.44	7.25
24	F	4-F	E					7.53	7.19

Contd...

No.	R_1	R_2	R_3	π_{R3}	σ_{R1}	σ_{R2}	$V_{w,R3}$ (10^2A^{03})	log ($1/IC_{50}$) Obsd[a]	Calcd, Eq.(3.15)
25	F	4-Cl	E	0.71	0.06	0.23	1.437	6.66	6.71
26	H	H	F	0	0	0	1.050	7.17	7.59
27	F	2-OH	F	-0.67	0.06	-0.37	1.050	7.30	7.69
28	H	H	G	.0	0	0	1.437	7.16	7.14
29	H	H	OCH_3	0	0	0	0.304	6.33	5.87
30	CH_3	H	OCH_3	0	-0.17	0	0.304	5.35	5.57
31	H	H	$OC_6H_5CH_2$	0	0	0	1.000	5.64	5.07
32	CH_3	H	$OC_6H_5CH_2$	0	-0.17	0	1.000	4.63	4.77
33[b]	H	H	$O(CH_2)_3COO$ Et	0	0	0	1.176	6.19	4.87
34	H	H	O-(m-$CO_2C_2H_5$)C_6H_4	0	0	0	1.532	4.55	4.46
35	H	H	O-(m-$CONHC_2H_5$)C_6H_4	0	0	0	1.561	4.17	4.43

[a] Taken from ref. 22 , [b] Not included in the derivation of equation (3.15)

A = N-1-ethyl-1-piperazine carboxamide
B = methylamino-piperazinyl methanethione
C = 4-piperidine carboxamide
D = ethyl-4-piperidine carboxylate
E = 3-methyl-5-(4-piperidinyl)-1,2,4-oxadiazole
F = 4-piperidine carbonitrile
G = 5-methyl-3-(4-piperidinyl)-1,2,4-oxadiazole

conducive to the activity. Rather its electronic nature–electron-withdrawing property as reflected by σ –will be highly favourable to the activity. Similarly, the electron-withdrawing property of R_1-substituent is also exhibited to be highly effective. However, the negative coefficient of $V_{w,R3}$, the van der Waals volume of R_3-substituent, indicates that the large size of R_3-substituent will not be advantageous, but all nitrogen-containing substituents, for which the indicator variable D has been used with a value of unity, seem to be quite favourable. All these substituents may be expected to have some constant electron-withdrawing effect.

In the derivation of equation (3.15), however, compound 33 was found to be slightly misfit, hence excluded. The equation predicts a very low activity for this compound as compared to its observed activity (Table 3.18). Its high activity may be attributed to its R_3-substituent, $O(CH_2)_3COOEt$, which is not a nitrogen-containing group but can produce some electron- withdrawing effect directly or indirectly.

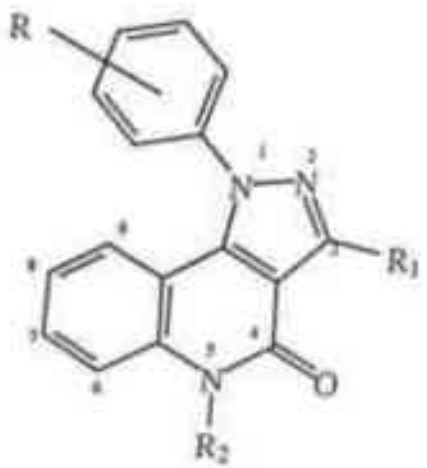
Now if we compare derivatives of **11** with those of **10**, we find that the R_1-substituent in the former and the R_7-substituent in the latter are at identical positions. Equation (3.13) suggests that a highly hydrophobic R_7-substituent ($\pi_{R7} > 0.34$) will not be beneficial. This is supplemented by equation (3.15) which indicates that instead of a hydrophobic substituent an electron-withdrawing substituent at that position (R_1-substituent) will be more advantageous. Thus R_7 in **10** or R_1 in **11** can be assumed to interact with a site at the receptor which is basically nonpolar in nature,

capable of interacting with small hydrophobic substituents, but can be polarized by large substituents, and thus strength of the binding will depend on the extent of the polarization of the active site which would be the function of the electronic charge over the substituents withdrawn from some electron-rich position of the molecule.

Similarly, a R_3-substituent is also indicated by equation (3.15) to interact with a polarizable or already a polar or cationic site, affecting the binding by its electron-withdrawing ability. Similar behaviour then can be expected from N1 in **8** and **9** and S9 in **10**. COR group has been already discussed to be involved in polar interaction with a better effect if R is an amine moiety. Thus the electronic interactions seem to dominate the activity of the compounds. However, there can also be assumed the presence of certain hydrophobic pockets, too, in the receptor, engulfing some hydrophobic moieties present in the compounds.[12]

3.10 Arylpyrazoloquinolinones and Arylbenzopyranopyrazolones

Some authors focussed the attention on the synthesis of 1-arylpyrazolo[4,5-c]quinolin-4-ones[25-29] (**12**) and 1-aryl- and 2-aryl[1]benzopyranopyrazol-4-ones[30,31] (**13**, **14**). All these compounds were tested for their ability to displace [^{3}H] flunitrazepam from bovine brain membrane. We have compiled all these compounds and listed the series of **12** in Table (3.19) and the series of **13** and **14** in Table (3.20) along with their IC_{50} values.



