"QSAR STUDIES ON DRUGS BINDING WITH BENZODIAZE'INES & CHOLECYSTOKININ RECEPTORS"

THESIS

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BY

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CERTIFICATE

This is to certify that the thesis entitled **"QSAR STUDIES ON DRUGS BINDING WITH BENZODIAZEPINES & CHOLECYSTOKININ RECEPTORS"** submitted by Ms. VEENA KISHINCHAND MULCHANDANI, ID No. 90PHXF026, for the award of the Ph.D. degree of the institute, embodies original work done by her under my supervision.

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Veena Mulchandani
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TO

MY REVERENT PARENTS
BHAGWANTI AND KISHIN MULCHANDANI

List of Publications

1. Quantitative Structure-Activity Relationship Studies on Benzodiazepine Receptor Binding : Recognition of Active Sites in Receptor and Modelling of Interaction.

   J. Molec. Recog.; 5; 75 (1992).

2. Quantitative Structure Activity Relationship Studies on Benzodiazepine Receptor Binding : Investigation of Interaction Model.

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3. Quantitative Structure-Activity Relationship Studies on Cholecystokinin Antagonists-Modelling of Ligand-Receptor Interaction.

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4. QSAR Studies on Some Drugs Binding to Benzodiazepine Receptors.

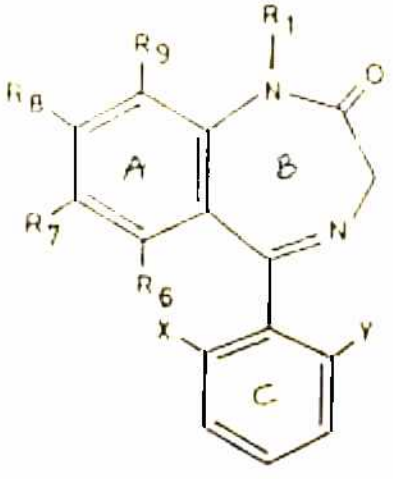
   In preparation.

Contents

CHAPTER - I
INTRODUCTION

A - BENZODIAZEPINES AND THEIR RECEPTORS

The discovery of Benzodiazepines (BZs) (1) has opened a new era in research of central nervous system (CNS) and drugs acting on it. The Benzodiazepines are a class of centrally acting drugs with wide range of therapeutic applications. They are used therapeutically as anxiolytics, hypnotics-sedatives, anticonvulsants, muscle relaxants, etc.[1] It is well established that Benzodiazepines and related ligands interact with a specific site that is closely associated with neuroinhibitory, postsynaptic γ - aminobutyric acid(GABA) receptors and a chloride ionophore channel[2,3]. The Benzodiazepines have been found to affect the dynamics of virtually all known neurotransmitors in CNS atleast at high doses. However, it was observed that these changes could not be due to a direct action of BZs on neurons that use catecholamines or acetylcholine as transmitters or to an action on their receptors.



(1)

The efforts to elucidate the mechanism of action of BZs and related compounds were not successful until a few years ago. GABA acts on atleast two different receptor types[4-8], the action of BZs seems to be restricted to synaptic effects of GABA, that are mediated by so called $GABA_A$ receptors. The direct consequence of $GABA_A$ receptor stimulation seems to be an increase of the permeability of neuronal membrane for anions, mainly for Cl^- anions. The term BZ is a chemical one in pharmacology and therapeutics. The term BZs has a more restricted meaning designating drugs belonging to the chemical class of BZs and having a pharmacological activity similar or identical to that of the early "classical" BZs, such as Diazepam. It would not be possible to completely avoid the use of the term BZs in the above defined sense although it is no longer correct, since BZs with antagonist activity have been found. The term BZR (benzodiazepine receptor) agonists will be used to include compounds of any chemical structure that interact in a similar way with BZR as BZ agonist and hence have similar pharmacological activities. BZR antagonists denote agents that bind to BZR, have no effect on GABA receptor function, but block the effect of BZR agonists. The term BZR of course no longer signifies a receptor that successfully interacts with ligands belonging to BZ class. The compounds of diverse structure and natures have been found to bind to BZR[9] and there are BZ derivatives that interact highly specifically with a completely different receptor such as tifluadom with opiate K receptor.[10-12]

The term receptor contains two absolutely necessary functions, namely that of recognition and binding of a ligand, and that of transduction of a stimulus forming function. A difficulty of using the terms binding site and receptor indiscriminately is that highly specific binding sites may be considered part of a receptor function when, infact, no pharmacological effect at all is initiated by the ligand binding site complex.

Conventional structure activity relationship (SAR) studies in the BZ series have not advanced our knowledge on mechanism of action of BZs and related compounds. However, they have contributed to increase the number of therapeutically useful drugs and have reached a practical goal. With the identification of a specific high affinity binding sites for BZs the interest in molecular mechanism of action of anxiolytic drugs increased considerably. The simple in vitro binding test is relatively well suited for the screening of large series of compounds and proved useful in detecting compounds acting directly on the receptor, distinguishing them from compounds requiring in vivo metabolic transformation in order to become active. It soon became clear that compounds from different structure classes may well act at the same receptor and have a similar mechanism as BZs. However among compounds with high affinity to the receptor not only agonists, but also antagonists and inverse agonists (ligands that differ dramatically in their intensive activity or efficacy) were

found. All these facts pointed to a pivotal role of the BZR in mediating the binding effects of a variety of different structures and stimulated SAR studies as an attempt to define the common structure features required for affinity to the BZR.

Since the detection of common structural features is nowadays greatly facilitated by computer graphics, it is important to keep in mind that an essential prerequisite for SAR is to compare compounds with identical molecular mechanism of action. For the establishment of meaningful SAR, it is necessary to distinguish between the agonistic, antagonistic and inverse agonistic activities of the ligand. Tentatively, it could be assumed that different ligands exert their effects by interacting at the same sites, each influencing differently the confirmation of the receptor glycoprotein environment, which modulates allosterically the supra molecular GABA receptor, the BZR and the chloride ionophore. Ligand structure and stereochemistry should correlate with the effector properties, but at present our understanding of these relationships is insufficient. Steric factors were shown to play an important role. In the BZ series of agonists and antagonists, the relevance of confirmation of ring B has been established. New molecular models are certainly needed; they should be shaped according to the particular SAR found for different types of ligands. Such refined models could reach a much greater predictive potential and usefulness

than those based solely on affinity. The present thesis reports QSAR (Quantitative Structure Activity Relationship) studies on some compounds that bind with BZR. Based on these QSAR studies attempts have been made to point out the active sites at the receptor and the mechanism of interaction.

B - CHOLECYSTOKININ AND ITS RECEPTORS

Cholecystokinin [CCK, H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2][13] is a gastrointestinal peptide hormone and putative central neurotransmitter. It is one of a growing list of peptides that play key roles in normal physiology as neurotransmitters and neurohormones.Cholecystokinin displays biological activities both in the peripheral and in the central nervous system.[14] In the peripheral system, the two major physiological actions of cholecystokinin are stimulation of gall bladder contraction and of pancreatic enzyme secretion.[15,16] In addition to its ability to cause stimulation of pancreatic enzyme secretion, CCK also causes desensitization, as well as residual stimulation, of enzyme secretion.[17,18] Beside the two major effects mentioned above, cholecystokinin stimulates glucose and amino acid transport, protein and DNA syntheses, energy metabolism and growth in the exocrine pancreas, and also affects secretion, absorption and motility in the stomach and intestine. It also stimulates pancreatic hormone secretion such as insulin, glucagon, somatostatin and pancreatic polypeptide, both in vivo and in vitro.[19] At least in two mammalian species, the rat and cat, CCK and related peptides are full agonists of gastric acid secretion, producing a maximal response similar to that of gastrin but with less potency. In contrast, in dogs and humans,CCK is a weak stimulant of acid secretion, and this has been explained by a stimulated secretion of somatostatin

which acts as an inhibitor of acid secretion by the parietal cell.

In the central nervous system, CCK induces hypothermia, analgesia, hyperglycemia, stimulation of pituitary hormone release and decrease in exploratory behaviour. CCK has also been found to induce satiety, either following central administration or even after peripheral administration, probably via activation of vagal afferent nerve endings. However, the mechanism by which CCK exerts its satiety effect is uncertain and appears to differ within species. Cholecystokinin was demonstrated to behave as a neuromodulator or neurotransmitter: (i) it is synthesized and stored by specific neurons in the brain; (ii) it is released under physiological conditions from nerve endings and can be inactivated after release; (iii) there are specific CCK receptors located in regions where the peptide is present; (iv) it can alter the firing rates of neurons when applied iontophoretically; and (v) it modifies the release and turnover of other neurotransmitters. Particularly, it has been demonstrated that CCK and dopamine (DA) coexist within mesolimbic and mesocortical dopoaminergic neurons. Experiments concerning functional interaction between CCK and dopamine indicate that CCK reduces or increases dopamine release depending on the brain region. In addition, CCK appears to increase dopamine-receptor affinity and reduce receptor density in the striatum. Behavioral studies showed that CCK can increase,

decrease or have no effect upon DA-mediated behavior such as sterotypy and locomotor activity. In other studies, CCK has been reported to increase, decrease or have little effect on the [^{3}H]DA release from striatal slices in vitro. However, despite the increasing number of studies, the consequence of CCK-DA interaction within the brain has still not been elucidated, and there seems to be a great deal of inconsistency within the literature regarding the interaction of CCK and DA.

There are two subtypes of CCK receptor.[20] The one which is found in the periphery to discrete regions of the CNS and mediates gall bladder contraction and pancreatic enzyme release is known as CCK-A receptor. This kind of receptor appears to be principally responsible for the satiety actions of peripherally administered CCK.[21] The other subtype of receptor is known as CCK-B receptor and is widely distributed in the brain and shows a pharmocological profile similar to that of gastrin receptor.[22,23] CCK-B agonists have been shown to cause panic attack in man[24] and CCK-B antagonists posses anxiolytic properties in animal models.[25] CCK-A and CCK-B receptors share many similar binding characteristics (for example affinity, acidic optimum pH and Mg^{2+} dependence), but they differ markedly in selectivity. The C-terminal octapeptide CCK-8 (CCK-27-33,H-Asp-Tyr(SO_3^-)-Met-Gly-Trp-Met-Asp-Phe-NH_2) is the minimum naturally occurring fragment that retains the full potency and complete spectrum of CCK activities, and also is the predominant form found in the

brain.[26,27] Numerous structure-activity studies starting with conservatively altered derivatives of CCK-8, eg., Boc-[Nle[28,29]]-CCK-7, have examined the effects of side chain and backbone modifications on binding to CCK-A and CCK-B receptors.[30,31] The present thesis gives an account of QSAR studies on different CCK- antagonists to further investigate the nature of binding of these antagonists to CCK-receptors.

C - QSAR METHODOLOGY

With a high demand of newer and better drugs on one side their discovery has become a challenging process on the other side due to complexity of the various biological systems. Infact, most of the discoveries of drugs so far have been either by sheer luck or creativity or a combination of these.

Trial and error methods usually employed for new drug development are highly uneconomical, as they require various predictions like pharmacokinetic, pharmacodynamic and toxic properties before the synthesis of a chemical compound. And moreover after synthesis these compounds must be tested on a suitable biological system. Finally after all this, it is observed that out of several thousand compounds synthesized and tested, hardly one or two or even none clicks.

To avoid all this, recent advances made in various branches of science have been employed in designing new chemical leads and optimization of activities with the congeneric series of compounds. Computers also have been used for this purpose and it is observed that computer aided techniques have been useful in reducing random synthesis and screening of various chemical compounds.

Long back 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 can explain variations in biological responses of those compounds. This has resulted in discovery, examination and interpretation of structure activity relationships in a more systematic way which led to the introduction of quantitative structure activity relationship (QSAR) studies. The quantitative approach to understand the drug action depends upon our ability to express structure by numerical values, and then relating these values to corresponding changes in activity. The QSAR study tries to explain the observed variations in biological activities of a group of congeners in terms of molecular variations caused by the change of the substitutents. The two important applications of QSAR analysis can be stated : the Predictive aspect and diagnostic aspect. The predictive aspect as the name suggests is used for the extrapolation of 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 aspect 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 tailor-made design of new drug of better

activity with lesser or no side effects. Several approaches used in QSAR studies are : the non-parametric methods - like Free-Wilson approach[32] and Fujita-Ban approach,[33] the parametric methods like Hansch approach,[34] discriminant analysis[35] and the pattern recognition technique.[36] Out of these techniques, while choosing the method, various factors have to be kept in mind, like, the quality of the biological data, number of compounds tested, degree of variance in the results, and the ratio of the time required for synthesis and biological testing. The most popular and widely used approach continues to be the linear free energy related model, the so called Hansch approach,[34] where the variance in biological effect (ΔBE) is explained by the variance of certain linear free-energy related substituent constants which describe the changes in lipophilic / hydrophilic (ΔL /Δ H), electronic (ΔEl), steric (ΔEs) and other properties of the parent molecule induced by the substituents. This model can be expressed as follows :

$$\Delta BE = f(\Delta L / \Delta H, \Delta El, \Delta Es, \ldots\ldots)$$

The change in lipophilicity can be described by the partition coefficient log P or the substituent constant π defined as π - log P_X - log P_H where X refers to the substituted derivative and H to the parent compound. Lipophilicity can also be described by Rm values obtained from reverse-phase chromatography and by log K obtained from HPLC. The change in electronic properties can be expressed by Hammett constant, pK_a, charge densities, spectroscopic properties like chemical

shift from IR or UV spectra, Field constant (F) and resonance constant (R). The steric influence of the substituents can be described by the Taft steric constant (Es), molar volume (MV) and molar refractivity (MR).

Besides many a drug activities have been found to depend exclusively upon the molecular size[48] which can be described by the van der Waals volume (Vw) and upon the molecular graph which is delineated by molecular connectivity index (χ).[47] In this thesis the extensive use has been made of these two parameters alongwith the hydrophobic constant measured in terms of octanol water partition coefficient of the compounds.

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

$$BA = a\pi(\text{or } \log P) + b\pi^2 \ (\text{or } [\log P]^2) + c\sigma + dEs + k \quad ..(1.1)$$

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 logarithmic of the concentration of drug leading to a desired response.

Equation 1.1 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 hydropohilic 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, for in vivo system is due to the nonlinear dependence of the rate constant of drug transport through aqueous and bio-organic phases on lipophilicity where as for in vitro systems, like drug-binding inhibition, such nonlinear relationships result from equilibrium distribution of the drug toward different areas at the receptor surface, from limited binding space at the active site or from limited solubility of more lipophilic congeners.

However, in many cases the relationships between activity and lipophilicity were found to be strictly linear[34] 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[39-45] were proposed, out of which Kubinyi's bilinear model was found, after Hansch's parabolic model, to be the most useful[46-52] model to describe the nonlinear relationships.

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[53]

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.[54,55] 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.[56] 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 affect 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 motion.[57-61] These motions may be involved in some molecular conformational changes on substrate or drug 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).[62] 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.[63] 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 - II
PARAMETERS USED
AND
THEIR CALCULATION

This Chapter discusses the methodology of calculation of various distinct parameters, on which most of the biological activities are found to be dependent on. Hence, they have been very useful in QSAR studies.

