QSAR Studies on Some Psychotomimetics, Enzyme Inhibitors and Cardiovascular Agents

Thesis
Submitted in Partial Fulfilment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

by

M. C. BINDAL

PHARMACY DISCIPLINE
BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN)

1981

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI (RAJASTHAN)

CERTIFICATE

This is to certify that the thesis entitled 'QSAR Studies on Some Psychotomimetics, Enzyme Inhibitors and Cardiovascular Agents' and submitted by Mr. M.C. Bindal, ID No. 77S81001, for the award of Ph.D. degree of the Institute, embodies original work done by him under my supervision.

Pilani.
Date: 18.4.1981

(S.P. GUPTA)
Assistant Professor
Chemistry Discipline.

PREFACE:

The present dissertation submitted by the author in partial fulfilment of his Ph.D. degree presents an account of quantitative structure activity relationship (QSAR) studies on some pharmacologically important compounds, such as psychotomimetics, enzyme inhibitors and cardiovascular agents. Being a graduate in pharmacy and a teacher in medicinal chemistry, the author developed slowly and slowly deep interest in theoretical aspect of drug design. But to work effectively in this field, an able and efficient guidance was required, which was very kindly and in a friendly spirit provided by Dr. S.P. Gupta, an Assistant Professor in Chemistry and actively engaged in the area at BITS. It was his supervision that made the entire work discussed in the thesis worth publishing. The thesis as such contains four Chapters and two Appendices. Chapter one presents a brief introduction to the importance of and approaches to QSAR studies, and the remaining three Chapters give an account of studies made by the author on some psychotomimetics, enzyme inhibitors, and cardiovascular agents including some miscellaneous drugs, respectively. A brief introduction to van der Waals force and its relation to molecular size, often referred to in the discussion on the mechanism of drug-receptor interaction has been put in Appendix A.

Appendix B describes the least square method, the basic approach adopted by the author.

While the author expresses his heartfelt gratitude to Dr. S.P. Gupta for his able supervision, he sincerely thanks Dr. C.R. Mitra, the Director of the Institute for providing the necessary facilities for the work. He then thanks Dr. B.M. Mithal, Professor in Pharmacy, Dr. V.K. Tewary, Dean, Research and Consultancy Division, Dr.H.L. Kundu, Dean, Educational Hardware Division, Dr. S.S. Mathur, Group Leader of Pharmacy Discipline, and Dr. R.C.Srivastava, Professor in Chemistry, for their cooperation and help in many other respects. However, without the help of Dr.Prithvi Singh, a valuable colleague of the author, the thesis would have not reached the completion so soon. He therefore thanks first Dr. Prithvi Singh for his this precious help and then puts on record the cooperation, encouragement and inspiration provided by his several other colleagues namely Dr. Kamal Kumar, Dr. R.P. Bhatnagar, Mr. D.G. Shewade, Mr. M.N.A. Rao, Mr. Datta Madamwar, and Dr. V.N. Sharma.

Finally, the author is full of appreciations for his wife, Mrs. Renu, and his little son, Master Ritesh, for permitting him to devote as much time as he could during the last phase of his work forgetting all family obligations. At this point he also takes the opportunity to express his gratefulness to his parents, other members

of his family, and friends, specially M/s Dhirendra Jaju and Dinesh Agarwal, for their support and encouragement.

At last but not least, the author thanks Mr. V.N. Sharma for careful typing and the staff of the Computer Centre at BITS for efficient handling of the programs throughout his work.

M. C. Bindal

(M.C. BINDAL)

Pharmacy Discipline
BITS, Pilani.

18th April, 1981.

C O N T E N T S

LIST OF PUBLICATIONS:

1. Quantitative interpretation of cholinesterase inhibition by carbamates, phosphates and amides.
   Indian J. Chem., 17B, 605 (1979).

2. The relationship of cellular respiration inhibition activity of 7-substituted 4-hydroxyquinoline-3-carboxylic acids with van der Waals volume.
   Res. Comm. Chem. Path. Pharmacol.(U.S.A.), 25, 111 (1979).

3. Quantitative correlation of anaesthetic potencies of halogenated hydrocarbons with boiling point and molecular connectivity.
   Arzneim.-Forsch. (Drug Research), 30(I), 234 (1980).