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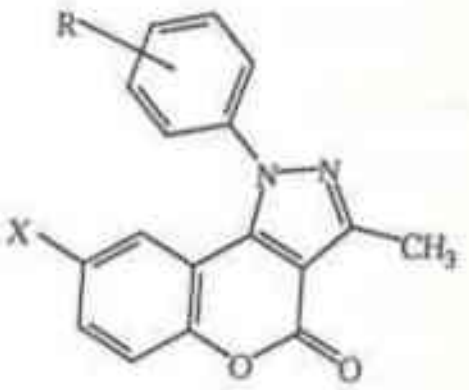

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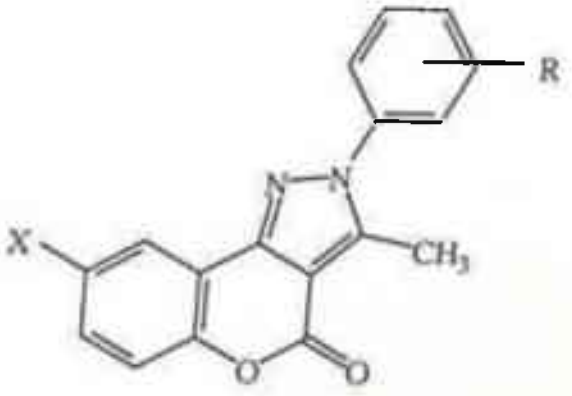


14

In the case of all the three series of compounds, the variation in substitution occurs mostly at the aryl ring present at 1 or 2 position. At other position in any series the substituents are limited e.g., in the series of **12** (Table 3.19), the R_1 – substituent at 3-position is either methyl or phenyl group and the R_2 –substituent at 5-position is either hydrogen or methyl group. Similarly, in the series of **13** and **14** (Table 3.20), the

additional substituent X at position 8 is H, Br, or NO_2. Thus the activity of the compounds has been mostly controlled by R-substituents at the aryl ring. We therefore tried to correlate the activity with the physicochemical properties of these substituents and used some indicator variables for R_1-, R_2-, and X-substituents. The variables I_1 and I_2 were used for R_1 and R_2, respectively, with the values of 0 and 1 each. $I_1 = 0$ for R_1 = Me and I_1 = 1 for R_1 = Ph; $I_2 = 0$ for R_2 = H and I_2 = 1 for R_2 = Me. Similarly, the variable I_x was used to indicate whether, in the series of **13** and **14** (Table 3.20), the X-substituent was H or Br/NO_2 with a value of 0 and 1, respectively.

A multiple regression analysis revealed that the activity of the compounds was primarily governed by the hydrophobic character of the meta-substituents and the steric property of the ortho-substituents at 1-aryl ring in the series of both **12** and **13**. Significant correlations were obtained for both the series, using the data of Tables (3.19) and (3.20), respectively, between their IC_{50} values and the hydrophobic constant π of meta-substituents and the steric constant E_s (Taft constant) of ortho-substituents (equations 3.16 and 3.17, respectively).

$$\log (1/IC_{50}) = 0.984\ (\pm 0.319)\ \pi_m + 0.443\ (\pm 0.203)\ E_{s,o} + 1.229\ (\pm 0.266)\ I_2 + 4.707 \qquad (3.16)$$

$$n = 35,\ r = 0.94,\ s = 0.32,\ F_{3,31} = 72.95$$

$$\log (1/IC_{50}) = 1.038\ (\pm 0.320)\ \pi_m + 0.902\ (\pm 0.301)\ E_{s,o} + 6.185 \quad (3.17)$$

$$n = 25,\ r = 0.91,\ s = 0.40,\ F_{2,22} = 53.03$$

Of the indicator variables used, only I_2, indicating the effect of Me group at N5 position in the series of **12**, could be found of some significance (equation 3.16). The I_1 parameter used to describe the effect of Me/Ph group at 3-position in the same series was found to be of little significance (equation 3.18) and so was the case with the I_x parameter used to indicate the effect of Br/NO_2 at position 8 in the series of **13** (equation 3.19). However, the high coefficient of I_2 indicates that in the case of 1-arylpyrazolo[4,5-c]quinolin-4-ones (**12**), the replacement of hydrogen from N5 by Me group would increase the activity of the compounds by a factor of nearly 15.

$$\log (1/IC_{50}) = 0.982\ (\pm 0.315)\pi_m + 0.407\ (\pm 0.208)E_{s,o} + 1.169\ (\pm 0.279)I_2 + 0.170\ (\pm 0.263)\ I_1 + 4.654 \quad (3.18)$$

$$n = 35,\ r = 0.94,\ s = 0.32,\ F_{4,30} = 56.46$$

$$\log (1/IC_{50}) = 0.973\ (\pm 0.375)\pi_m + 0.883\ (\pm 0.310)E_{s,o} + 0.154\ (\pm 0.453)I_x + 6.155 \quad (3.19)$$

$$n = 25,\ r = 0.91,\ s = 0.41,\ F_{3,21} = 34.72$$

A comparison of equations (3.16) and (3.17) with each other shows that the coefficient of π_m in both the equations is almost identical, but

that of $E_{s,o}$ in equation (3.17) is almost double of that in equation (3.16). These observations suggest that in the series of both 1-arylpyrazolo[4,5-c]quinolin-4-ones (1-APQs) and 1-aryl[1]benzopyranopyrazol-4-ones (1-ABPs), the substitution of any group at the meta position of the aryl ring will lead to an identical effect but at the ortho position it will produce 3 times more effect in 1-ABPs than in 1-APQs.

In the derivation of equation (3.17), the compound 19 of the Table (3.20) was not included, as it was found to be an outlier. The predicted activity of this compound was found to be much higher than its observed activity. It was hard to explain why this compound possesses so low activity. If we compare its activity (4.96) with that of compound 1 (6.52), which differs only in terms of the X-substituent at position 8, an interesting anomaly is found. While the former has bromine at this position, the latter is completely unsubstituted at this position. An examination of Table (3.20) does not show that there should be any drastic reduction in the activity of compound by substitution at this position. In fact the quantitative analysis of the effect (equation 3.19) suggests that, although I_x parameter used to account for this effect is statistically of little significance, the substitution of Br or NO_2 group at this position should lead to an increase in the activity. Hence it is found difficult to explain the low activity of compound 19. It can be attributed to an experimental error.