A. Van der Waals Volume :

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

To calculate V_W of molecules, spherical shapes are assumed for all atoms according to Bondi[1] because of the absence of generally accepted pear shapes. The values of the van der Waals radii used and calculated volume of atoms are listed in Table 2.1. Since van der Waals radii are greater than covalent radii, a correction 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 2.2. A correction for branching in the molecule is also included in the V_W calculation. Such correction is also mentioned in the Table 2.2. All these values have been taken from the literature.[2]

Table 2.1 :van der Waals radius and volume of atoms.*

Atom		Radius (A)	Sphere volume (10^2 A^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 reference 2

Table 2.2: Correction values of van der Waals volume, for sphere over lapping due to covalent bonding, and branching

Bond	Bond length (A)	Correction value (10^2 A^3)
C-C	1.5	-0.078
C-H	1.1	-0.043
C-N	1.4	-0.065
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

Table 2.2 Continued . . .

Bond	Bond length (A)	Correction value (10^2 A^3)
O-B	1.5	-0.079
S-H	1.3	-0.040
S-S	2.0	-0.062
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 reference 2.

B. Molecular Connectivity Index :

Kier and Hall[3] 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- suppressed 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 that particular χ.

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

$$^{1}\chi = \Sigma C_{ij} = \Sigma(\delta_i \delta_j)^{-\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

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 :

$$\delta^v_i = Z^v_i - N_H \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.2)$$

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 :

$$^1\chi^v = \Sigma C_{ij} = \Sigma(\delta^v_i \, \delta^v_j)^{-\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.3)$$

The application of Eq.2.2 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) together with atomic number(Z) and the number of hydrogen atoms (N_H) attached to that atom will give appropriate δ^v value for atoms beyond second row in the periodic table.[4] The mathematical expression for this is :

$$\delta^v = (Z^v - N_H) \; / \; (Z - Z^v) \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.4)$$

According to this equation $\delta^v_{Cl} = 0.70$ and $\delta^v_{Br} = 0.25$. The δ^v value for some heteroatoms including halogens are listed in Table 2.3

Only the above discussed connectivity indices are used in our studies. Higher order connectivity indices are discussed by Kier and Hall in their monograph.[3]

Table 2.3: 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	(-)20.000
NH_4^+	1	Cl	0.690;(.7)[a]
N^+	6	Br	0.254;(0.25)[a]
$=NH_2^+$	3	I	0.085;(0.162)[a]

* Taken from reference 3

a Obtained from Eq.2.4.

C. Hydrophobic parameter : [log P]

The fragment method suggested by Hansch and Leo[5] for calculating logP, where P is the partition coefficient of the solute in octanol/water system, is known as constructionist or synthetic approach. Experimentally determined logP values can often be reproduced or approached theoretically with the help of this approch. The basic assumption of this approach is : the logP 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.

$$\log P = \sum_{1}^{n} a_n f_n + \sum_{1}^{m} b_m F_m \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.5)$$

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 $-\overset{1}{C}=$ in $CH_2=CH_2$ is an IC but not in $H_2C=O$. Fragments are of two types: (1) fundamental fragments defined as fragments whose free valency will lead to isolating carbons; (2) derived fragments, a derivative of fundamental fragments (e.g. CF_3). A fundamental fragment can be either a single atom or a 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 :

(1) Non-polar fragments : these are simple ICs and hydrogens attached to ICs; (2) 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.); and (3) 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 the Factors table. The superscripts are applicable also to fragments. They are listed as:

(1) None = aliphatic structural attachment

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

(3) 1/Ø = as 2 but attachment from right as written

(4) ØØ = two aromatic attachments

(5) X = aromatic attachment, value enhanced by second, electron - withdrawing substitutent($\sigma_I \geq \pm 0.35$)

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 compounds containing hetero atoms, into fundamental fragments rather than into derived fragment. Some important fragments values and Factor values are listed in Tables 2.4 and 2.5 respectively. A simple example for logP calculation is shown below.

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

$6f^{\emptyset}_{C} + f_C + 8f_H =$ logP (Toulene)

6(0.13) + 0.20 + 8(0.23) = 2.82 (Cacld.) 2.80(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:

$$f^{\emptyset}_{C_6H_5} + f_{CH_3} = \text{logP (Toluene)}$$

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

Sometimes calculated logP values of compounds deviate very much from the experimentally determined values. For example, observed logP 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.[5]

Table 2.4 : Some common Fragment Constants.*

Without Carbon	f	$f^{\emptyset}$	$f^{\emptyset\emptyset}$	With Carbon	f	$f^{\emptyset}$	$f^{\emptyset\emptyset}$
-Br	0.20	1.09		C	0.20	0.20	
-Cl	0.06	0.94		$-CF_3$[a]		1.11	
-F	-0.38	0.37		-CN	-1.27	-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	$f^{\emptyset}$	Without Carbon	$f^{\emptyset}$	With Carbon	$f^{\emptyset}$	With Carbon	$f^{\emptyset}$
-N=	-1.12	-N=N-	-2.14	C	0.13	-CH-	0.35_5
-N<	-1.60	-O-	-0.08	C	0.22_5[c]	-C(O)-	-0.59
$-N<^{\emptyset}$	-0.56	-NH-	-0.65	$\overset{*}{C}$	0.44[d]	-OC(O)-	-1.40

* Taken from reference 4. [a] Derived fragement. [b] For methyl ethers and ethylene oxide, use -1.54. [c] For ring fusion carbon. [d] For ring fusion - hetero.

Table 2.5 List of Some Factors*

	Unsaturation Double	Triple	Geometric Proportional to Length: x(n-1) Short Chains: 1-time	
Involving bonds				
Normal	$F(=) = -0.55$	$F(\equiv) = -1.42$	Chain: $F_b = -0.12$	Alkane Chain: $P_{cBr} = 0.13$
Conjugate to $\emptyset$	$F^{\emptyset}(=) = -0.42$		Ring[a]: $F_b = -0.09$	H-polar Fragment: $F_{qBr} = -0.22$
Conjugate to $2\emptyset$	$F^{\emptyset\emptyset}(=) = 0.0$	$F^{\emptyset\emptyset}(\equiv) = 0.0$	Branching: $F_{bYN} = -0.20$[b]	Ring Cluster = $F_{rCl} = -0.45$
			$F_{bYP} = -0.31$[c]	
Involving multiple halogenation[d]				
On same Carbon (geminal) F_{mhCm}	$(n=2) = 0.30$	$(n=3) = 0.53$	$(n=4) = 0.72$	On adjecent Carbon (vicinal) $F_{mhVn} : 0.28\ (n-1)$
Involving H-polar proximity				
Chain :	$F_{p1} = -0.42\ \Sigma f_1 \cdot f_2$	$F_{p2} = -0.26\ \Sigma f_1 \cdot f_2$	$F_{p3} = -0.10\ \Sigma f_1 \cdot f_2$	
Aliphatic ring :	$F_{p1} = -0.32 \Sigma f_1 \cdot f_2$	$F_{p2} = -0.20 \Sigma f_1 \cdot f_2$		
Aromatic:	$F^{\emptyset}_{p1} = -0.16\ \Sigma f_1 \cdot f_2$	$F^{\emptyset}_{p2} = -0.08\ \Sigma f_1 \cdot f_2$		
Involving intramolecular H-bond				
$F_{HBN} = 0.60$ for nitrogen			$F_{HBO} = 1.0$ for oxygen	

* Taken from reference 5.
a Aromatic rings are excluded
b For amine
c For Phosphorus esters
d Value per halogen atom

D. Hydrophobic constant (π) of substituents:

Although logP 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.[6,7] 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

$$\pi_X = \log P_X - \log P_H \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.6)$$

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

$$\pi_{Cl} = \log P_{C_6H_5Cl} - \log P_{C_6H_6} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.7)$$

$$2.84 - 2.13 = 0.71$$

A positive value for π means that relative to H the substituent favours the octanol phase. A negative value indicates its hydrophillic character relative to H. The value of π varies somewhat from system to system. Certain π values are given in table 2.6.

E. Electronic Parameter (σ):

The development of electronic parameter is one of the most important break throughs for mechanistic organic chemistry which came in 1935 when L.P.Hammet proposed [8] the following equation to define an electronic parameter σ.

$$\sigma = \log K_X - \log K_H \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.8)$$

In equation 2.8, K_H is the ionisation constant of benzoic acid in water at 25°C and K_X is the ionisation 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 2.6.

F. 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.

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

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 confomational change in a macromolecular binding site. If the conformational change favoured the process under study, one would certainly expect a positive coefficeient with the MR term, however, if the conformational change were detrimental, a negative coefficient could result for the MR term. Negative coefficients with MR have also been assumed to reflect steric hindrance of one kind or another. Some MR value used are tabulated in Table 2.6.

For QSAR studies in this thesis, standard values for different parameters for various substituents have been taken from literature.[5]

Table 2.6: Data on physicochemical parameters of some important substituents

No.	Substituent	π	σ_m	σ_p	MR
1	H	0.0	0.0	0.0	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_6H_5	1.96	0.06	-0.01	25.36
20	CN	-0.57	0.56	0.66	6.33

continued . . .

Table 2.6 continued . . .

No.	Substituent	π	σ_m	σ_p	MR
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
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	0.0	0.0	7.19
28	$CHOHCH_3$	-0.86	0.0	-0.07	11.82
29	CH_2OCH_3	-0.78	0.02	0.03	12.07
30	SCH_3	0.61	0.15	0.0	13.82
31	NHCHO	-0.98	0.19	0.0	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 reference 5.

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CHAPTER - III
RESULTS AND DISCUSSION

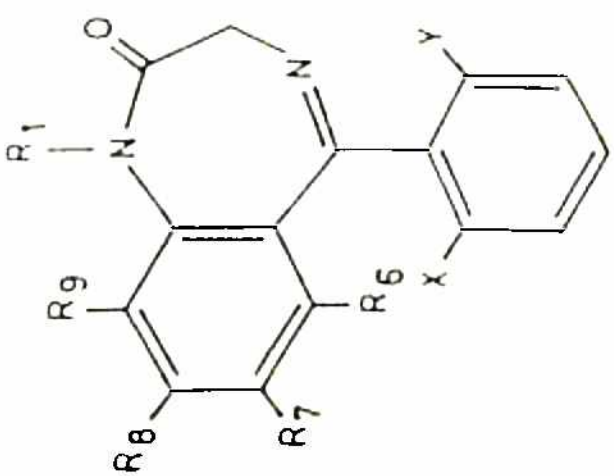
A : DRUGS BINDING TO BENZODIAZEPINE RECEPTORS

As already discussed in chapter I, the discovery of benzodiazepines (BZs) has opened a new era in research of the central nervous system (CNS), and drugs acting on it. It has been well established that BZs and related ligands interact with a specific site, that is closely associated with a neuroinhibitory postsynaptic Γ-aminobutyric acid receptor and a chloride ionophore channel.[1] Various compounds have been suggested as possible 'endogenous ligands'that physiologically act on benzodiazepine receptors.[2] Initial observations have shown that β-carboline derivatives and esters of β-carboline-3-carboxylic acid, cyclopyrrolones,pyrazoloquinolines,benzylpurines, etc., bind to BZ-receptors.[3-5] Some of the compounds binding to BZ-receptors possess BZ-like agonist activity while others act as antagonists or inverse agonists.[6,7] Many structure activity relationship studies have been made on binding of ligands to BZ-receptors, but no complete model of their interaction has been yet presented. A receptor model hitherto suggested by Hollinshead et al.[8] for the binding of two prototypes of BZs,diazepam and flunitrazepam, has not been found to be fully satisfactory to account for the binding of all types of BZ ligands. It was therefore proposed to carry out a QSAR study on ligands that belong to different categories. This study was found to be of great help in the investigation of the various active sites of the receptors and the interaction mechasnism between ligands and those sites. Ligands subjected to QSAR were varying series of BZ

derivatives, come β-carbolines and a series of 9-benzylpurines.

Materials and Methods

For a large number of 'classical'1,4-BZs (I) the data on the inhibition of [^{3}H]diazepam binding with BZ-receptor were compiled by Haefely et al.[6] These compounds with their IC_{50} values (the molar concentration of the compound leading to 50% inhibition) are listed in Table 3.1. The inhibitin data for β-carbolines (II) as listed in Table 3.2 were taken from the study of Cain et al.[9] These data also were against [^{3}H]diazepam binding. The inhibition data shown in Table 3.3 for some β-carbolines (III) against [^{3}H]flunitrazepam binding with the BZ receptor are those studied by Hollinshead et al.[8] Series of tetracyclic 1,4-BZ derivatives (IV) and a series of 9-benzylpurines (V) were also taken for the QSAR study.The BZ-receptor binding data for the tetracyclic 1,4-BZ derivatives have been taken from the compilation of Haefely et al.[6] and those for the 9-benzylpurines from a recent study made by Kelley et al.[10] The last two series with their activity data, IC_{50}, the molar concentration leading to 50% inhibition of [^{3}H]diazepam binding , are listed in Tables 3.4 and 3.5, respectively. The physicochemical parameters, particularly the hydrophobic constant π and the electronic constant σ (Hammett constant), used were those as listed in Table 2.6. A least square method[11] was used to derive various correlation equations.



(I)

Table 3.1 : 'Classical' BZs (I) and their binding affinity ($-\log IC_{50}$) for the receptor

Compd. no.	R_1	R_6	R_7	R_8	R_9	X	Y	-log IC_{50}	
								Obs[a]	Calc. [Eqn.3.1]
1.	H	H	F	H	H	H	H	7.40	7.43
2.	CH_3	H	F	H	H	H	H	7.77	7.43
3.	H	H	F	H	H	F	H	8.13	8.17
4.	CH_3	H	F	H	H	F	H	8.29	8.17
5.	H	H	Cl	H	H	H	H	8.03	7.72
6.	CH_3	H	Cl	H	H	H	H	8.09	7.72
7.	H	H	Cl	H	H	F	H	8.70	8.46
8.	H	H	Cl	H	H	F	F	8.80	8.46
9.	CH_3	H	Cl	H	H	F	F	8.39	8.46
10.	H	H	Cl	H	H	Cl	H	8.74	8.52
11.	H	H	Cl	H	H	Cl	F	8.52	8.52
12.	H	H	Cl	H	H	Cl	Cl	8.15	8.52
13.	CH_3	H	Cl	H	H	Cl	Cl	8.26	8.52
14.	CH_3	H	Br	H	H	F	F	8.62	8.55
15.	CH_3	H	I	H	H	F	H	8.54	8.62
16.	H	H	CF_3	H	H	H	H	7.89	7.86
17.	H	H	N_3	H	H	F	H	8.28	8.23
18.	H	H	NO_2	H	H	H	H	8.00	7.65
19.	H	H	NO_2	H	H	F	H	8.82	8.39
20.	CH_3	H	NO_2	H	H	F	H	8.42	8.39
21.	H	H	NO_2	H	H	Cl	H	8.74	8.46
22.	CH_3	H	NO_2	H	H	Cl	H	8.66	8.46

continued . . .

Table 3.1 continued . . .