4. The relationship of vasodilator activity of adenosine analogs with molecular connectivity and van der Waals volume.
   Arzneim.-Forsch. (Drug Research), 30(I), 924 (1980).

5. Effect of molecular size on insecticidal activities of dinitrophenols and alkyl thiocyanates.
   Indian J. Chem., 19B, 322 (1980).

6. Structure-activity studies on LSD analogs using van der Waals volume.
   Eur. J. Med. Chem., in press.

7. Correlation of toxicity and pyretogenic activity of lysergamides with van der Waals volume.
Indian J. Biochem. Biophys., in press.

8. Correlation of serotonin uptake inhibition activity of tryptamine derivatives in thrombocytes with van der Waals volume.
Arzneim.-Forsch.(Drug Research), in press.

9. Effect of molecular size on carbonic anhydrase inhibition by sulfonamides.
Int. J. Quant. Chem., in press.

10. Correlations of sulfhydryl reactivity of unsaturated acylphenoxyacetic acids with electronic parameters.
Indian J. Pharm. Sci., in press.

11. Effect of molecular size on inhibition of synaptosome dopamine uptake by antihistaminic pheniramines.
Biochem. Pharmacol., communicated.

I. QSAR STUDIES - AN INTRODUCTION.

QSAR Studies - An Introduction:

Despite all the scientific efforts made in the pharmaceutical industry, universities and Government research laboratories, the finding of new useful drugs remains essentially an inspired hit and miss procedure. Typically only 1 in 15,000 compounds tested ever emerges as a commercial drug and the average cost for one noble drug to emerge on the U.S. market is of the order of $ 40 million. Thus it is a small wonder that any rationalization of activity which will reduce the number of compounds to be tested is attractive, not only from the scientific point of view but also commercially.

The term 'drug design', introduced recently in chemical pharmacology, represents mainly the effort to develop new drugs on rational basis so as to decrease the trial and error factors and predict the biological activity of compounds before their synthesis. A scientific approach to drug design is possible only if one knows how drugs act. Most of the drug molecules bind to some highly specific and selective sites in tissues, called receptors which form part of the three dimensional structure of a specific macromolecule[1]. The drug-receptor interaction leads to a chain of events, finally resulting in response at the macroscopic level. Therefore, attempts to design drugs involve

consideration of the following points : (i) molecular structure of the drug, (ii) behaviour of the drug in the biophase, (iii) geometry of the receptor, (iv) drug - receptor interaction, (v) changes in structure on binding and (vi) the resulting biological response.

Of a number of procedures involved in drug design, the first step is the detection of some biological action in a group of compounds so as to serve as a lead. This is followed by molecular manipulations to increase or modify the activity. This in turn requires knowledge of the type of action and the activity desired; this is usually done by correlating the chemical structure with biological activity. Among the methods used to study quantitative structure activity relationship (QSAR) are the Hansch method[2-4], the Free Wilson method[5], factor analysis[6], cluster analysis[7], discriminant analysis[8], pattern recognition[9,10], substructural analysis[11], Darvas simplex method[12], and molecular orbital methods[13,14]. Out of these methods the Hansch method has been most widely adopted for QSAR studies. In this method, the biological activities are analysed (statistically) in relation to various physicochemical properties of drug molecules. The parameters which are used to obtain the correlation equations can be divided into two groups: (i) those which describe mainly the physical properties of a compound, such as water

solubility, molecular weight, surface tension, partition coefficient, molar refraction constants and chromatographic Rf values, and (ii) those which are related to the chemical activity, such as Hammett's electronic constants, Taft's steric constants, dipole moment, charge densitities and electron donor - acceptor properties.

In the present thesis only the Hansch approach is adopted but some additional parameters like van der Waals volume[15] and molecular connectivity indices[16] have been used. The study has been made on some psychotomimetics, enzyme inhibitors and cardiovascular agents. Besides, some miscellaneous series of drugs have also been treated.

REFERENCES:

1. A. Korolkovas, Essentials of molecular pharmacology (Wiley - Interscience Inc., New York), 1970.

2. R.F. Gould, Biological correlations - The Hansch Approach : Advances, chemical series No. 144, (American Chemical Society, Washington, D.C.), 1972.