If we combine compounds 1-20 of series **12** (Table 3.19) and compounds 1-18 of series **13** (Table 3.20) both groups having the substituents only at 1-aryl ring, and use a dummy parameter D equal to zero for the former and 1 for latter, we get a correlation as expressed by

$$\log (1/IC_{50}) = 0.707\ (\pm 0.288)\pi_m + 0.658\ (\pm 0.179)E_{s,o} + 1.228\ (\pm 0.247)D + 4.879 \qquad \textbf{(3.20)}$$

$$n = 38,\ r = 0.93,\ s = 0.36,\ F_{3,34} = 68.16$$

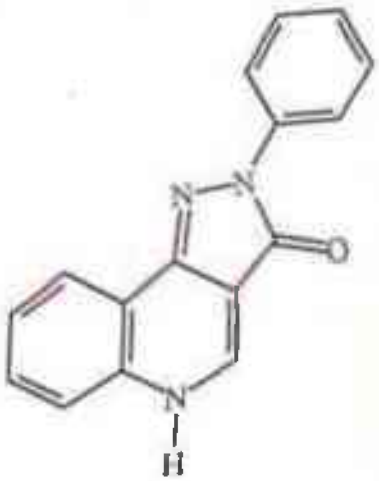
This correlation is highly significant and suggests that 1-APQs and 1-ABPs can be combined with a dummy parameter indicating their difference at 5-position. The high coefficient of D in equation (3.20) indicates that 1-ABPs would be much superior to 1-APQs for the binding with BZ receptor.

For the series of **14**, i.e., 2-ABPs (compounds 27-34 of Table 3.20), we could not find any significant correlation so that we suggest that the substitution at the aryl ring when it is at 2-position produces irregular effect.

Now the questions arise as to how: (i) 1-APQs and 1-ABPs bind to the BZR, (ii) the latter exhibit better binding than the former, (iii) the replacement of N5 hydrogen by CH_3 group in 1-APQs leads to better activity, and (iv) a substituent at the meta position of 1-aryl ring increases the activity while at the ortho position decreases it.

this fact was used in early pharmacophoric elucidation work was considered.

This molecule possesses two hydrogen-bond acceptor sites. One is carbonyl oxygen and other is N4 nitrogen. Similarly, another potent BZR ligand discovered in 1982, CGS 8216 (15), which is structurally quite different from flunitrazepam, also possesses two hydrogen-bond acceptor sites : the carbonyl oxygen and pyrazole nitrogen at position 1. In fact



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some reviews of molecular modelling studies[32,33] suggested that, in order to have a good BZR ligand, two hydrogen-bond acceptor sites must be present on the molecule. In quite agreement to this proposition, both 1-APQs (12) and 1-ABPs (13) contain two hydrogen-bond acceptor sites : the carbonyl oxygen at position 4 and the pyrazole nitrogen at position 2. Therefore, in an orientation identical to that of flunitrazepam, the 1-APQs

and 1-ABPs can be shown to bind with two hydrogen-bond donor sites H_1 and H_2 which may be present at the BZR as shown in Figures (3.1) and (3.2), respectively.

The superiority of 1-ABPs over 1-APQs is now attributed to the participation of O5 oxygen also in the hydrogen bonding with the receptor. As shown in Figure (3.2), this oxygen along with the carbonyl oxygen forms a three-centre hydrogen bond with H_1 donor site of the receptor and thus reinforces the binding of the compound with the receptor.

Now the presence of a methyl group at N5 in 1-APQs (R_2=CH_3) makes the N5 position identical to the N1 position of flunitrazepam. Flunitrazepam is a member of BZ series and some fundamental SAR studies on BZs have suggested that the substitution at N1 position is advantageous[32]. Therefore, the CH_3 group at this position in flunitrazepam and likewise at N5 in 1-APQs must be expected to participate in the binding with the receptor, involving most likely the hydrophobic interaction. Therefore, a lipophilic region L_1 is defined at the receptor for the interaction of N5 methyl in 1-APQs, giving a positive effect to the activity of the ligands.

Similarly, to account for the positive effect of meta-substituents at 1-aryl ring, we can define two more lipophilic regions L_2 and L_3 at the receptor and assume that, since correlations have already suggested