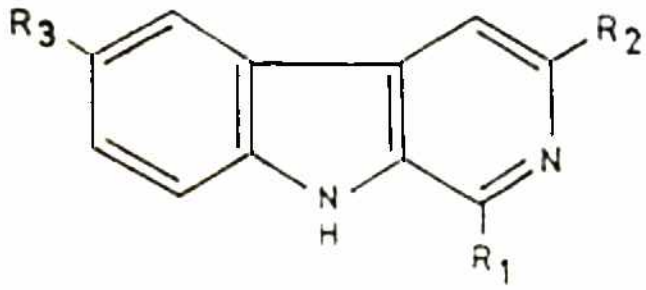
Compd. no.	R_1	R_6	R_7	R_8	R_9	X	Y	-log IC_{50}	
								Obs[a]	Calc. [Eqn.3.1]
23.	H	H	NO_2	H	H	CF_3	H	8.46	8.59
24.	CH_3	H	NHOH	H	H	F	H	7.02	7.08
25.	H	H	NH_2	H	H	H	H	6.41	6.26
26.	CH_3	H	NH_2	H	H	H	H	6.34	6.26
27.	CH_3	H	NH_2	H	H	F	H	7.19	7.00
28.	H	H	NH_2	H	H	Cl	H	7.12	7.06
29.	CH_3	H	CN	H	H	H	H	6.42	7.36
30.	CH_3	H	CN	H	H	F	H	-7.52	8.09
31.	H	H	CH_2CH_3	H	H	H	H	7.44	7.37
32.	H	H	$CH=CH_2$	H	H	H	H	7.62	7.41
33.	H	H	CHO	H	H	H	H	7.37	7.09
34.	H	H	$COCH_3$	H	H	F	H	7.74	7.90
35.	H	H	H	H	H	H	H	6.46	6.99
36.	H	H	H	H	H	F	H	7.68	7.73
37.	H	H	H	H	H	F	F	7.72	7.73
38.	CH_3	H	H	H	H	F	H	7.85	7.73
39.	CH_3	H	H	H	H	Cl	H	8.42	7.79
40.	H	Cl	H	H	H	H	H	6.49	6.12
41.	CH_3	Cl	H	H	H	F	H	6.82	6.86
42.	H	H	H	Cl	H	F	F	7.55	7.73
43.	H	H	H	CH_3	H	F	H	7.72	7.73
44.	CH_3	H	H	H	Cl	F	H	7.14	7.73

continued . . .

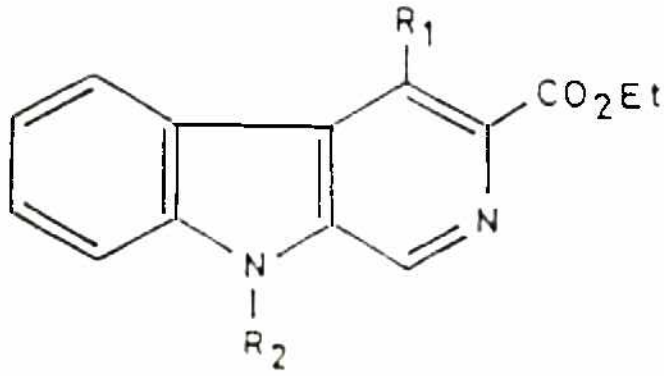
Table 3.1 continued . . .

Compd. no.	R_1	R_6	R_7	R_8	R_9	X	Y	-log IC_{50}	
								Obs[a]	Calc. [Eqn.3.1]
45.	CH_3	Cl	H	Cl	H	F	H	6.52	6.86
46.	CH_3	H	Cl	Cl	H	H	H	7.40	7.72
47.	H	H	Cl	Cl	H	F	H	8.44	8.46
48.	H	H	CH_3	Cl	H	F	H	7.85	7.90
49.	H	H	Cl	H	Cl	H	H	7.43	7.72
50.	H	H	Cl	H	CH_3	H	H	7.28	7.72

[a]Taken from ref.6.



(II)



(III)

Table 3.2 : β-Carbolines (II) and their in vitro BZ receptor binding affinity ($-\log K_i$)

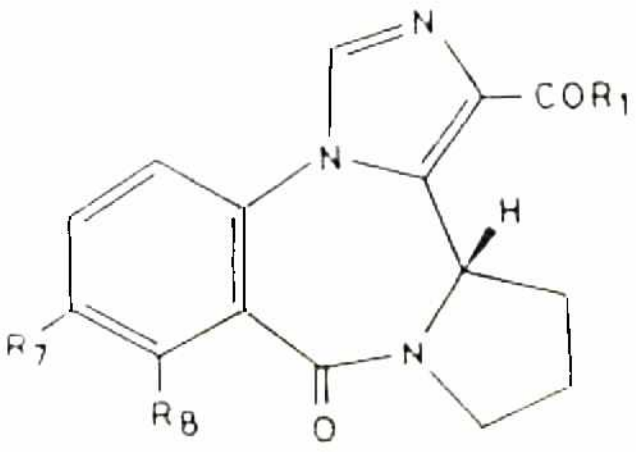
Compound no.	R_1	R_2	R_3	$-\log K_i$	
				Obs.[a]	Cal.[Eqn.3.3]
1.	C_2H_5	$COOCH_3$	OH	5.24	5.35
2.	C_2H_5	$COOCH_3$	H	5.12	5.35
3.	C_6H_5	$COOCH_3$	H	5.41	5.35
4.	C_2H_5	H	H	3.60	3.59
5.	CH_3	H	H	4.91	4.51
6.	H	$COOCH_3$	H	8.98	8.22
7.[b]	H	COOH	H	4.62	8.22
8.	H	$COCH_3$	H	7.24	7.74
9.	H	CHO	H	7.21	7.50
10.	H	$COOCH_3$	OH	8.58	8.22
11.	H	$COOC_2H_5$	H	8.96	8.73
12.	H	$COOC_3H_7$	H	9.00	9.27
13.	H	CH_2OH	H	5.83	5.45
14.	H	$CHOHCH_3$	H	5.50	5.62
15.	H	H	H	5.79	6.46

[a]Taken from ref.9.

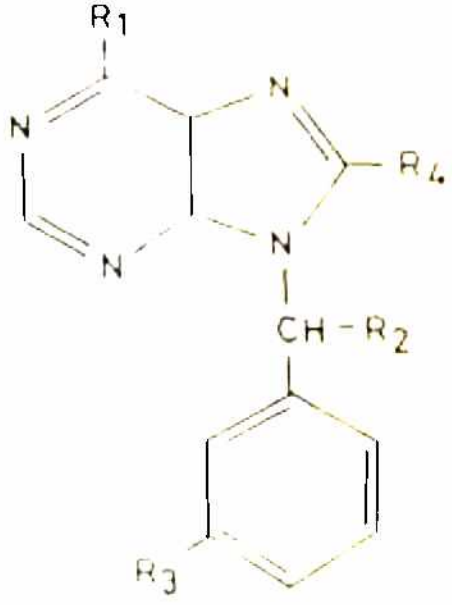
[b]Not used in the derivation of eqn.3.3

Table 3.3 : Hollinshead's data on β-carbolines (III) for binding with a BZ receptor.

Compound no.	R_1	R_2	R_3	IC_{50},(nM)
1.	CH_2OCH_3	H	OCH_2Ph	1.00
2.	CH_2OCH_3	H	OH	0.90
3.	CH_2OCH_3	CH_3	OCH_2Ph	945.00
4.	CH_2OCH_3	H	OCH_3	0.50
5.	CH_2OCH_3	H	H	2.30
6.	CH_2CH_3	H	OCH_2Ph	22.00
7.	CH_2CH_3	CH_3	OCH_2Ph	75000.00
8.	H	H	OCH_2Ph	8.90
9.	H	CH_3	OCH_2Ph	75000.00



(IV)



(V)

Table 3.4 : Tetracyclic 1,4-benzodiazepines (IV) and their BZ-receptor binding affinity along with calculated hydrophobic constant of COR_1 substituent. For other substituents see Table 2.6.

Compd. no.	Substituent R_1	R_7	R_8	π [a] COR_1	-log IC_{50} Obsd.[b]	Cald.[c]	Cald.[d]
1.	NH_2	H	Cl	-1.49	5.52	5.91	5.84
2.	OC_2H_5	H	H	0.52	8.19	8.13	8.17
3.	OC_2H_5	H	Cl	0.52	8.77	8.13	8.17
4.	OC_2H_5	Cl	H	0.52	7.21	6.92	6.91
5.[e]	O-t-C_4H_9	H	OCH_3	1.58	7.34	8.54	8.60
6.	O-t-C_4H_9	H	C_2H_5	1.58	8.00	8.54	8.60
7.	O-t-C_4H_9	H	CH_3	1.58	8.49	8.54	8.60
8.	O-t-C_4H_9	H	H	1.58	8.49	8.54	8.60
9.	O-t-C_4H_9	H	SCH_3	1.58	8.47	8.54	8.60
10.	O-t-C_4H_9	H	F	1.58	8.21	8.54	8.60
11.	O-t-C_4H_9	F	H	1.58	8.11	8.31	8.35
12.	O-t-C_4H_9	H	Cl	1.58	8.60	8.54	8.60
13.	O-t-C_4H_9	F	Cl	1.58	8.51	8.31	8.35
14.	O-t-C_4H_9	Cl	H	1.58	7.05	7.34	7.33
15.	O-t-C_4H_9	H	Br	1.58	8.66	8.54	8.60
16.	O-t-C_4H_9	H	I	1.58	8.68	8.54	8.60
17.	O-t-C_4H_9	H	CF_3	1.58	8.48	8.54	8.60
18.	O-t-C_4H_9	H	NO_2	1.58	8.55	8.54	8.60
19.	O-n-C_3H_7	H	Cl	1.06	8.85	8.41	8.46
20.	O-i-C_3H_7	H	Cl	1.05	8.60	8.40	8.45

continued . . .

Table 3.4 continued . . .

Compd. no.	Substituent R_1	R_7	R_8	π COR_1 [a]	-log IC_{50} Obsd.[b]	Cald.[c]	Cald.[d]
21.	OC_3H_5	H	Cl	0.51	8.77	8.12	8.17
22.	O-n-C_4H_9	H	Cl	1.60	8.59	8.55	8.60
23.	O-i-C_4H_9	H	Cl	1.59	8.20	8.55	8.60
24.	O-s-C_4H_9	H	Cl	1.59	8.54	8.55	8.60
25.	O-CH_2-cyclopropyl	H	Cl	1.20	8.64	8.46	8.51
26.	O-n-C_6H_{13}	H	Cl	2.68	8.59	8.44	8.47
27.	O-cyclohexyl	H	Cl	2.37	8.40	8.52	8.56
28.	O-cycloheptyl	H	Cl	2.94	8.47	8.32	8.34
29.	O-cyclooctyl	H	Cl	3.51	8.28	7.96	7.97
30.	$OCH_2C_6H_5$	H	Cl	1.77	8.82	8.56	8.61
31.	OC_6H_5	H	Cl	1.46	8.28	8.52	8.58

[a]Calculated from fragment constants as suggested by Hansch and Leo.[12] For substituents at R_7 and R_8 positions see Table 2.6

[b]Taken from ref.6.

[c]Calculated using eqn.3.4.

[d]Calculated using eqn.3.5.

[e]Not used in deriving eqn.3.5.

Table 3.5 : 9-Benzylpurines (V) and their BZ-receptor binding affinity. Substituent's physicochemical parameters used are given in Table 2.6.

Compd. no.	Substituent				-log IC_{50}		
	R_1	R_2	R_3	R_4	Obsd.[a]	Cald.[b]	Cald[c]
1.	$N(CH_3)_2$	H	H	H	4.89	5.03	5.08
2.	$N(CH_3)_2$	H	NH_2	H	6.05	6.72	6.58
3.	$N(CH_3)_2$	H	H	Br	5.52	5.03	5.08
4.	$N(CH_3)_2$	H	NH_2	Br	6.96	6.72	6.58
5.[d]	$N(CH_3)_2$	H	NHCHO	Br	7.96	6.89	6.64
6.	$N(CH_3)_2$	CH_3(S)	H	H	5.68	5.87	5.92
7.	$N(CH_3)_2$	CH_3(R)	H	H	4.00	4.19	4.24
8.	$N(CH_3)_2$	CH_3(RS)	NH_2	H	6.80	6.72	6.58
9.	$N(CH_3)_2$	CH_3(RS)	NH_2	Br	6.28	6.72	6.58
10.	$N(CH_3)_2$	H	OH	H	5.92	5.91	5.86
11.	$N(CH_3)_2$	H	$OCOCH_3$	H	6.36	6.38	6.18
12.	OH	H	H	H	4.72	5.03	5.08
13.	SCH_3	H	H	H	5.48	5.03	5.08
14.	$N(CH_3)_2$	CH_3(RS)	OH	H	6.32	5.91	5.86
15.	OH	CH_3(RS)	OH	H	5.66	5.91	5.86
16.	SCH_3	CH_3(RS)	OH	H	5.92	5.91	5.86
17.	$N(CH_3)_2$	CH_3(RS)	$OCOCH_3$	H	6.42	6.38	6.18
18.	SCH_3	CH_3(RS)	$OCOCH_3$	H	5.77	6.38	6.18

[a] Taken from ref.10.

[b] Calculated using eqn.3.10.

[c] Calculated using eqn.3.11.

[d] Not used in deriving eqn.3.11.

Result and Discussion

A multiple regression analysis of the data of Table 3.1 has revealed that the physico-chemical properties of only the R_7 substituent and those of the Y substituent in the phenyl moiety were important for the activity of BZs. In fact, these are the only two substituents which have been varied in most of the compounds. The remaining substituents have not been altered much; only occasionally have they been changed from H to CH_3 or to a halogen. The best correlation, therefore, that the regression asnalysis has revealed for BZs is:

$$-\log IC_{50} = 0.449\ (\pm 0.143)\pi_{R7} + 1.114\ (0.361)\sigma_{R7} + 2.174\ (\pm 0.537)\sigma_Y - 0.870\ (0.387)I_6 + 6.988 \quad . \ . \ . \ (3.1)$$

$$n = 50, \quad r = 0.91, \quad s = 0.31, \quad F_{4,45} = 52.38$$

Where 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. The data in parentheses are 95% confidence intervals. I_6 is an indicator parameter used to account for the effect of chlorine present at the 6-position. It was given a value of unity or zero for the presence or absence of chlorine, respectively. It appears to be an important parameter, as a notable decrease in the significance of the correlatin occurs if it is dropped [Eqn.(3.2)]. Its negative coefficient in Eqn.(3.1) suggests that the presence of chlorine at the 6-position will reduce the activity approximately to one-eighth. How this negative

effect is actually produced will be discussed later.

$$-\log IC_{50} = 0.443\ (\pm 0.170)\ \pi_{R7} + 1.314\ (\pm 0.417)\ \sigma_{R7} + 2.164\ (\pm 0.641)\ \sigma_Y + 6.887 \quad \ldots\ldots\ldots\ldots (3.2)$$

$n = 50, \quad r = 0.86, \quad s = 0.37, \quad F_{3,46} = 44.37$

Coefficients of all the variables on the right-hand side in Eqn.(3.1) are significant at the 95% confidence interval and the F-value is significant at the 99% level [$F_{4,45}$ (0.01) = 3.77]. Thus Eqn. (3.1) exhibits quite a significant correlation accounting for about 83% variance ($r^2 = 0.83$) in the activity. The use of parameters related to other substituents in the compound were not found to have any further effect on the significance of the equation. Therefore Eqn.(3.1) suggests that a highly hydrophobic and electron withdrawing substituents at the 7-position and a highly electron-withdrawing group at the Y-position of the phenyl moiety are desirable for high inhibition of the compounds in the series of BZs.