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

4. C. Hansch in 'drug design', Vol. 1, E.J. Ariëns, ed., (Academic Press Inc., New York), 1971, p. 271.

5. S.M. Free and J.M. Wilson, J. Med. Chem., 7, 395 (1964).

6. M.L. Weiner and P.H. Weiner, J. Med. Chem., 16, 655 (1973).

7. C. Hansch, S.H. Unger and A.B. Forsythe, J. Med. Chem., 16, 1217 (1973).

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

9. K.C. Chu, Analyt. Chem., 46, 1181 (1974).

10. B.R. Kowalski and C.F. Bender, J. Am. Chem. Soc., 94, 5632 (1972).

11. R.D. Cramer, G. Redl and C.E. Berkoff, J. Med. Chem., 17, 533 (1974).

12. F. Darvas, J. Med. Chem., 17, 799 (1974).

13. L.B. Kier, Molecular orbital theory in drug research (Academic Press Inc., New York), 1971.

14. B. Pullman and A. Pullman, Quantum biochemistry (Wiley - Interscience Inc., New York), 1961.

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

16. L.B. Kier and L.H. Hall, Molecular connectivity in chemistry and drug research (Academic Press, Inc., New York), 1976.

II. QSAR STUDIES ON PSYCHOTOMIMETICS.

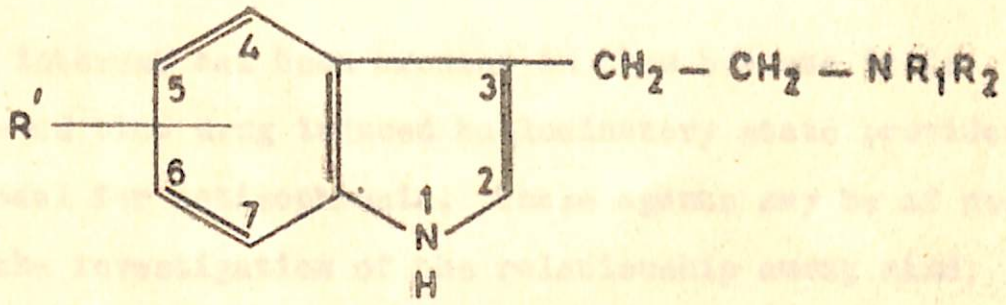
2.1. Introduction:

Psychotomimetics drugs are most appropriately defined[1] as substances which will consistently produce changes in thought, perception, and mood, occuring alone or in concert without causing major disturbances of the autonomic nervous system or other serious disability. They have also been referred to as psychedelic (mind manifesting)[2] agents to express the general activation of psychic phenomenon without the connotation of negative or morbid components. Since the extraordinary, unexpected, colourful, world encompassing, or frightening visions conjured up by these agents are comparable to autogenous hallucinations, they have been also called hallucinogens.

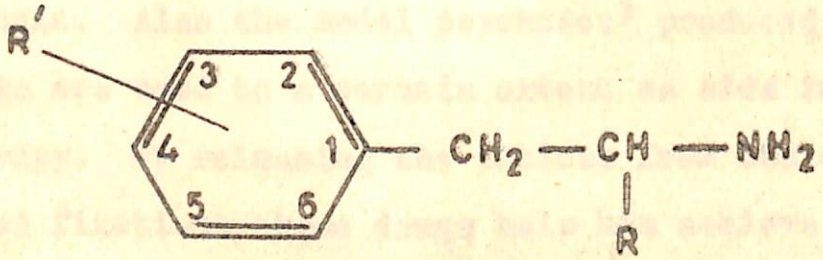
Presently several compounds are known which cause psychoses. However, the true hallucinogens, which have been used deliberately to produce psychosis belong to the following chemical classes:

(a) Indole alkylamines (I)
(b) Phenyl alkylamines (II)
(c) Lysergic acid derivatives (III).