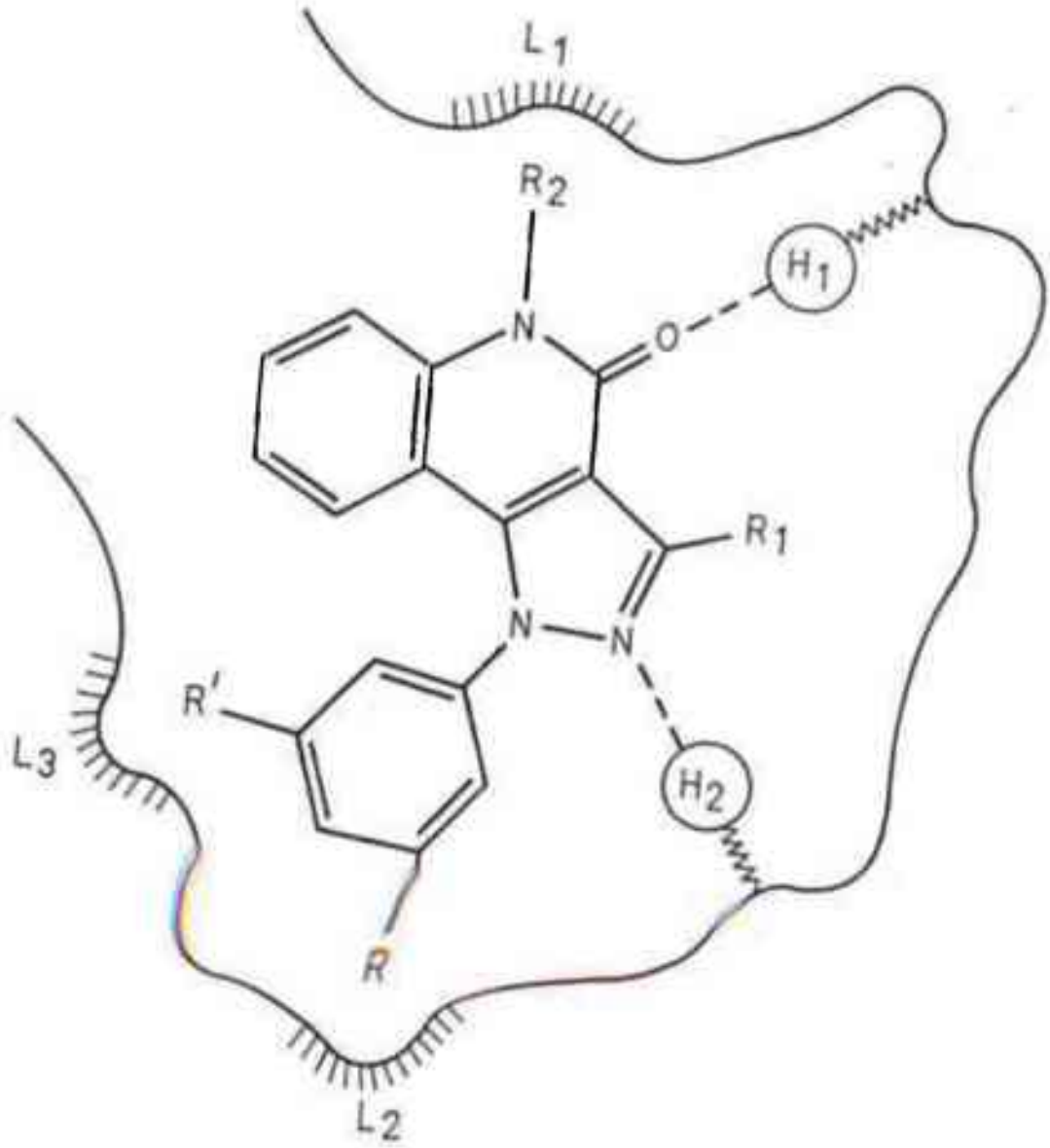

Figure (3.1): The proposed model for the binding of 1-arylpyrazolo[4,5-c]quinolin-4-ones with the BZR. H_1 and H_2 refer to the hydrogen-bond donor sites and L_1, L_2, and L_3 to the lipophilic sites at the receptor.

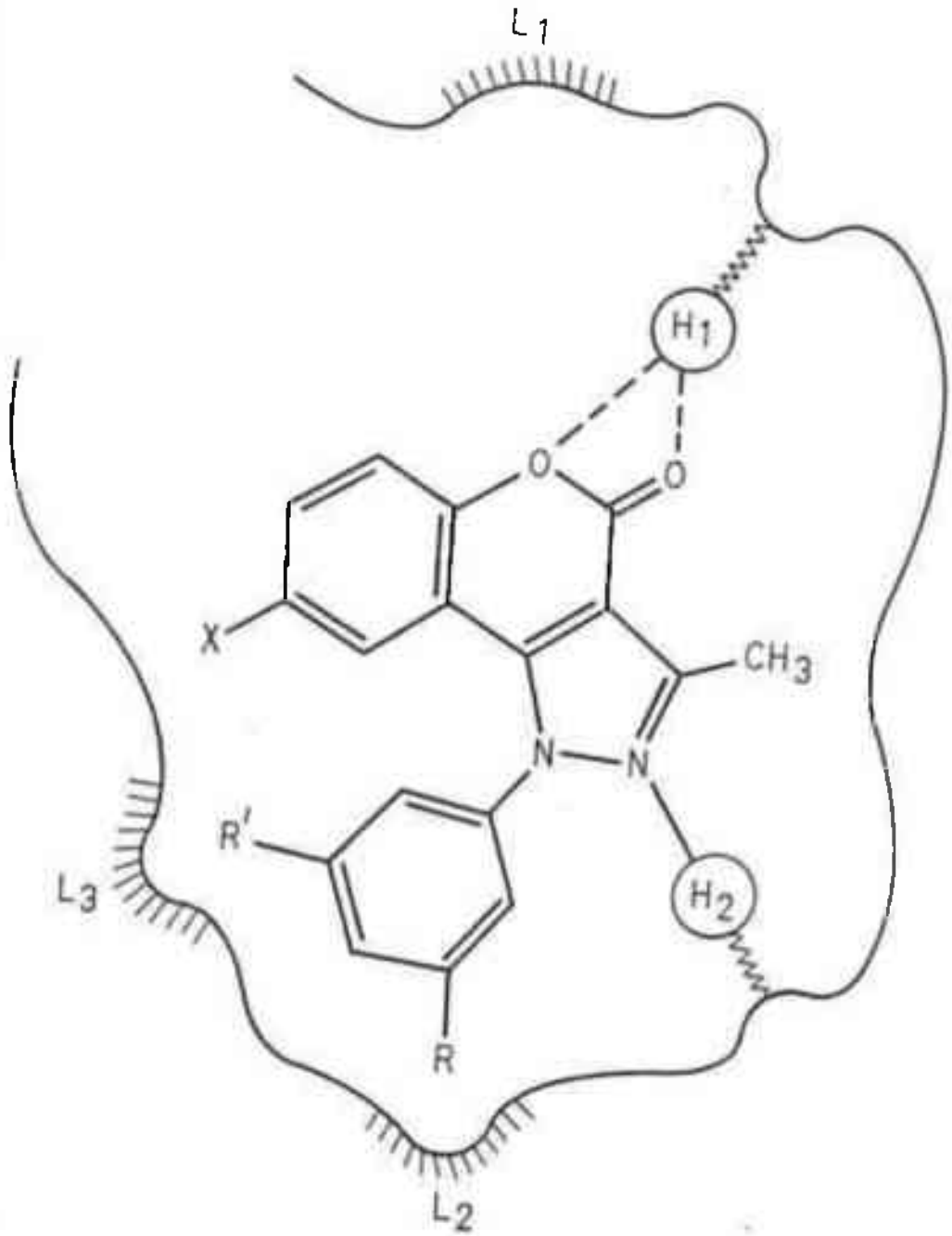

Figure (3.2): The proposed model for the binding of 1-aryl[1]benzo-pyranopyrazol-4-ones with the BZR.

that these substituents will affect the activity by their lipophilic character, the substituents, being at either or both of the meta positions, will be interacting with these lipophilic regions, strengthening further the binding of the compounds.