For β-carbolines (Table 3.2), the best correlation that has been obtained is shown by Eqn.(3.3). In the derivation of

$$-\log K_i = 6.457 - 4.282\ (\pm 1.442)\ \pi_{R1} + 1.438\ (\pm 0.863)\ (\pi_{R1})^2 + 4.792\ (\pm 1.840)\ \sigma_{R2} + 0.974\ (\pm 0.611)\ \pi_{R2} \quad \ldots\ldots (3.3)$$

$n = 14, \quad r = 0.98, \quad s = 0.47, \quad F_{4,9} = 39.38$

Eqn.(3.3), compound 7 was not included since it behaved as an outlier. As can be seen in Table 3.2, its observed activity value is very low compared to the value predicted by the equation. The anomalous behaviour of this compound will be discussed later. Equation (3.3), represents a highly significant correlation and exhibits the role of hydrophobic and electronic properties of R_1 and R_2 substituents in the binding of compounds with BZ-receptors. A dummy parameter used to account for the effect of the R_3 substituent which was either H or OH was not found to have any effect on the significance of the correlation.

These QSAR studies on BZs and β-carbolines lead us to suggest the mode of interaction of these drugs with BZ-receptors and to recognize the important active sites in the latter. Previous studies on the binding of pyrazoloquinolines[13] and purines and amino acid derivatives[14] with BZ-receptors have indicated that a BZ-receptor should have a strong nucleophilic centre, a polar site and a hydrophobic pocket to accomodate any hydrophobic group present in the drug molecule. This receptor model is very near to the one shown in Fig. 3.1[8] for the binding of diazepam or flunitrazepam, the two BZs against which the binding affinity of other ligands are studied. In Fig.3.1, the receptor is shown to possess two hydrogen bond donor sites, H_1 and H_2, one interacting with imine nitrogen N_4 and the other with carbonyl oxygen attached to C_2 in diazepam/flunitrazepam, a cationic site E^+ to interact with

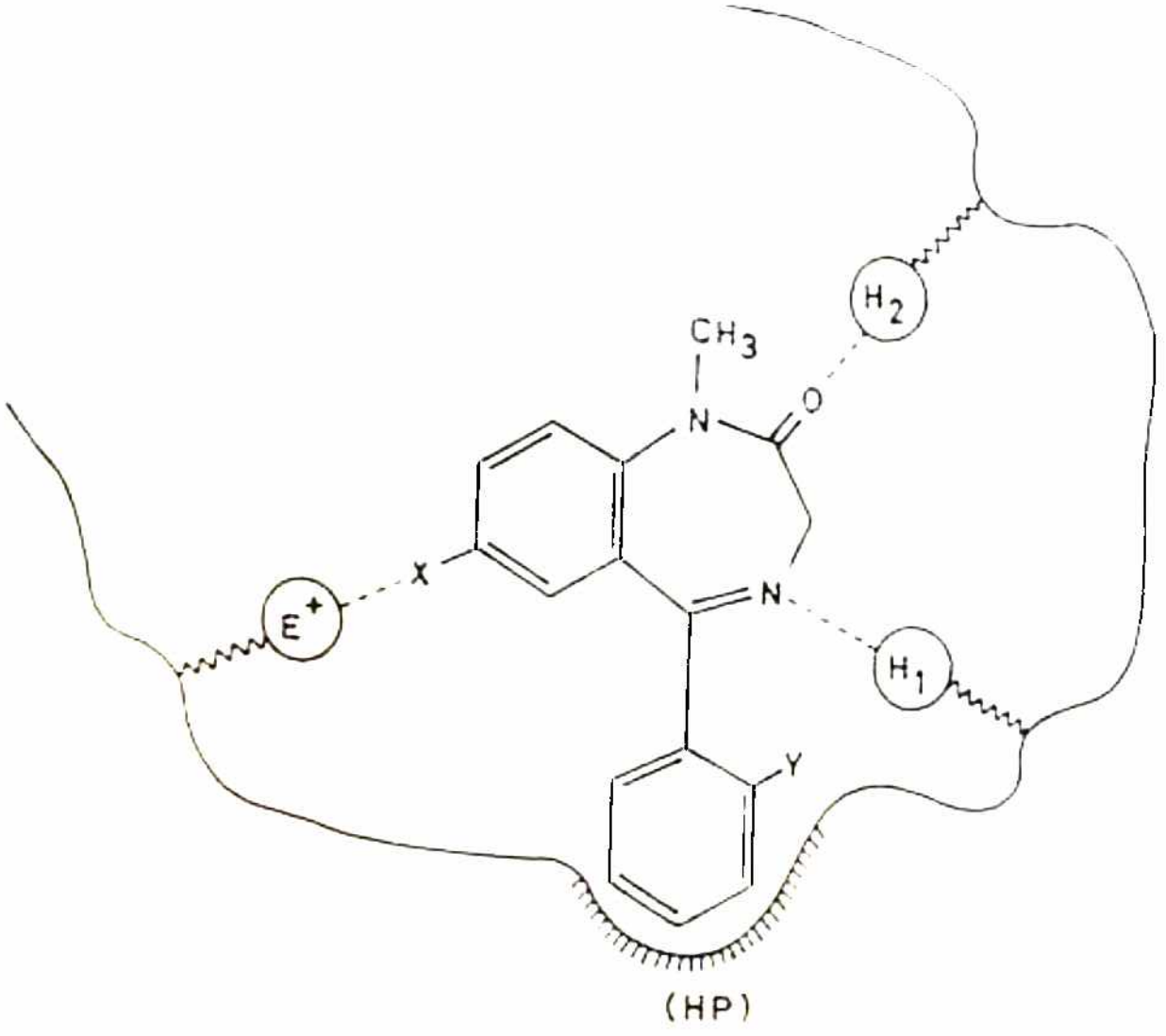


Fig.3.1. Hollinshead's model for the interaction of diazepam (X = Cl, Y = H) and flunitrazepam (X = NO_2, Y = F) with BZ receptor.

chlorine in diazepam or with the NO_2 group in flunitrazepam, and a large hydrophobic pocket to engulf the phenyl moiety attached at the 5-position. This receptor model however is not fully satisfactory to account for the binding of 'classical' BZs whose QSAR study has been presented here. For the BZs which have acted fully as agonists, Eqn.(3.1) shows that if the substituent is varied at the 7-position, which is occupied by chlorine in diazepam and by the NO_2 group in the flunitrazepam, the binding affinity would be affected by both the hydrophobic and electronic properties of the substituent. Similarly, if the Y-substituent of the phenyl moiety, which is H in diazepam and F in flunitrazepam, is also varied, the binding affinity of the molecule, according to Eqn.(3.1), would be a function of its electronic property. In Fig.3.1,this Y-substituent has not been shown to interact with the receptor. The electronic property of the Y-substituent and of R_7 (substituent at the 7-position) which is shown to affect the activity is related to the electron-withdrawing nature and hence may lead to an increase in the electronic charge and enable the substituents to interact more strongly with cationic sites of the receptor. As shown in Fig.3.1, cationic site is available for R_7 (denoted by X). Thus, a strong electrostatic interaction can take place between the cationic site and the R_7 substituent. Now here the attention can be drawn to the role played by the chlorine present at the 6-position. Chlorine is a strong electron-withdrawing group and thus can withdraw the electron from the R_7 substituent. This will result in a decrease in the electronic charge of the

substituent and thus in the strength of its electrostatic binding with the receptor. This surmise explains the negative role played by the chlorine at the 6-position and suggests further that any electron-withdrawing group at this position will likewise reduce the activity. It can therefore be expected that any electron-donating group will, on the other hand, enhance the activity.

To explain how the hydrophobic nature along with the electronic property of the R_7-substituent affects the activity, we assume that the cationic site E^+ of the receptor has some hydrophobic group which interacts hydrophobically with R_7 in the vicinity. As already pointed out, the Y-substituent in BZs should also be involved in the electrostatic interaction, but the Hollinshead model (Fig.3.1) does not show any polar site at the receptor in the vicinity of this substituent. We therefore assume that the hydrophobic pocket engulfing the phenyl moiety is not completely hydrophobic but is slightly polar (cationic) at one end to permit the interaction of Y. Since, unlike the Y-substituent, the X-substituent of the phenyl ring in 'classical' BZs (I) is not found to have any effect on the binding affinity, the hydrophobic pocket of the receptor can be assumed to have no axial conformational symmetry around the phenyl ring, thus excluding any possibility of interaction with the X substituent. Taking all these points into consideration, the model for the interaction of BZs with their receptors can be

best represented by Fig.3.2. Based on this model, the binding of β-carbolines can also be explained.

Figure 3.3 represents the binding of β-carbolines (Table 3.2) for which Eqn.(3.3) has been derived. According to Eqn.(3.3), the binding affinity of this series of compounds is affected by both hydrophobic and electronic properties of the R_2 substituent. This is very well explained by the model showing the possibility of both types of interaction, hydrogen bonding and hydrophobic, for the group.

A R_2 group like CO_2CH_3 will exhibit hydrogen bonding at both hydrogen bonding centres as shown in Fig.3.3 (bonding with H_1 is a three-centre hydrogen bond stabilizing the hydrogen bond with imine nitrogen) and will be involved in hydrophobic interaction too, but a group like CHO will have only hydrogen bonding and no hydrophobic interaction. Consequently, these three groups show their effects in decreasing order. A group which has no hydrogen bonding ability would lead to a very low activity. Compound 7, which was excluded from the regression analysis, has very poor activity because R_2 is a carboxylic group which is extensively ionized at the pH used and the resulting COO^- group may be expected to be sufficiently hydrated in solution to be incapable of participating in hydrogen bonding or hydrophobic interactions with the receptor.

Equation (3.3) also suggests that the hydrophobic nature of

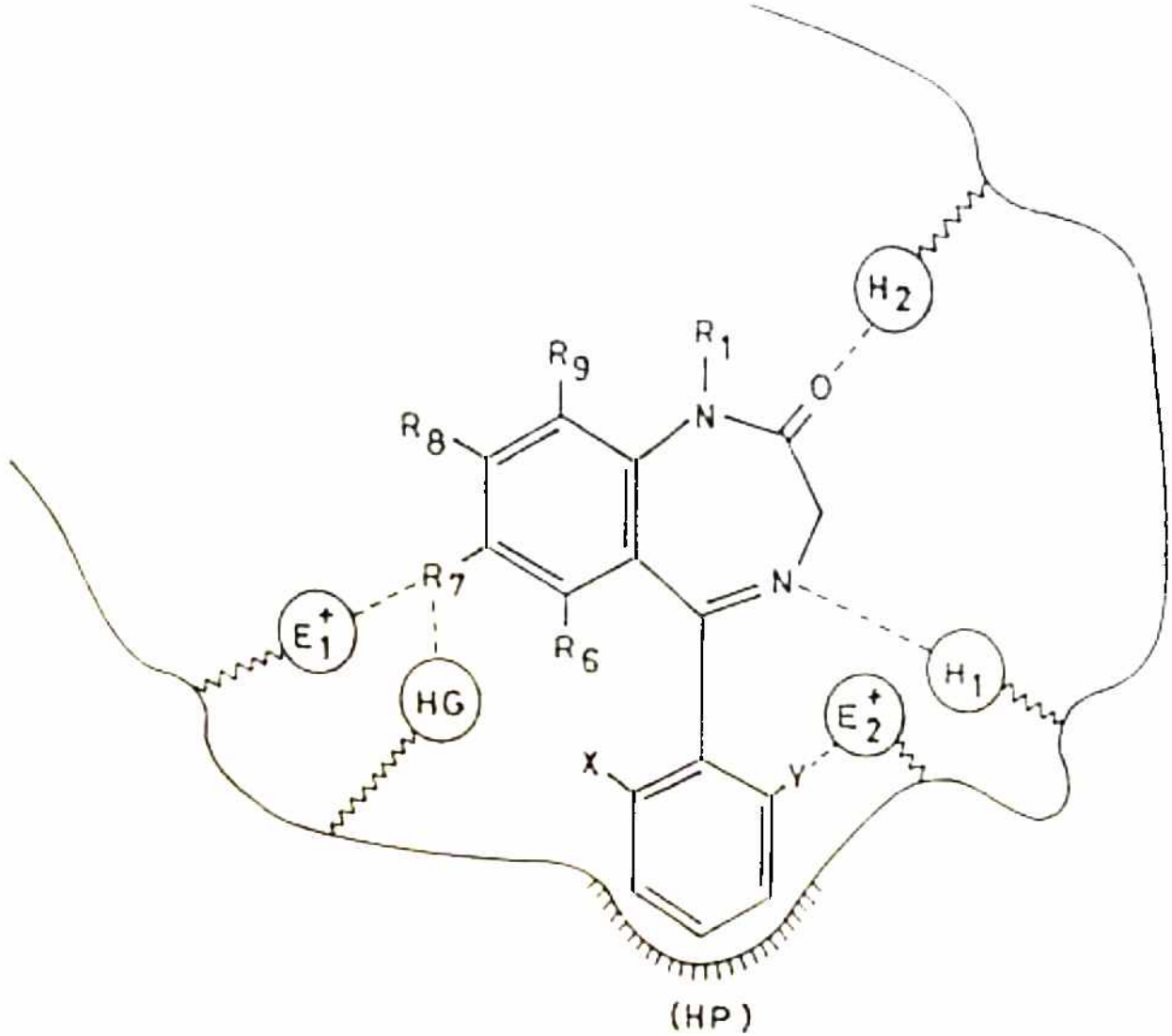


Fig.3.2. Proposed model for the interaction of BZs with the receptor.

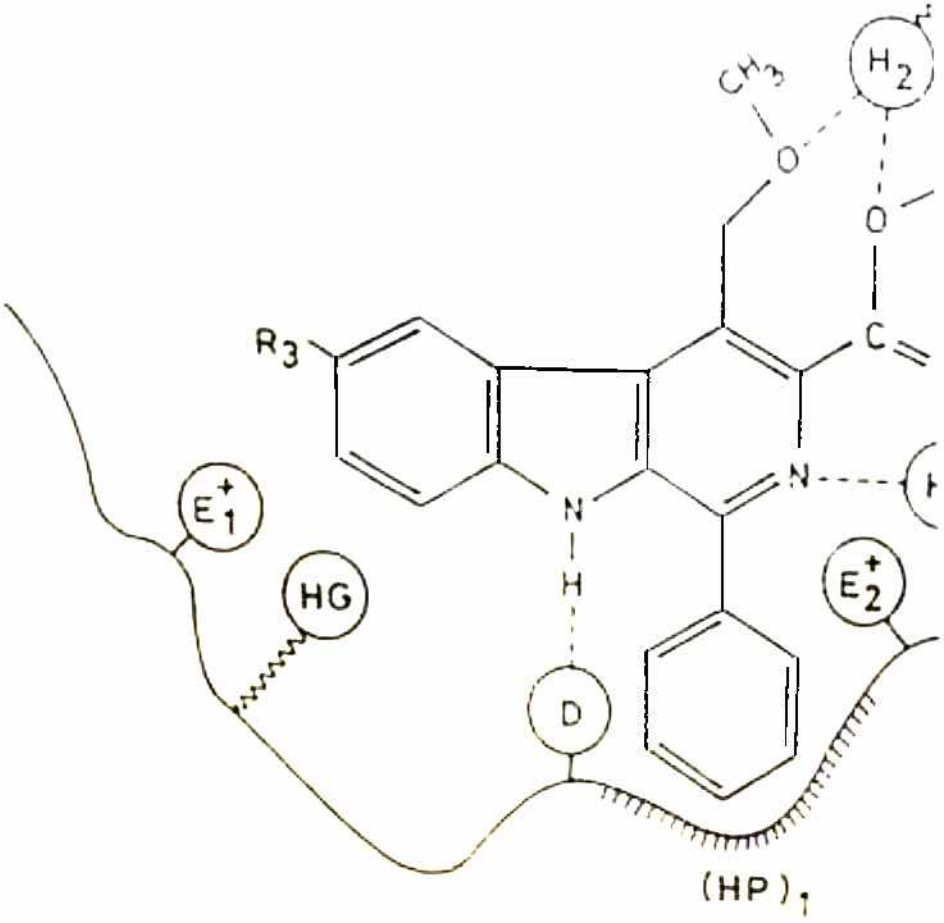


Fig.3.3. Proposed model for the inter
with the BZ receptor.

action of β-carbolines

the R_1 group will play a negative role till the hydrophobic constant for the group attains an optimum value of 1.49. Only a few substituents have been tried at the R_1 position and they have all led to a decrease in activity (Table 3.2). An alkyl group and a phenyl group have been used. Their negative effect can be attributed to their electron-releasing ability. By releasing an electron, they can increase the basicity of N_2 nitrogen which can consequently be protonated and thus loose its ability to undergo hydrogen bonding with the H_1 site of the receptor (Fig.3.3). The slightly less negative effect of the C_6H_5 group is due to its larger size which enables it to reach the hydrophobic pocket, $(HP)_1$, of the receptor (Fig.3.3). It shows a hydrophobic interaction with π value (1.96) which is greater than the optimum value (1.49). The smaller alkyl groups, like CH_3 and C_2H_5, will have no access to the hydrophobic pocket and thus would produce only a negative effect.