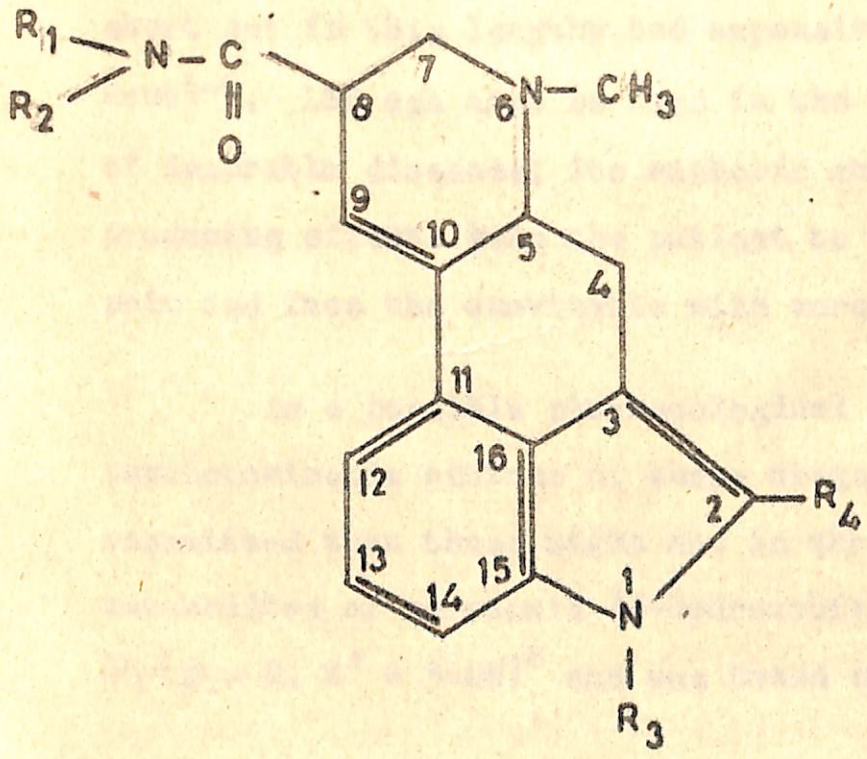
Except for some unknown ritualistic uses, these drugs were primarily of toxicological interest but recently


R'
4
5
3
6
7
2
1
N
H
CH2 — CH2 — N R1R2

I


R'
3
2
4
1
5
6
CH2 — CH — NH2
R

II


R1
N — C
R2
O
7
8
6
N — CH3
9
10
5
4
11
3
16
12
2
R4
13
15
14
1
N
R3

III

the interest has been aroused in them because it is assumed that drug induced hallucinatory state provided a model for schizophrenia. These agents may be of use in the investigation of the relationship among mind, brain, and biochemistry, with the purpose of elucidating the etiology of mental diseases, such as schizophrenia. Also the model psychoses[3] produced by these drugs are used to a certain extent as aids in psychotherapy. By releasing the patient from his self centered fixations, these drugs help him achieve a better response to psychotherapeutic treatment. Repressed memories may be activated, and even earliest childhood experiences may be remembered. Such recall is of great importance in psychoanalysis and provides a short cut in this lengthy and expensive type of treatment[4-6]. LSD can also be used in the terminal stages of incurable diseases; its euphoric and detachment producing effects help the patient to tolerate chronic pain and face the enevitable with more equanimity[7].

As a possible pharmacological basis for the psychotomimetic actions of these drugs, one theory postulated that these might act in the brain as antimetabolites of serotonin (5-hydroxytryptamine, 5HT; I: $R_1 = R_2 = H$, $R' = 5\text{-}OH$)[8] and was based on the finding

that d-lysergic acid diethylamide (LSD; III : R_1= R_2 = C_2H_5, R_3= R_4= H) in low concentration antagonized the contractile effects of serotonin on smooth muscle[9]. However, 2 bromo-LSD which has 50% more antiserotonin (anti-5HT) activity than LSD on smoothh muscle and which readily enters the brain has no hallucinogenic activity[10]. Mescaline (II : R = H, R'= 3,4,5-tri OCH_3) an effective hallucinogen is devoid of antiserotonin activity on rat uterus[11]. Thus it is very difficult to find the mode of action of hallucinogens. Correlation of structure of drugs with their pharmacological activities provides an insight into the mechanism of drug action, but such correlation studies on hallucinogens have been rather difficult, as there were no chemical or 'in-vitro' systems for the evaluation of their pharmacological activity and as 'in vivo' studies of hallucinogenic action might not always reflect their true potency at receptor site. The first attempt was made by Karreman, Isenberg, and Szent Györgyi[12] to correlate the electronic structure of a few CNS agents with their potency. They performed molecular orbital calculations on chlorpromazine, LSD and serotonin, and concluded that these drugs were potent electron donors. Later Synder and Merril[13] found the energy of the highest occupied molecular orbital (E_{HOMO}) related with the potency of

some hallucinogens. However, extensive opportunity was provided for structure-activity relationship (SAR) studies on psychotomimetics after Shulgin, Sargent and Naranjo[14] compiled the relative potencies studied in humans for a fairly large list of psychotomimetic drugs. Shulgin et al's data were found to be correlated not only with E_{HOMO}[15] but with hydrophobicity[16] and a topological parameter[17] also.