Thus the interaction models represented by Figures (3.1) and (3.2) explain all the questions surfaced from QSAR studies. The ortho-substituents may be assumed to produce the steric effects by tilting the plane of the aryl ring.

Table (3.19): 1-Arylpyrazolo[4,5-c]quinolin-4-ones (**12**) and their Benzodiazepine Receptor Binding Affinity and Physicochemical parameters.

No.	R	R_1	R_2	π_m(R)	$E_{s,m}$(R)	log (1/IC_{50}) Obsd[a]	log (1/IC_{50}) Calcd, Eq. (3.16)
1	H	Me	H	0.00	0.00	4.64	4.71
2	2-Cl	Me	H	0.00	-0.97	4.40	4.28
3	3-Cl	Me	H	0.71	0.00	5.36	5.41
4	4-Cl	Me	H	0.00	0.00	4.66	4.71
5	2-Me	Me	H	0.00	-1.24	4.44	4.16
6	3-Me	Me	H	0.56	0.00	5.05	5.26
7	4-Me	Me	H	0.00	0.00	4.68	4.71
8	2-OMe	Me	H	0.00	-0.55	4.25	4.46
9	3-OMe	Me	H	-0.02	0.00	5.13	4.69
10	4-OMe	Me	H	0.00	0.00	4.40	4.71
11	2-Br	Me	H	0.00	-1.16	4.70	4.19
12	3-Br	Me	H	0.86	0.00	5.60	5.55
13	3-F	Me	H	0.14	0.00	4.85	4.84
14	4-F	Me	H	0.00	0.00	5.00	4.71
15	3,5-Me_2	Me	H	1.12	0.00	5.52	5.81
16	2,4-Me_2	Me	H	0.00	-1.24	4.35	4.16
17	2,3-Me_2	Me	H	0.56	-1.24	4.03	4.71

Contd...

No.	R	R_1	R_2	$\pi_m(R)$	$E_{s,o}(R)$	log (1/IC_{50})	
						Obsd[a]	Calcd, Eq. (3.16)
18	3,4-Me_2	Me	H	0.56	0.00	5.43	5.26
19	2,6-Me_2	Me	H	0.00	-2.48	3.66	3.61
20	2,5-Me_2	Me	H	0.56	-1.24	4.33	4.71
21	H	Ph	H	0.00	0.00	4.68	4.71
22	3-Cl	Ph	H	0.71	0.00	5.52	5.41
23	3-Br	Ph	H	0.86	0.00	5.82	5.55
24	3-Me	Ph	H	0.56	0.00	5.52	5.26
25	4-Cl	Ph	H	0.00	0.00	4.52	4.71
26	4-Me	Ph	H	0.00	0.00	4.41	4.71
27	H	Ph	Me	0.00	0.00	5.51	5.94
28	3-Cl	Ph	Me	0.71	0.00	7.15	6.63
29	3-Br	Ph	Me	0.86	0.00	7.00	6.78
30	3-Me	Ph	Me	0.56	0.00	7.05	6.49
31	4-Cl	Ph	Me	0.00	0.00	5.94	5.94
32	4-Me	Ph	Me	0.00	0.00	5.96	5.94
33	3-Me	Me	Me	0.56	0.00	6.58	6.49
34	3-Cl	Me	Me	0.71	0.00	6.00	6.63
35	4-Cl	Me	Me	0.00	0.00	5.57	5.94

[a] Taken from ref. 27, 28 and 29.

Table (3.20): 1-Aryl- and 2-Aryl[1]benzopyranopyrazol-4-ones (13 and 14) and their *Benzodiazepine Receptor Binding Affinity* and Physicochemical parameters.
Compounds 1 – 26 belong to 13 and 27 –34 to 14.

No.	R	X	π_m(R)	$E_{s,o}$ (R)	log (1/IC_{50}) Obsd[a]	log (1/IC_{50}) Calcd. Eq.(3.17)
1	H	H	0.00	0.00	6.52	6.19
2	4-Me	H	0.00	0.00	5.70	6.19
3	3-Me	H	0.56	0.00	6.55	6.77
4	2-Me	H	0.00	-1.24	4.52	5.07
5	4-Cl	H	0.00	0.00	6.14	6.19
6	3-Cl	H	0.71	0.00	7.05	6.92
7	2-Cl	H	0.00	-0.97	4.80	5.31
8	3-Br	H	0.86	0.00	7.00	7.08
9	4-NO_2	H	0.00	0.00	6.00	6.19
10	3-NO_2	H	-0.28	0.00	6.49	5.89
11	2-NO_2	H	0.00	-2.52	4.37	3.91
12	4-OMe	H	0.00	0.00	6.15	6.19
13	3-OMe	H	-0.02	0.00	6.12	6.16
14	2-OMe	H	0.00	-0.55	5.10	5.69
15	3,5-Me_2	H	1.12	0.00	7.19	7.35
16	4-NH_2	H	0.00	0.00	5.89	6.19
17	3-NH_2	H	-1.23	0.00	5.47	4.91

Contd...

No.	R	X	π_m(R)	$E_{s,o}$ (R)	log (1/IC_{50}) Obsd[a]	log (1/IC_{50}) Calcd, Eq.(3.17)
18	2-NH_2	H	0.00	-0.61	6.20	5.64
19[b]	H	Br	0.00	0.00	4.96	6.19
20	3-Br	Br	0.86	0.00	7.19	7.08
21	3-Me	Br	0.56	0.00	6.85	6.77
22	3,5-Me_2	Br	1.12	0.00	7.89	7.35
23	H	NO_2	0.00	0.00	5.74	6.19
24	3-Br	NO_2	0.86	0.00	6.80	7.08
25	3-Me	NO_2	0.56	0.00	6.72	6.77
26	3,5-Me_2	NO_2	1.12	0.00	7.92	7.35
27	H	H	0.00	0.00	5.51	
28	3-Me	H	0.56	0.00	5.52	
29	3-Br	H	0.86	0.00	4.60	
30	3-OMe	H	-0.02	0.00	5.30	
31	H	Br	0.00	0.00	4.80	
32	3-Br	Br	0.86	0.00	4.96	
33	3-Me	Br	0.56	0.00	5.44	
34	3-Me	NO_2	0.56	0.00	4.48	