The R_3 group, which is an OH group, was not found to have any effect on the binding affinity. This can be easily explained by assuming that the receptor has no corresponding active site for the interaction of this group. However, we assume that there is one more active site, a hydrogen- bond acceptor site D, at the receptor that might be involved in the hydrogen bonding with N_9 nitrogen. The basis of assuming hydrogen bonding with this nitrogen is that when the hydrogen of this nitrogen was replaced by a CH_3 group, the activity drastically

decreased (Table 3.3). It can be pointed out here that this hydrogen bond acceptor site may also be involved in hydrogen bonding with the R_6 substituent of BZs (Fig.3.2, D not shown), provided R_6 is a hydrogen bond donor. Thus if R_6 is a hydrogen bond donor and an electron-donating group, it will doubly affect the binding of BZs with the receptor. With all these considerations, Fig.3.3 appears to be an appropriate model for the interaction of β-carbolines with BZ-receptors.

Because of the little variation in substituents of compounds in Table 3.3, it was not possible to carry out a QSAR study. A group such as CH_2OCH_3 at the 4-position in β-carbolines (R_1 in III) may show three-centre hydrogen bonding with H_2 site stablizing the hydrogen bonding of the group at the 3-position (Fig.3.3).

To further verify the proposed active sites at the BZ-receptor, we made a few more QSAR studies. A series of tetracyclic 1,4-BZs and a series of 9-benzylpurines were further treated. For all the 31 compounds of tetracyclic 1,4-BZ series (Table 3.4), the best correlation that could be obtained is as shown by Eqn.(3.4).

$$-\log IC_{50} = 0.881\ (\pm 0.255)\ \pi_{COR1} - 0.232\ (\pm 0.087)\ (\pi_{COR1})^2 - 1.696\ (\pm 0.800)\ \pi_{R7} + 7.733 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

$$n = 31, \quad r = 0.848, \quad s = 0.38, \quad F_{3,27} = 23.12$$

This Eqn. expresses a significant correlation between the

activity and the hydrophobic parameter π of COR_1 group and R_7 substituent. No physicochemical parameter for R_8 substituent was found to be of any importance in the correlation. Though Eqn.(3.4) accounts for about 72% of the variance in the activity (r^2 = 0.72), it predicts very high activity for compound 5 as compared to its observed activity. If this compound is deleted, the correlation is significantly improved to account for 83% of the variance in the activity. In both Eqns.(3.4) and (3.5), the F-value is significant at 99% level [$F_{3,27}$ (0.01) = 4.60; $F_{3,26}$ (0.01) = 4.64]. No electronic parameter for any substituent was found to play any role in the correlation.

$$-\log IC_{50} = 0.909\ (\pm 0.203)\ \pi_{COR1} - 0.243\ (\pm 0.070)\ (\pi_{COR1})^2 - 1.777\ (\pm 0.636)\ \pi_{R7} + 7.766 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

$n = 30, \quad r = 0.905, \quad s = 0.30, \quad F_{3,26} = 39.20$

In case of 9-benzylpurines too (Table 3.5), no electronic parameter was found to affect the activity, and the best correlation that was obtained using hydrophobic characteristic of substituents was as shown by Eqn.(3.6). The hydrophobic parameter for R_1-substituent was found to be insignificant, and so were found the two indicator parameters used to account for

$$-\log IC_{50} = 5.112 - 1.400\ (\pm 0.645)\ \pi_{R3} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.6)$$

$n = 18, \quad r = 0.755, \quad s = 0.60, \quad F_{1,16} = 21.18$

the effect of the presence of CH_3 at R_2-position and Br at R_4-position. Instead a parameter I_s to account for the configurational effect of CH_3 group at R_2-position was found to be of considerable significance [Eqn.(3.7)]. It was given a value of 1 for R-configuration, 1 for S-configuration and zero for the RS-configuration. However, correlation expressed by

$$\log IC_{50} = 5.112 - 1.400\ (\pm 0.582)\ \pi_{R3} - 0.840\ (0.814)\ I_s \quad \ldots\ldots\ldots\ldots\ldots (3.7)$$

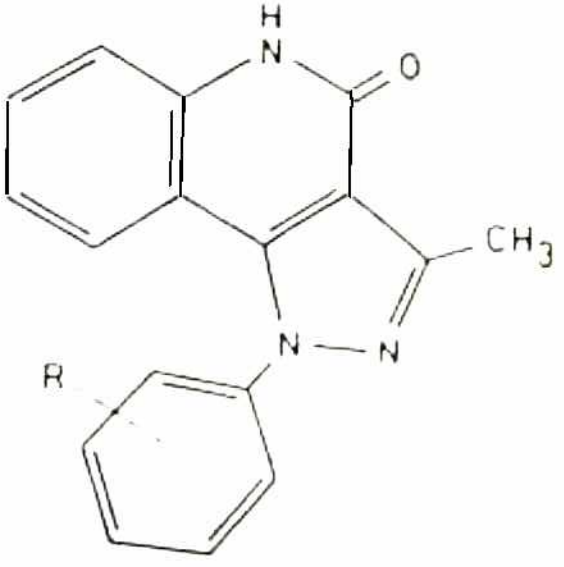
$$n = 18, \quad r = 0.821, \quad s = 0.54, \quad F_{2,15} = 15.54$$

Eqn.(3.7) [or Eqn.(3.6)] shows the negative effect of hydrophobic property of R_3-substituent. This finding is neither in confirmity to the previous study on arylpyrazoloquinolines[13], nor does it make any sense in the interpretation of drug-receptor interaction. In arylpyrazoloquinolines (VI), the hydrophobic property of substituent at 3- or 5- position, identical to R_3-position, in aryl ring was found to produce the positive effect [Eqn.(3.8)] and, as obvious from Eqn.(3.8), a steric effect was shown to be produced by substituent at 2- or 6- position. Since in this

$$-\log IC_{50} = 0.481\ (\pm 0.188)\ E_s(2,6) + 0.606\ (\pm 0.372)\ \pi(3,5) + 4.814 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.8)$$

$$n = 20, \quad r = 0.87, \quad s = 0.27, \quad F_{2,17} = 24.76$$

case, π was dependent upon the size of substituent, a single parameter V_w, the van der Waals volume, was found to account



(VI)

well for the change in the activity [Eqn.(3.9)]. The negative coefficient of V_w (2,6) in Eqn.(3.9) describes the steric

$$-\log IC_{50} = 4.864 - 2.60\ (\pm 1.14)V_w(2,6) + 1.62(\pm 1.15)V_w(3,5) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.9)$$

$$n = 20, \quad r = 0.86, \quad s = 0.28, \quad F_{2,17} = 22.56$$

effect produced by substituent at 2- or 6- position. Assuming that the positive coefficient of $V_w(3,5)$ in Eqn.(3.9) accounts for the dispersion interaction rather than hydrophobic interaction with the receptor, it was attempted to correlate the activity of 9-benzylpurines with molar refractivity index (MR) of the substituents and obtained a better correlation [Eqn.(3.10)] than that expressed by Eqn.(3.7). A still better correlation was obtained [Eqn.(3.11)] when compound 5 was deleted, as it behaved slighlty as an outlier. Now, as expected, Eqn.(3.11) accounts very well for the dispersion in-

$$-\log IC_{50} = 0.632\ (\pm 0.256)\ MR.R_3 - 0.038\ (\pm 0.019)\ (MR.R_3)^2 - 0.840\ (\pm 0.714)\ I_s + 4.416 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.10)$$

$$n = 18, \quad r = 0.877, \quad s = 0.47, \quad F_{3,14} = 15.59$$

$$-\log IC_{50} = 0.565\ (\pm 0.204)\ MR.R_3 - 0.035\ (\pm 0.015)\ (MR.R_3)^2 - 0.840\ (\pm 0.554)\ I_s + 4.534 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.11)$$

$$n = 17, \quad r = 0.901, \quad s = 0.36, \quad F_{3,13} = 18.67$$

teraction but optimizes the MR value (MR_{opt} = 8.07) beyond which there would be a steric effect from the substituent at the same position.

Now Eqn.(3.11) and Eqn.(3.5) which are meant for two entirely different series of BZ-receptor ligands suggest that a slight modification is needed in the proposed active sites, particularly those involved in electrostatic interaction at the BZ-receptor. The previous model proposed for the BZ-receptor based on QSAR studies on the series of 'classical' BZs and β-carbolines as shown in Fig.3.3 has two cationic sites E_1' and E_2^+, two hydrogen bond donor sites H_1 and H_2, a hydrogen bond acceptor site D, two hydrophobic pockets $(HP)_1$ and $(HP)_2$, and a hydrophobic group HG. In case of classical BZs(I), the substituent at R7 position equivalent to that of R7 in (IV) was speculated to have electrostatic intreraction with E_1^+ site of the receptor, as the activities of the compounds was found to be well correlated with electron withdrawing ability and hydrophobic property of this substituent. But in the present case of tetracyclic 1,4-BZs, only the hydrophobic parameter of R7 substituent is found to be related with the activity and as Eqn.(3.5) exhibits, its role is negative. Unfortunately, there are only two different R7 substituents, F and Cl. In Table 3.4 if one compares the activity of compound 11 and 14 with that of 8, it is found that chlorine reduced the activity to a greater extent than fluorine present at R7 position. Since chlorine is bigger in size than fluorine and since there are not many substituents at this position to have any further idea, it can be simply assumed that they produce some steric effects. Similarly, OCH_3 group at R8-position also appears to produce the steric effect

lowering the activity of compound 5, while other substituents at this position have hardly any effect. This explains why compound 5 behaved as an outlier. Substituents at R_8-position would have produced a positive effect, provided they had been a hydrogen bond donor and thus capable of forming a hydrogen bond with site D as suggested in the previous model (Fig.3.3). But unfortunately none of R_8 substituents shown in Table 3.4 has this property and thus produces no effects.

The hydrophobic property of COR_1 group at imidazole ring was however found to play a positive role but to an optimum (Eqn.3.5, π_{opt} = 1.88), as there is a parabolic correlation between it and the activity. This in fact accords to the interaction model proposed earlier (Fig.3.3). Its interaction with the receptor is shown in Fig.3.4. The carbonyl oxygen will have the hydrophobic interaction with $(HP)_2$ site which is indicated to possess a limited bulk tolerance.

In case of 9-benzylpurines, it has been already discussed on the basis of Eqn.(3.11) that R_3 substituent may be involved in dispersion interaction and if bigger in size would produce steric effect. This substituent can be assumed to interact with E_1^+ site, but this site should not be purely cationic but polar in nature and may be symbolized by E_1^{+-}. That R_3 substituent interacts with E_1^{+-} site, as shown in Fig.3.5, gets support from the configurational effect of CH_3 group at R_2-position. Eqn.(3.11) suggests that S-configuration in which CH_3 group will be on the right side of the chiral carbon as

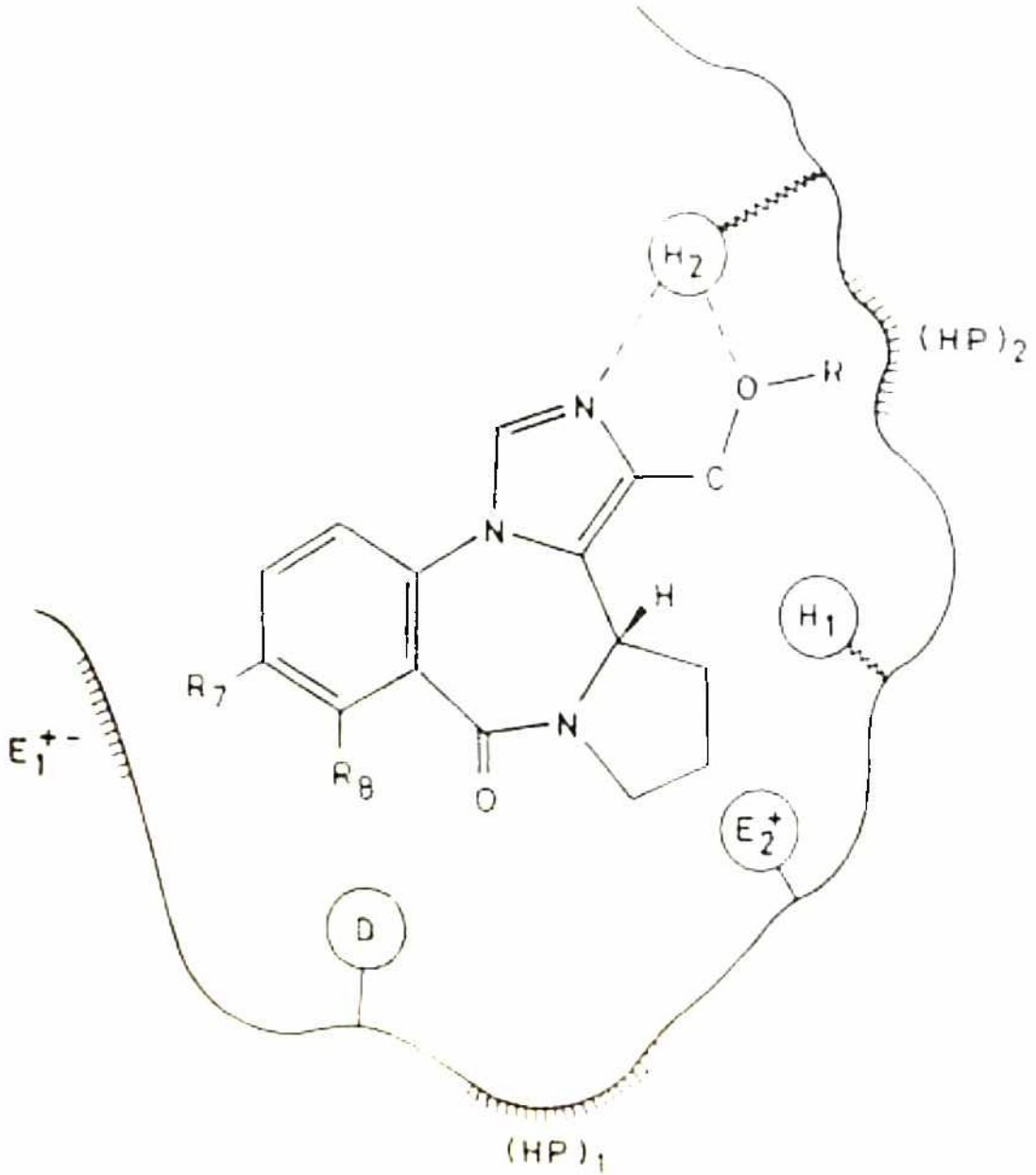


Fig.3.4. The binding of tetracyclic 1,4-BZs with BZ-receptors.