[a]Taken from ref. 30 and 31. [b]Not included in the derivation of equations (3.17) and (3.19)

3.11 *An Overview*

As already discussed in Section 1.1.4, the benzodiazepine receptors exhibit a very high specificity for pharmacologically and clinically active benzodiazepines and, as shown in Figure (1.4), the minimum structural requirement for binding in benzodiazepines are only the aromatic ring A and the carbonyl group in position 2. The substituents at ring A, the 4, 5-(methyleneimino) group, the substituted or unsubstituted N1, and the 5-phenyl ring have been shown to produce little effect on in vitro binding. The aromatic ring A is believed to undergo π/π stacking, probably with amino acid residues, within the receptor, and the carbonyl oxygen acts as a proton acceptor and is arbitrarily labelled as π_1. If these are only two kinds of interaction which take place between a BZR and its ligand, then all nonbenzodiazepines (**1-11**) discussed here must be as potent as benzodiazepines, but they widely differ in their activities from benzodiazepines as well among themselves.

It shows therefore that benzodiazepine receptors may have several binding sites and the activity of a ligand may depend upon how many of these sites the ligand would interact with. The following two models (Figures 3.3 and 3.4) proposed by Hollinshead et al.,[23] and Ananthan et al.,[24] respectively, exhibit that even benzodiazepine derivatives may have as many interactions as possible with the receptor.

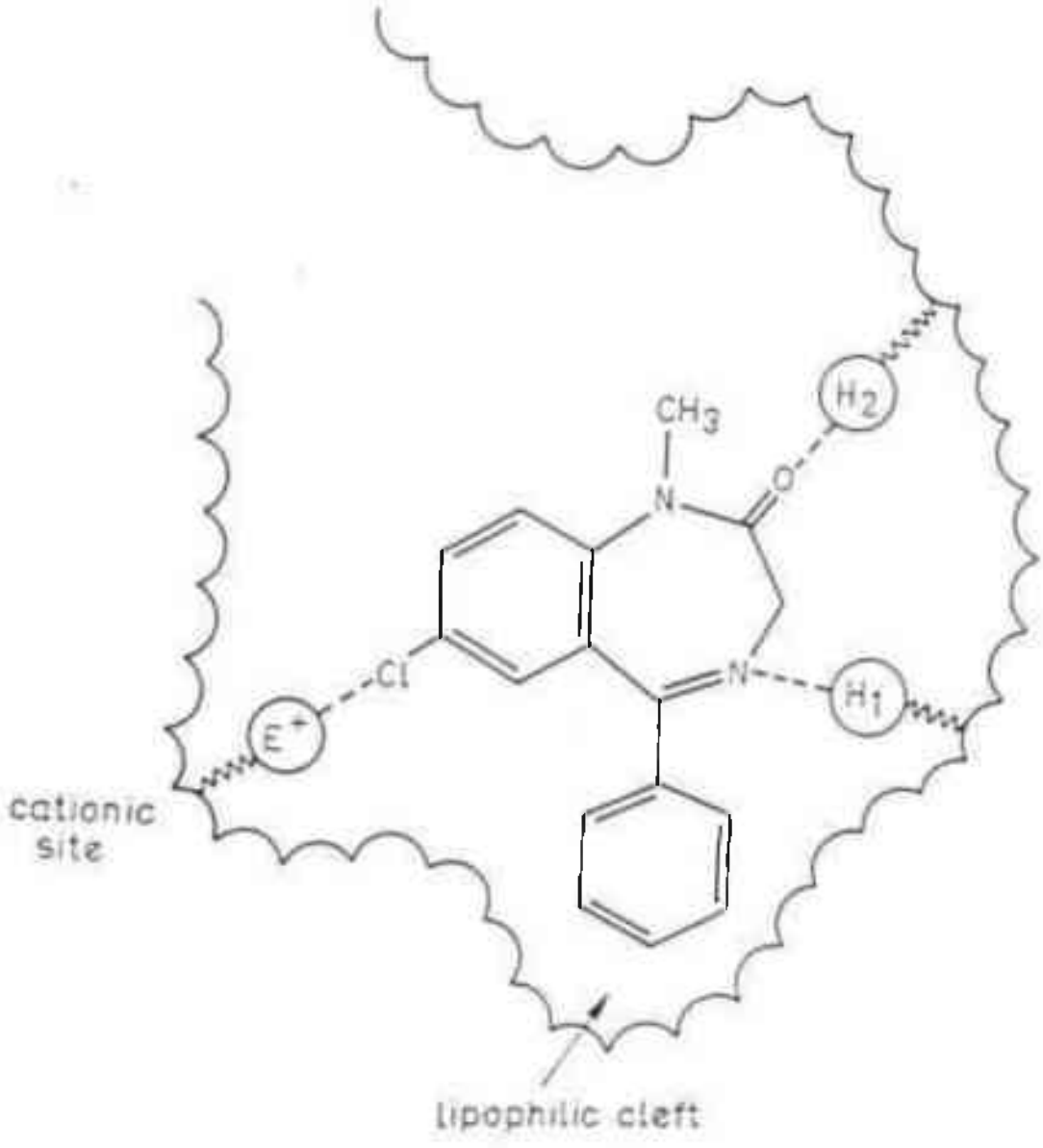

Figure (3.3): Interactions of Benzodiazepines with agonist pharmacophore of the BZR[23]

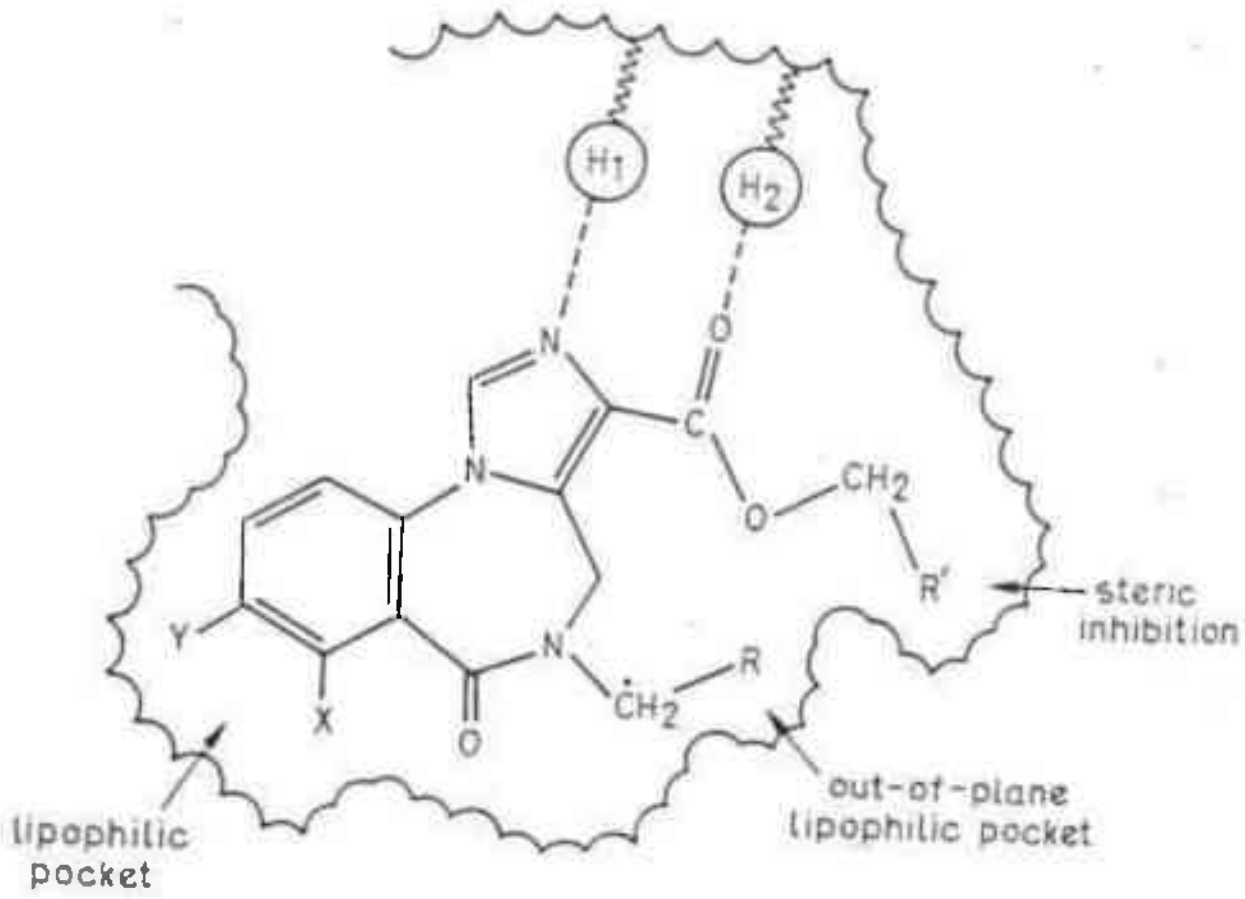

Figure (3.4): A model for the interaction of imidazobenzodiazepine carboxylic esters at the BZR agonist site.[24]

However, the hydrogen bonding and hydrophobic interactions seem to be major forces involved in binding with BZRs. Polar or electrostatic interactions are of secondary importance. Except in the case of pyridobenzimidazoles (5) and 2-aryl-4-piperidinoquinolines (11), in no other case discussed here could we find any role of electronic parameters. The two final equations (equations 3.9 and 3.10) obtained for pyridobenzimidazoles exhibit that only an ortho substituent in aryl R moiety can affect the activity through its electron-withdrawing ability, otherwise the overall lipophilic character of R group is a major controlling factor. Equations (3.9) and (3.10) also indicate that R′ group at N9 may also have hydrophobic interaction if sterically allowed. A model for the interaction of a pyridobenzimidazole with receptor, therefore, can be proposed as shown by Figure (3.5).

In the case of 2-aryl-4-piperidinoquinolines (11) also, only the electron-withdrawing substituents of both the phenyl rings have been shown to be conductive (equation 3.15). It means that cationic sites are more frequently available in BZRs. Equation (3.15) suggests that only electronic nature of R_2 substituent will be advantageous; its lipophilic character will have an adverse effect.

In all other cases, however, the hydrophobic interactions seem to be the next effective binding force after the hydrogen bonding. In cases like fused imidazopyridines (6, 7), there appear ample opportunities of