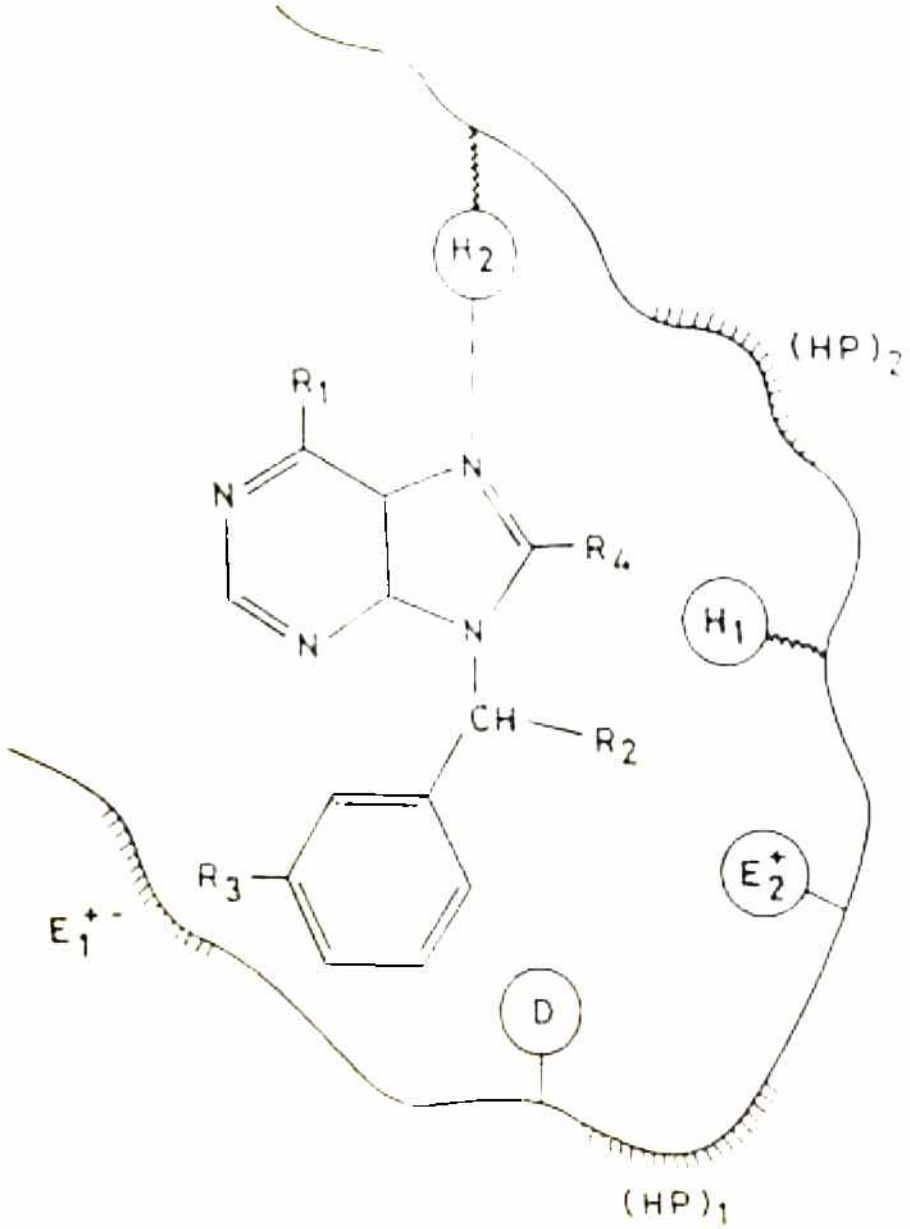


Fig.3.5. The binding of 9-benzylpurines with BZ-receptors.

shown in Fig.3.5 would enhance the activity. This is very obvious, as in this configuration the aryl ring and R_3-substituent will be towards E_1^{+-} site of the receptor.In the other case, i.e., in R-configuration the aryl ring would be on the right side of the chiral carbon and thus away from the active site of the receptor.

As obvious from Fig.3.5, none of other substituents in 9-benzylpurines is approaching to any active site, hence whatever be their physicochemical characteristics, they have not been able to affect the activity and consequently any parameter related to them remained insignificant in the correlation equation.

This study points out that the earlier interaction model [Fig.3.3] needs only a slight modification. However, further study is needed to provide a perfect model.

B : CHOLECYSTOKININ ANTAGONISTS

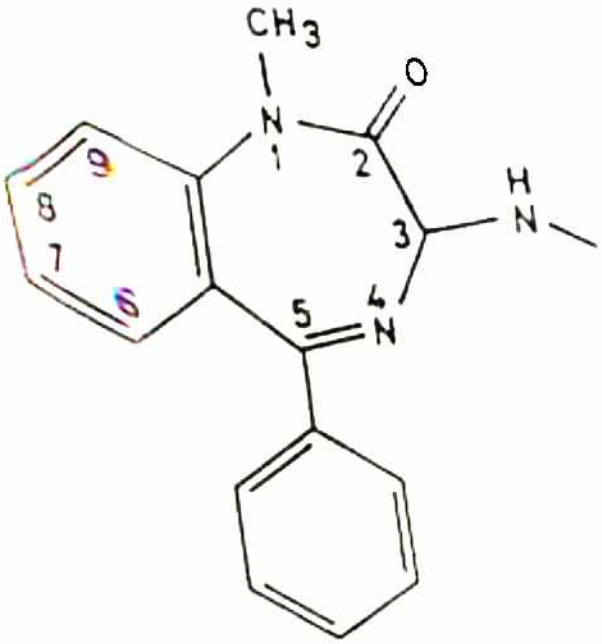
As already discussed in chapter I, cholecystokinin (CCK) is a gastrointestinal peptide harmone and putative central neurotransmitter. It exerts a variety of actions on peripheral target tissues such as gall bladder contraction and pancreatic exocrine secretion, and may function as a neurotransmitter or neuromodulator in the central nervous system. These effects are mediated by two receptor subtypes designated as CCK-A and CCK-B. CCK-A is found in peripheral target tissues and CCK-B in central nervous system. The latter exhibits ligand specificifies similar to the gastrin receptor.

Synthetic studies using benzodiazepine core of the natural product asperlicin[15] have yielded the highly potent CCK-A antagonist MK-329(VII)[16,17] and the CCK-B/gastrin antagonist L-365, 260(VIII).[18,19] However, so far hardly any study has discussed the nature of binding of CCK antagonists to the receptors, which would have facilitated investigations of the role of CCK in normal physiology and diseased states and the design of simpler but more effective nonpeptide antagonists. Gupta and Saha[20] did make some study in this direction through a quantitative structure-activity relationship (QSAR) study on analogues of (VII) and (VIII), but got only a rough idea of ligand-receptor interaction. To have further insight into the nature of binding and the active sites in the receptor, further QSAR studies are needed. In this communication, we present QSAR studies on three different series of CCK

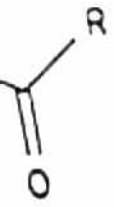
antagonists and discuss their implications with regard to the nature of drug receptor interactions.

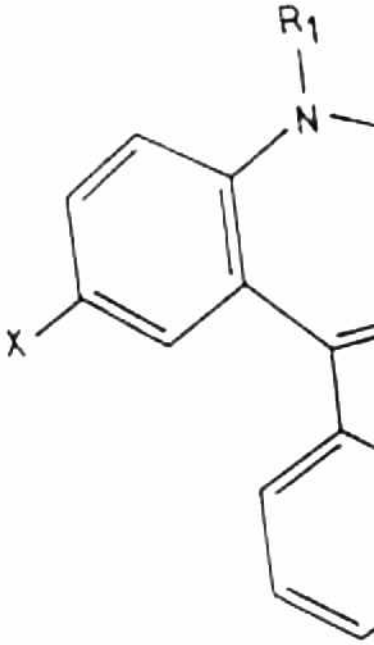
MATERIALS AND METHODS

The three different series of CCK antagonists that were subjected to QSAR studies are: (1) a series of 3-(3-indolylmethyl)benzodiazepines [IBZs,(IX)] studied by Evans et al.[17], (2) a series of glutamic acid (GA) analogues (X) studied by Freidinger et al.[21], and (3) a series of quinazolinone (QZ) derivatives (XI) studied by Yu et al.[22] These three series are listed along with their CCK-receptor binding affinities in Tables 3.6 - 3.8, respectively. The structural physicochemical parameters of substituents controlling the binding affinities were either calculated or taken from the literature.[12] Amongst the structural parameters, the van der Waals volume V_W, the first-order valence connectivity index $^1\chi^v$ were calculated as discussed in chapter II. The receptor binding affinity parameter is in terms of IC_{50}, the molar concentration of compound required for half-maximal inhibition of binding of ^{125}I-labeled CCK to CCK receptors in rat pancreas or guinea pig brain, or for half-maximal inhibition of binding of ^{125}I-labeled gastrin to guinea pig gastric glands. It is specified, as the case may be, in the footnotes of the Tables.



(VII), R = 2-indolyl (S-configu
(VIII), R = m-Me-phenylamino (





ration)

R - configuration)

(IX)

Table 3.6 :3-(3-Indolylmethyl)benzodiazepines (IX) and their CCK-receptor binding affinity and structural parameters.

Sr.No.	X	Y	Z	R_1	R_2	3-Stereo	$^{v}_{w.R1}$ (10^2 A^3)	$^{1}\chi^{v}_{R2}$	-log IC_{50}[a], Pancreas Obsd.[b]	Cald[c]
1.	Cl	H	H	H	H	R	0.056	0.0	5.47	5.37
2.	Cl	H	H	H	H	S	0.056	0.0	4.50	4.34
3.	H	H	H	H	H	R	0.056	0.0	5.92	6.24
4.	H	F	H	H	H	R	0.056	0.0	6.30	6.24
5.	Cl	Cl	H	H	H	R	0.056	0.0	5.30	5.37
6.	H	F	H	H	H	S	0.056	0.0	4.98	5.20
7.	H	COOH	H	H	H	R	0.056	0.0	4.75[d]	6.24
8.	H	H	5-Br	H	H	RS	0.056	0.0	5.54	5.72
9.	H	F	5-F	H	H	RS	0.056	0.0	5.85	5.72
10.	H	F	6-F	H	H	RS	0.056	0.0	5.89	5.72
11.	Cl	H	H	CH_3	H	R	0.245	0.0	5.85	5.70
12.	H	H	H	CH_3	H	R	0.245	0.0	6.52	6.57
13.	H	F	H	CH_3	H	R	0.245	0.0	6.57	6.57
14.	H	F	H	C_2H_5	H	R	0.399	0.0	6.52	6.46
15.	H	F	H	CF_3CH_2	H	R	0.537	0.0	5.44	6.06
16.	H	F	H	n-C_5H_{11}	H	R	0.861	0.0	4.00	4.06

continued . . .

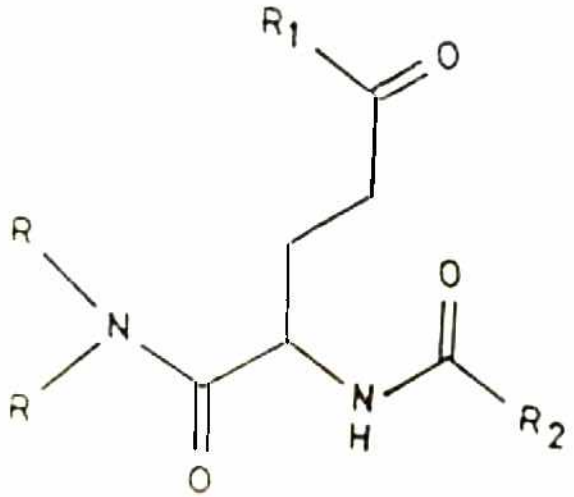
Table 3.6 continued . . .

Sr.No.	X	Y	Z	R_1	R_2	3-Stereo	$V_{w,R1}$ (10^2 A^3)	$^1\chi^v_{R2}$	-log IC_{50}[a], Pancreas Obsd.[b]	Cald[c]
17.	H	F	H	$(CH_3)_2CH(CH_2)_2$	H	R	0.861	0.0	4.00	4.06
18.	H	F	H	c-$C_3H_5CH_2$	H	R	0.603	0.0	5.66	5.77
19.	H	F	H	$(CH_3)_2N(CH_2)_2$	H	R	0.822	0.0	4.00	4.38
20.	H	F	H	CH_2COOEt	H	R	0.809	0.0	5.08	4.49
21.	H	F	H	CH_2COOH	H	R	0.488	0.0	6.52	6.25
22.	H	F	H	CH_2CONH_2	H	R	0.519	0.0	5.68	6.13
23.	H	F	H	$(CH_2)_2CN$	H	R	0.576	0.0	6.16	5.90
24.	H	F	H	$(CH_2)_2COOH$	H	R	0.642	0.0	5.85	5.58
25.	H	H	H	CH_3	CH_3	R	0.245	0.0	7.00	6.57
26.	H	F	H	CH_3	CH_3	R	0.245	0.0	6.44	6.57
27.	Cl	H	H	CH_3	$PhCH_2$	R	0.245	2.264	3.82	4.16
28.	H	F	H	CH_3	p-ClC_6H_4CO	R	0.245	2.772	4.96	4.68

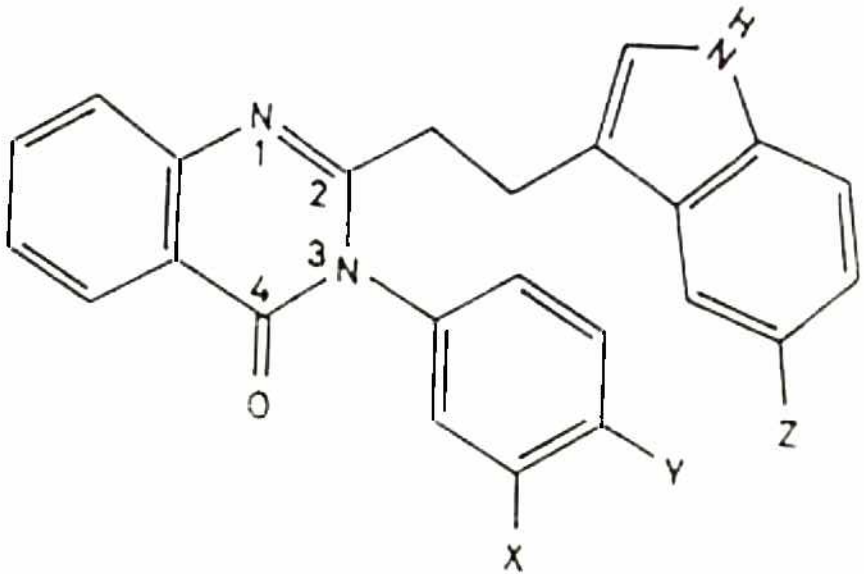
[a] IC_{50} : the molar concentration of the compound required for half-maximal inhibition of binding of [^{125}I] CCK-33 to rat pancreas.