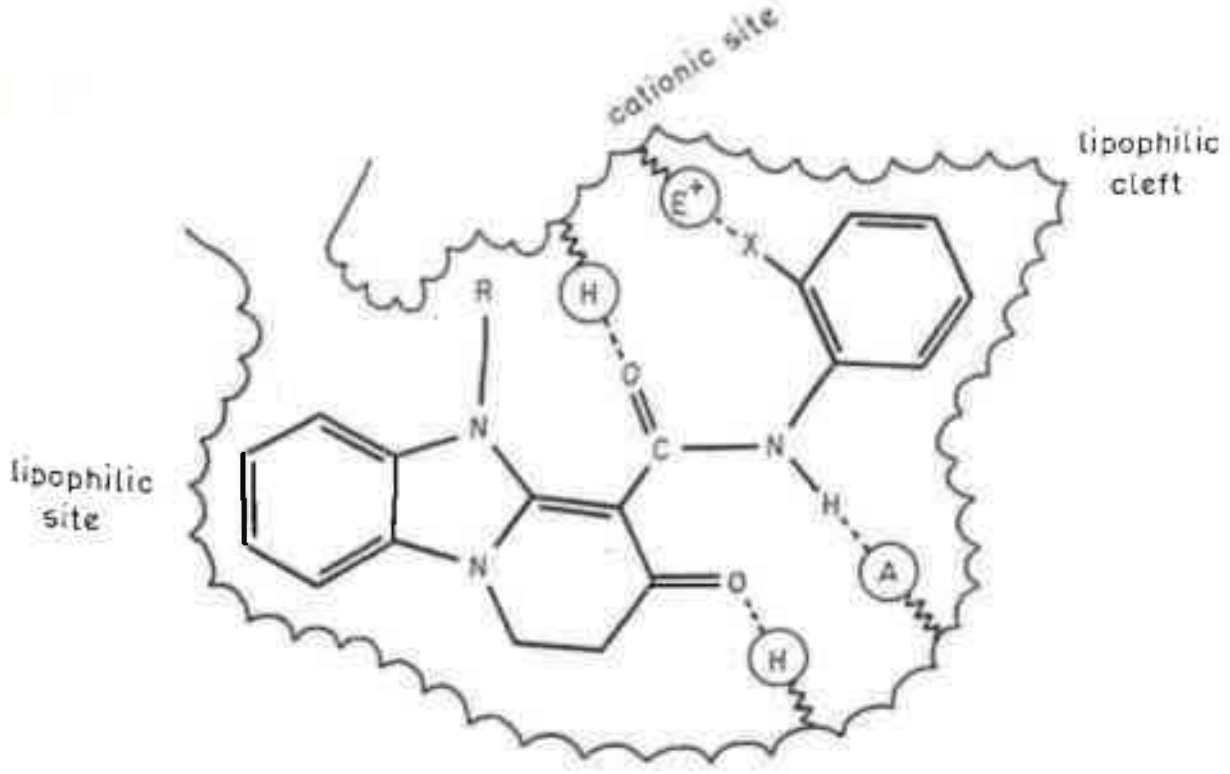

Figure (3.5): A proposed model of binding of a pyridobenzimidazole with BZR

hydrogen bonding and a strong hydrophobic interaction of the benzene ring with the receptor such that no substituents at any ring (A or D studied) are found to be tolerated. A Fujita-Ban analysis (Table 3.12 or 3.13) has revealed that no substituent has any positive contribution to the activity, rather they all have steric effects as exhibited by equation (3.11) or (3.12). A model for the binding of 6 or 7 with the receptor can be visualized to be as shown in Figure (3.6). Likewise the binding of all other series of compounds can be shown with a predominance of hydrogen bonding and hydrophobic interactions and thus Figures (3.1) and (3.2) are good models for the binding of 13 and 14.

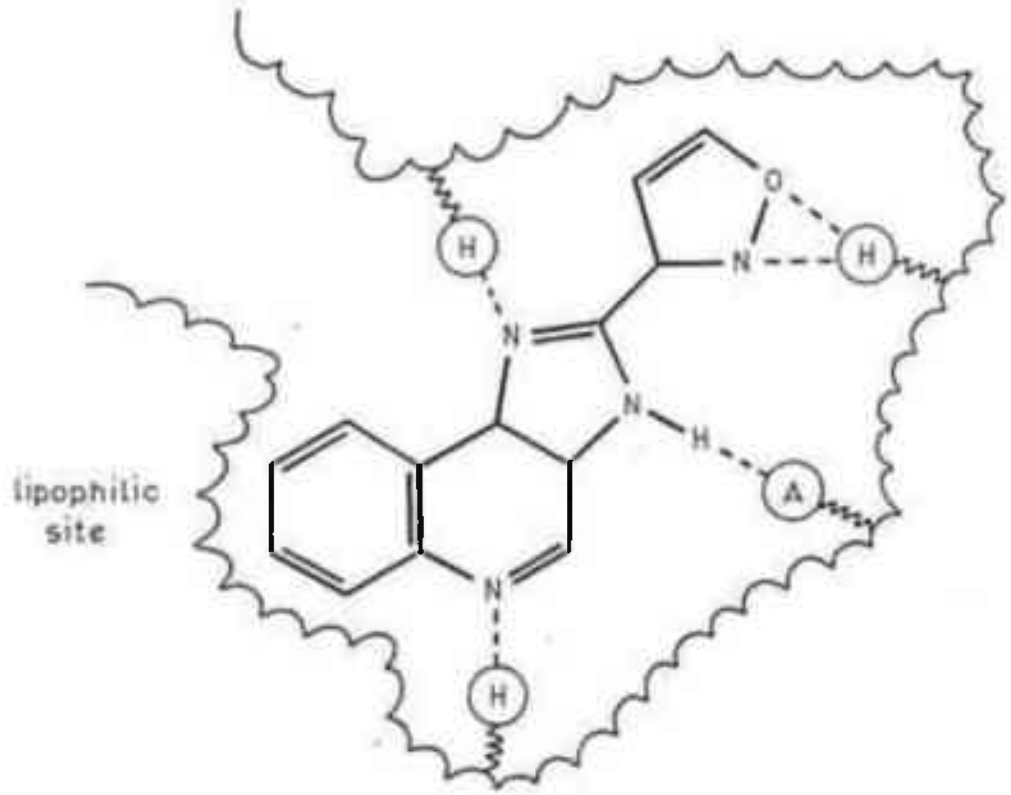

Figure (3.6):A proposed model for the binding of a fused imidazopyridine (6 or 7) with BZR.

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List of Publications

1. Quantitative Structure-Activity Relationship Studies on Benzodiazepine Receptor Binding of Some Nonbenzodiazepine Series of Ligands. *Quant. Struct.-Act. Relat. 15, 12-16 (1996)*

2. Quantitative Structure-Activity Relationship Studies on Some Nonbenzodiazepines Binding to the Benzodiazepine Receptor. *Quant. Struct.-Act. Relat. 16, 367-371 (1997)*

3. Quantitative Structure-Activity Relationship Studies on Non-benzodiazepines Binding to Benzodiazepine Receptor. *Proc. Indian natn. Sci. Acad., 63, A, No.5, 365-381 (1997)*

4. Quantitative Structure-Activity Relationship Studies on Some Nonbenzodiazepine Series of Compounds Acting at the Benzodiazepine Receptor. *Bioorg. Med. Chem. 6, - (1998)*