[b] Taken from ref.17. [c] Calculated from Eqn.(3.13).

[d] Not used in deriving Eqn.(3.13).



(X)



(XI)

Table 3.7 : Glutamic acid analogues (X) and their CCK-receptor and gastric glands binding affinities.

S. No.	R	R_1	R_2	Stereo	$V_w(10^2$ Å$^3)$			-log IC_{50}					
								Pancreas		Brain		Gas.Glands	
					$N(R)_2$	R_1	R_2	Obsd[b]	Cald[c]	Obsd[b]	Cald[d]	Obsd[b]	Cald[e]
1.	n-C_3H_7	OH	Ph	RS	1.117	0.137	0.785	3.60	3.47	3.10	3.34	3.05	3.26
2.	n-C_5H_{11}	OH	3,4-Cl_2Ph	RS	1.733	0.137	1.115	7.75	7.40	5.65	6.26	5.72	6.16
3.	n-C_3H_7	OH	2-Indolyl	S	1.117	0.137	1.054	5.43	5.40	4.06	3.89	4.77	4.14
4.	n-C_3H_7	OH	2-Indolyl	R	1.117	0.137	1.054	5.96	6.63	4.57	4.91	4.41	4.99
5.	n-C_3H_7	OCH_2Ph	2-Indolyl	R	1.117	0.998	1.054	6.09	5.62	5.34	4.91	5.16	4.99
6.	n-C_5H_{11}	OH	2-Indolyl	R	1.733	0.137	1.054	8.12	7.44	6.64	6.53	6.77	6.29
7.	c-C_6H_{11}	OH	2-Indolyl	R	1.833	0.137	1.054	7.23	7.57	5.47	5.00	5.39	4.69
8.	c-C_6H_{11}	OCH_2Ph	2-Indolyl	R	1.833	0.998	1.054	6.70	6.56	4.52	5.00	4.00	4.69
9.	n-C_5H_{11}	OH	p-Cl-Ph-NH	RS	1.733	0.137	1.044	6.85	6.73	6.20	5.98	6.16	5.82
10.	n-C_5H_{11}	OCH_2Ph	p-Cl-Ph-NH	RS	1.733	0.998	1.044	5.03	5.72	4.80[f]	5.98	4.36[g]	5.82
11.	n-C_5H_{11}	OC_2H_5	o-Cl-Ph-NH	RS	1.733	0.458	1.044	5.92	6.35	6.00	5.98	5.35	5.82
12.	n-C_5H_{11}	Pyrro-lidinyl	p-Cl-Ph-NH	RS	1.733	0.705	1.044	6.17	6.06	6.60	5.98	6.37	5.82
13.	n-C_5H_{11}	OH	m-OMe-Ph-NH	S	1.733	0.137	1.114	6.27	6.77	5.01	5.75	4.89	5.73

continued . . .

Table 3.7 continued . . .

S. No.	R	R_1	R_2	Stereo	$V_w(10^2$ Å$^3)$			-log Ic_{50}					
								Pancreas		Brain		Gas.Glands	
					$N(R)_2$	R_1	R_2	Obsd[b]	Cald[c]	Obsd[b]	Cald[d]	Obsd[b]	Cald[e]
14.	n-C_5H_{11}	OH	m-OMe-Ph-NH	R	1.733	0.137	1.114	8.35	8.01	6.15	6.77	6.51	6.58
15.	n-C_5H_{11}	Pyrro-lidinyl	m-OMe-Ph-NH	S	1.733	0.705	1.114	6.77	6.10	6.32	5.75	6.07	5.73
16.	n-C_5H_{11}	Pyrro-lidinyl	m-OMe-Ph-NH	R	1.733	0.705	1.114	6.92	7.34	7.20	6.77	6.72	6.58

[a] IC_{50} :the molar concentration of the compound required for half maximal inhibition of binding of [^{125}I]CCK-33 to rat pancreas and guinea pig cortex, and for half-maximal inhibition of binding of [^{125}I]gastrin to guinea pig gastric glands.

[b] Taken from ref.21.

[c] Calculated from Eqn.(3.14).

[d] Calculated from Eqn.(3.15).

[e] Calculated from Eqn.(3.16).

[f] Not used in deriving Eqn.(3.15).

[g] Not used in deriving Eqn.(3.16).

Table 3.8 : Quinazolinone analogues (XI) and their CKK B receptor binding affinity.

S.No.	X	Y	Z	$V_{w,x}$	-log IC_{50}[a]	
					Obsd.[b]	Cald[c]
1.	H	H	H	0.056	6.17	6.72
2.	F	H	H	0.115	6.14	6.23
3.	Cl	H	H	0.244	6.16	6.32
4.	Br	H	H	0.287	6.43	6.33
5.	Me	H	H	0.245	6.82	7.03
6.	Et	H	H	0.399	7.14	7.20
7.	MeO	H	H	0.304	6.80	6.79
8.	i-PrO	H	H	0.612	7.59	7.14
9.	CF_3	H	H	0.383	6.32	6.37
10.	MeO	H	Me	0.304	7.26	7.12
11.	MeO	H	MeO	0.304	7.17	6.78
12.	MeO	H	F	0.304	6.96	6.87
13.	MeO	H	Cl	0.304	7.33	7.21
14.	MeO	H	Br	0.304	7.42	7.30
15.	MeO	H	Br	0.304	7.47	7.30
16.	n-PrO	H	Br	0.612	7.24	7.66
17.	i-PrO	H	Br	0.612	8.03	7.66
18.	Et	H	Br	0.399	7.34	7.71
19.	MeS	H	Br	0.423	7.34	7.38
20.	CF_3	H	Br	0.383	6.64	6.88
21.	NMe_2	H	Br	0.501	7.80	7.95
22.	MeO	MeO	Br	0.304	6.89	7.30

continued . . .

Table 3.8 continued . . .

S.No.	X	Y	Z	$V_{w,x}$	-log IC_{50}[a]	
					Obsd.[b]	Cald[c]
23.	H	MeO	Br	0.056	7.51	7.23
24.	H	EtO	Br	0.056	7.06	7.23
25.	H	i-PrO	Br	0.056	6.96	7.23
26.	H	Et	Br	0.056	7.55	7.23
27.	H	i-Pr	Br	0.056	7.43	7.23
28.	H	MeS	Br	0.056	7.43	7.23
29.	H	NMe_2	Br	0.056	7.48	7.23

[a] IC_{50} :the molar concentration for half-maximal inhibition of binding of [^{125}I]CCK-8 sulfate to mouse brain membranes.

[b] Taken from ref.22.

[c] Calculated from Eqn.(3.17).

RESULTS AND DISCUSSION

A multiple regression analysis was performed and the best correlation that was obtained for the first series of CCK-antagonists (Table 3.6) is as shown by Eqn.(3.12):

$$-\log IC_{50} = 5.290\ (\pm 2.631)\ V_{w,R1} - 8.375\ (\pm 2.883)\ (V_{w,R1})^2 - 0.668\ (\pm 0.265)\ {}^{1}\chi^{v}\ R2 - 0.723\ (\pm 0.464)\ I_X + 0.362\ (\pm 0.342)\ D + 5.316 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.12)$$

$n = 28,\quad r = 0.91,\quad s = 0.42,\quad F_{5,22} = 19.96$

This equation represents a highly significant correlation between the CCK-receptor affinity of IBZs (IX) and the structural parameters of the substituents. Two dummy parameters I_X and D have also been used in the equation. I_X with a value of unity indicates that X-substituent is chlorine, otherwise with a value of zero it indicates that X is simply a hydrogen atom. The parameter D is concerned with the configuration of the indolylmethyl group at the 3-position. It has been assigned a value of unity for R-configuration, -1 for S-configuration, and zero for RS-configuration. Though Eqn. (3.12) exhibits a highly significant correlation, it predicts a very high value for compound 7 as compared to its observed activity. Hence on exclusion of this compound, a much better correlation was obtained [Eqn. (3.13)]. In Eqns. (3.12) and (3.13), we have used the van der Waals volume of R_1-substituent and first order valence connectivity index for R_2-substituent. A replacement of ${}^{1}\chi^{v}$ gave inferior result. No physicochemical or

$$-\log IC_{50} = 3.933\ (\pm 2.074) V_{w,R1} - 7.236\ (\pm 2.225)\ (V_{w,R1})^2 - 0.680(\pm 0.198)\ {}^{1}\chi^{v}_{R2} - 0.864\ (\pm 0.353)\ I_X + 0.518\ (\pm 0.266)\ D + 5.518 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.13)$$

$n = 27, \quad r = 0.95, \quad s = 0.31, \quad F_{5,21} = 38.32$

structural parameters for Y- and Z-substituetns were found to have any relation with the activity.

For GA analogues of Table 3.7, the best correlations obtained were as follows,

$$-\log IC_{50}(\text{pancreas}) = 1.312\ (\pm 1.220)\ V_{w,N} - 1.173\ (\pm 0.847)\ V_{w,R_1} + 9.449\ (\pm 4.457)\ V_{w,R_2} + 0.618\ (\pm 0.392)\ D - 5.251 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.14)$$

$n = 16, \quad r = 0.92, \quad s = 0.53, \quad F_{4,11} = 16.13$

$$-\log IC_{50}(\text{brain}) = 73.774\ (\pm 44.431)\ V_{w,N} - 24.970\ (\pm 15.382)\ (V_{w,N})^2 + 3.929\ (\pm 4.873)\ V_{w,R_2} + 0.510\ (\pm 0.448)\ D - 50.982 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.15)$$

$n = 15, \quad r = 0.91, \quad s = 0.56, \quad F_{4,10} = 11.47$

$$-\log IC_{50}(\text{gas.glands}) = 73.696(\pm 48.598) V_{w,N} - 25.125\ (\pm 16.824)\ (V_{w,N})^2 + 4.842\ (\pm 5330)\ V_{w,r2} + 0.426(\pm 0.490)\ D - 51.503 \quad \ldots\ldots\ldots (3.16)$$

$n = 15, \quad r = 0.88, \quad s = 0.61, \quad F_{4,10} = 8.43$

In Eqns. (3.14)-(3.16) $V_{w,N}$ refers to the van der Waals volume of $N(R)_2$ group in (X) and D is a dummy parameter to indicate

the configuration of R_2 group. It has been assigned a value of unity for R-configuration, -1 for S-configuration, and zero fro RS-configuration. In the derivation of Eqns. (3.15) and (3.16), compound 10 was not included as it behaved as an outlier. These equations predicted its activity values much higher than the corresponding observed ones. The reasons of such differences for compound 10 in Table 3.7 and for 7 in Table 3.6 will be discussed later.

Although the van der Waals volume for R_2-substituent does not appear to be statistically significant at 95% confidence intervals in Eqns. (3.15) and (3.16), its deletion reduces the overall significance of the correlation (r becomes 0.87 for Eqn. (3.15) and 0.82 for Eqn. (3.16). The deletion of D from Eqn. (3.16), which is also not statistically significant there, further reduces the value of r leading to 0.76. Thus these two parameters appear to be important in controlling the binding affinity of compounds. No physicochemical or structural parameters related to R_1-substituent were found to have any influence on the significance of the correlations, when included in Eqns. (3.15) and (3.16).

For quinazolinone analogues (Table 3.8), the CCK-B receptor binding affinity was found to be correlated to also hydrophobic parameter π and the electronic parameter σ (Hammett constant) of some substituents in addition to V_w. The best correlation that was obtained in this case is as shown by Eqn. (3.17). This equation exhibits a fairly

$$-\log IC_{50} = 0.598\ (\pm 0.282)\ \pi_Z + 1.056\ (\pm 0.621)\ V_{w,X} - 1.622\ (\pm 0.760)\ \sigma_X + 6.660 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.17)$$

$n = 29, \quad r = 0.85, \quad s = 0.28, \quad F_{3,25} = 21.28$

significant-correlation. No parameter for Y-substituent was found to be effective.

Now these QSAR equations can be used to investigate the active sites at the receptors. For 3-(3-indolylmethyl) benzodiazepines (IX), Eqns. (3.12) and (3.13) suggest that R_1 group attached to N^1 will have some dispersion interaction with the receptor, as the activity is correlated to its van der Waals volume. But since there is a parabolic correlation of activity with V_w, R_1, the interaction would be optimized with a value of $V_w = 0.272 \times 10^2$ A^3 [Eqn. (3.13)], which is only slightly more than the V_w of CH_3 group, a group present at N^1 in highly potent known CCK antagonists (VII) and (VIII). It therefore means that the active site at the receptor interacting with R_1 group will have a limited bulk tolerance. Similarly, the negative coefficient of $^1\chi^v_{R2}$ in Eqns. (3.12) and (3.13) suggests that the longer or bigger is the R_2 group, the more would be the steric effect. It has been recently pointed out[20] that the whole indolyl group can react with a large hydrophobic pocket present in CCK-A and gastrin receptors. Thus it is in conformity to the previous finding that any substituent present at indolyl ring will hinder its interaction with the receptor. Moreover, Eqns. (3.12) and (3.13) also express that indolyl ring will have positive

effect only when it is in R-configuration.

Steric effect in inhibition mechanism will also be produced by chlorine group present at 7-position of benzodiazepine moiety as I_X indicating the presence of chlorine has the negative coefficients in Eqns. (3.12) and (3.13). It indicates either the whole phenyl moiety or a part of it around 7-position interacts with the receptor and, if a bulky group like chlorine is present at the 7-position, this interaction is hindered. Since no physicochemical or structural parameters for Y- and Z- substituents were found to be important in the correlations, it can be assumed that they have no oppertunity to have any kind of interaction with the receptor.

In GA analogues (X), R_2-substituent is equivalent to indolyl group in IBZs. Eqns. (3.14)-(3.16) obtained for GA analogues clearly exhibit that R_2 group will interact with the receptor but may have the dispersion interaction. Thus the assumption of Gupta and Saha[20] that indolyl group interacts with the receptor is verified, but this assumption is rectified in the sense that there might not be hydrophobic interaction but dispersion interaction. Further Eqns. (3.14)-(3.16) also show that, just like indolyl group in IBZs, the R_2-substituent in GA analogues will also have better interaction when in R-configuration.

The involvement of $N(R)_2$ group also in dispersion interaction with receptors is suggested by Eqns. (3.14)-(3.16). But while for CCK receptor in pancreas (CCK-A) there is only a linear

relationship between the activity and $V_{w,N}$, for that in brain (CCK-B) and for gastrin receptor there are parabolic correlations between the two. Thus, CCK-B and gastrin receptors are found to have limited bulk tolerance for $N(R)_2$ group. The optimum value of $V_{w,N}$ for both the receptors is around 1.47×10^2 Å^3, which is less than V_w for $N(n\text{-pentyl})_2$ group. This suggests that R should not contain more than 4 carbon atoms. If this $N(R)_2$ group is compared with the thick-line moiety of IBZs, as shown in Fig. 3.6, it becomes obvious why X produces the steric effect in IBZs when it is chlorine and not when it is hydrogen. It comes after the fourth carbon atom in the chain after N^4. These discussions however do not throw any light as to why compound 7 in the series of IBZs and compound 10 in the series of GA analogues behaved as an outlier.

For QZ analogues (XI), Eqn. (3.17) exhibits that the Z-substituent of the indolyl ring may be involved in the hydrophobic interaction with the receptor. This may be true, as while the whole indolyl ring may be involved in the dispersion interaction, a substituent on it may approach to a hydrophobic region at the receptor and may bind to it hydrophobically. However, the X-substituent of N^3-phenyl ring is shown [Eqn. (3.17)] to be involved in dispersion interaction only and further its electron donating ability is found to have a positive effect on the binding. The electron donation may make the substituent acquire the positive charge

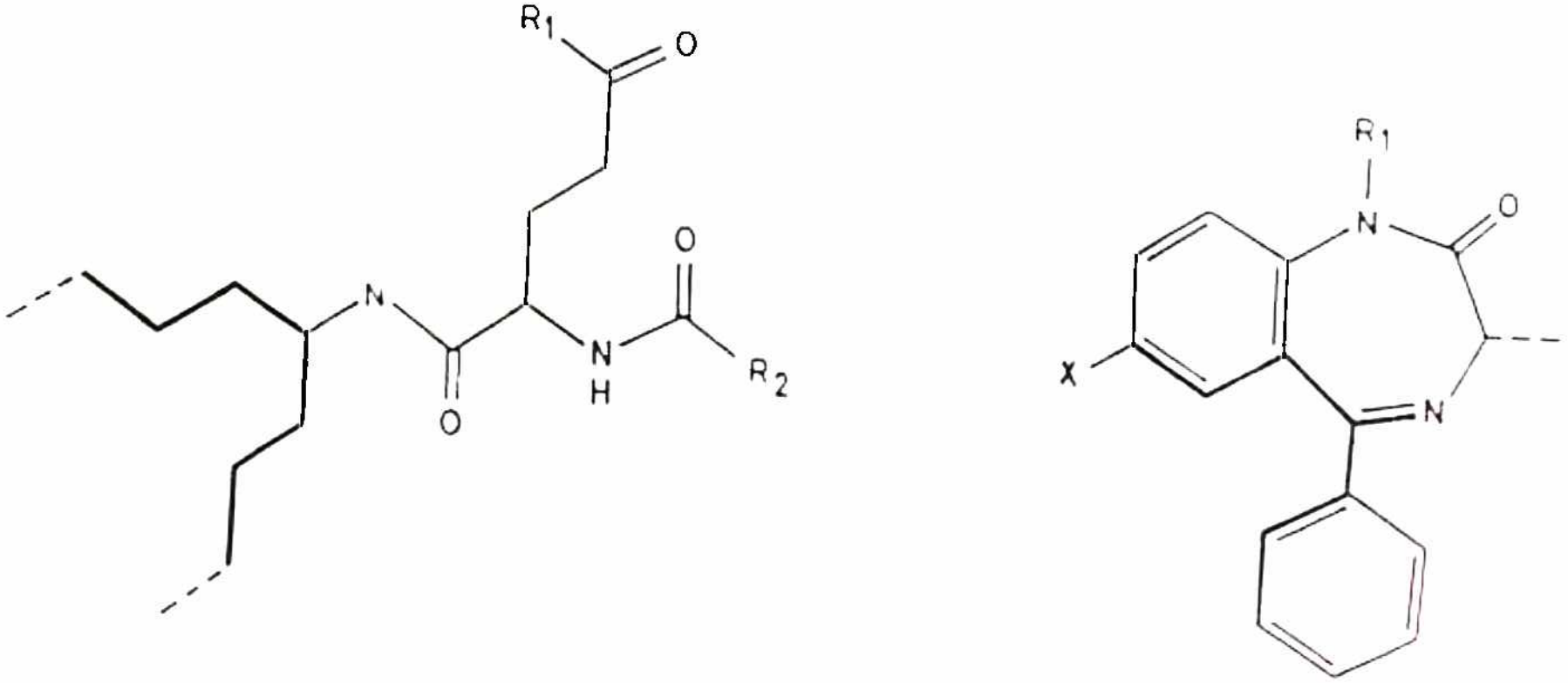


Fig.3.6. Matching of thick-line portions in glutamic acid analogues and 3-(3-indolylmethyl)benzodiazepines.

and thus instead of a weak dispersion interaction, there can be strong charge-dipole interaction. From the correlation analysis the Y-substituent is not indicated to have any kind of interaction with the receptor.

From the above findings it becomes clear that CCK and gastrin receptors each possesses two large polar sites where indolyl or equivalent group and a group on the other end of the molecule may have strong dispersion interaction. In addition to these two sites, there can be some secondary sites where some small substituents of the molecule might interact. These sites may be polar or hydrophobic in nature. Figs. 3.7-3.9 show the binding of the three different series of CCK-antagonists to the receptors. In Fig. 3.7, the binding of R_1 group with a small active site (presumably polar) is in agreement to the previous model.[20] This R_1 group, as already discussed, should not be larger than CH_3 group, a group that is present in potent antagonists, MK-329 (VII) and L-365,260 (VIII). CH_3 group at this position appears to be the most appropriate group not only from the point of view of its size but also from the point of view of its some other characteristics, as R_1 group in GA analogues, which is expected to interact with the same active site with which R_1 in IBZs interacts (Fig. 3.7 and 3.8), makes a negative contribution to the activity [Eqn. (3.14)] even if it is not a larger group but a smaller group like OH. CH_3 is an electron-donating group and OH is an electron-withdrawing group. It can be therefore assumed that R_1 should not be only a small group

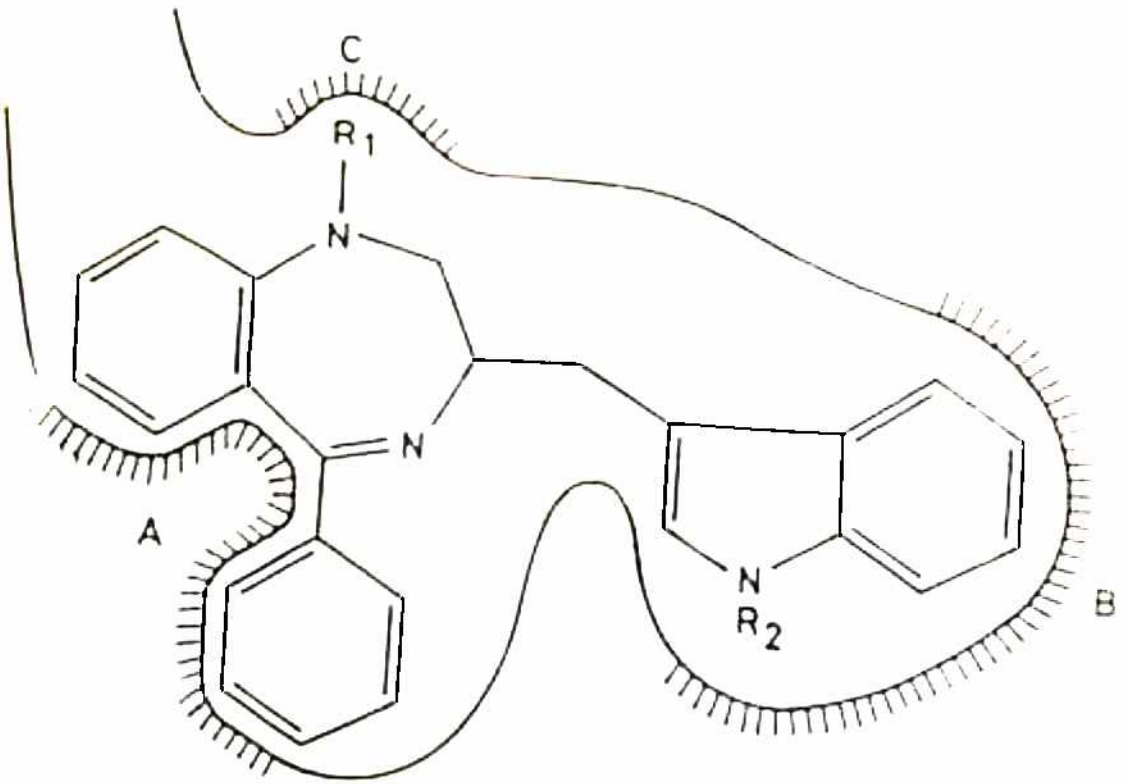


Fig.3.7. A proposed model for the binding of 3-(3-indolylmethyl)benzodiazepines to CCK receptors in *pancreas (CCK-A)*. A,B,C all sites are assumed to be polar in nature.

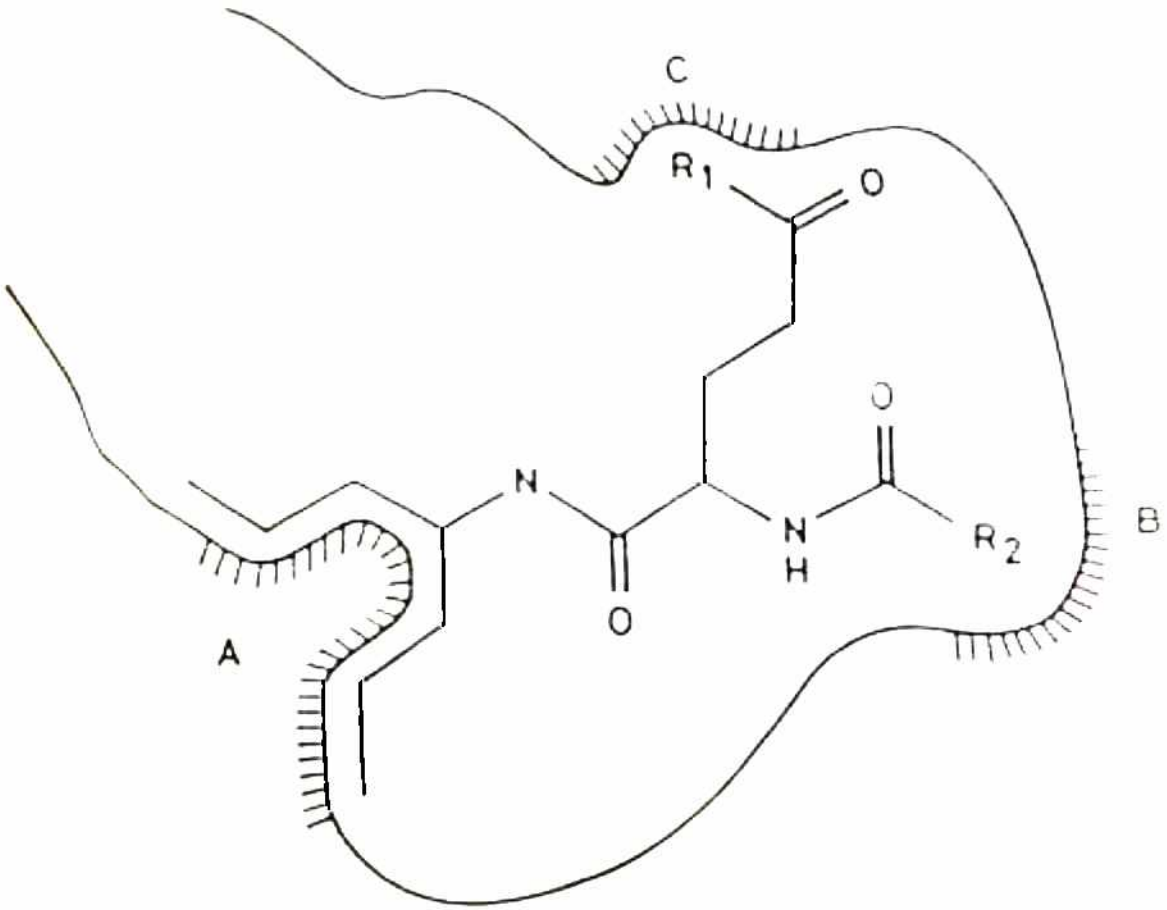


Fig.3.8. A proposed model for the binding of glutamic acid analogues to CCK-A receptors. A,B,C all sites are assumed to be polar in nature. In CCK-B (CCK receptors in brain) and gastrin receptors, the site C is assumed to be absent.

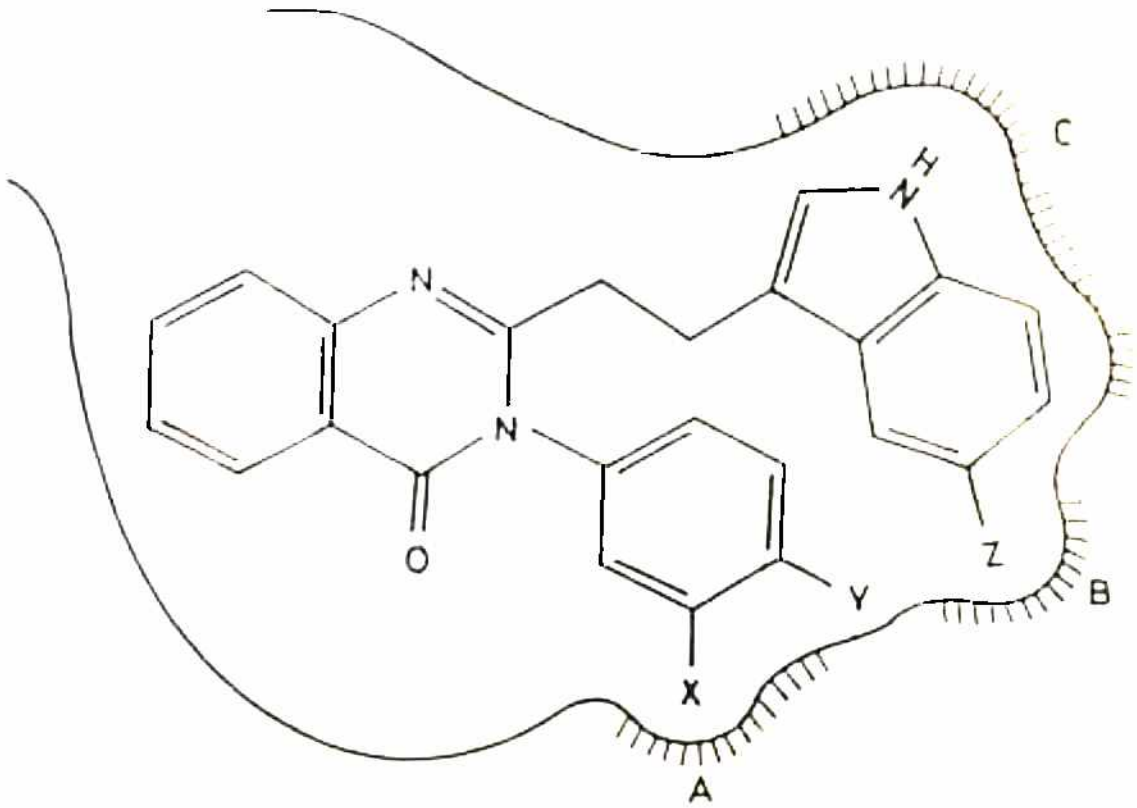


Fig.3.9. A proposed model for the binding of quinazolinone analogues to CCK-B receptors. Sites A and C are assumed to be polar in nature, while site B is assumed to be hydrophobic.

but also an electron-donating group. A comparison of Fig. 3.7 with Fig. 3.8 suggests that the nature of binding of benzodiazepine derivatives and that of simple GA analogues to CCK-A receptors are almost identical. However, the binding of GA analogues to CCK-B and gastrin receptors is slightly different from their binding to CCK-A receptors in the sense that R_1 group is not found to be bonded with the former [Eqns. (3.15) and (3.16)]. Thus in CCK-B and gastrin receptors, no such active site with which R_1 can interact should be assumed to be present. Also the study on the binding of QZ analogues to CCK-B receptor does not indicate the presence of any such active site at this receptor (Fig. 3.9).

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