In the present Chapter we have analysed the hallucinogenic and other related activities of varying classes of psychotomimetics in relation to some other parameter.

2.2. Hallucinogenic and Antiserotonin Activities of LSD Analogs:

As already pointed out, the basic structure activity studies with the use of quantum mechanical methods on hallucinogens, such as LSD, phenylisopropylamines (II : R = CH_3) and tryptamines*(I) have suggested that these drugs exert their biological effects through the formation of charge transfer complex with the receptors[12,13,15,18,19]. The electronic parameter to which the hallucinogenic activity was found to be related

---

* Tryptamine - I : $R_1 = R_2 = R' = H$.

was E_{HOMO}. This parameter reflects the ability of compounds to donate electrons in a charge transfer type of interactions. More recently, however, this relationship between E_{HOMO} and activity has been questioned with respect to LSD and its analogs[20-22] and N,N-dialkyltryptamines[23]. To date, no direct electronic correlate relating the activity of hallucinogens has been reported. Instead, some physical property such as hydrophobicity, and a topological parameter known as molecular connectivity have been found to be successful in analysing the hallucinogenic activity data in some cases[16,17,24-27].

In case of LSD analogs, however, Kumbar and Siva Sankar[20] and Siva Sankar and Kumbar[21] found the total orbital energy (E_T) of compounds to be related with their hallucinogenic (H) and antiserotonin (anti-5HT) activities as shown by the equations (2.2.1) and 2.2.2) respectively.

$$\log H = -11.8596 + 7.3951 \log E_T$$
$$n = 12,\ s = 0.40 \qquad (2.2.1)$$

$$\log (\text{anti-5HT}) = -16.2811 + 10.2838 \log E_T$$
$$n = 15,\ r = 0.889,\ s = 0.271 \qquad (2.2.2)$$

where n is the number of data points, r the correlation coefficient and s the standard deviation.

In eq. (2.2.1), the value of r was not reported. However, on the basis of these equations, Kumbar and Siva Sankar postulated that some kind of strain in molecules was responsible for these two activities. Among the various factors responsible for this strain in the molecules, one may be the size of the substituents. It was therefore proposed to make a study on the correlation of the hallucinogenic and antiserotonin activities of LSD analogs with van der Waals volume, Vw.

2.2.(a) Calculation of Vw:

The Vw 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. It has been recently shown to be related with hydrophobic behaviour of drug molecules[28] and consequently with various biological activities[29].

To find Vw of molecules, spherical shapes are assumed for all atoms according to Bondi[30] 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

TABLE 2.1: VAN DER WAALS RADIUS AND VOLUME OF ATOMS*

Atom		Radius, A^{o}	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 28.

TABLE 2.2: CORRECTION VALUES OF VAN DER WAALS VOLUME FOR SPHERE OVERLAPPING DUE TO COVALENT BONDING AND FOR BRANCHING*

Bond	Bond length Å	Correction value (10^2 Å^3)	Bond	Bond length Å	Correction value (10^2 Å^3)
C-C	1.5	-0.078	O-H	1.0	-0.034
C-H	1.1	-0.043	O-B	1.5	-0.079
C-N	1.4	-0.065	S-H	1.3	-0.040
C-O	1.4	-0.056	S-S	2.0	-0.062
C-S	1.8	-0.066	S-F	1.6	-0.052
C-F	1.4	-0.056	C=C	1.3	-0.094
C-Cl(aliphatic)	1.8	-0.058	C=N	1.3	-0.072
C-Cl(aromatic)	1.8	-0.066	C=O	1.2	-0.068
C-Br(aliphatic)	1.9	-0.060	C=S	1.6	-0.081
C-Br(aromatic)	1.9	-0.068	N=N	1.2	-0.061
C-I(aliphatic)	2.1	-0.063	N=O	1.2	-0.053
C-I(aromatic)	2.1	-0.072	S=O	1.5	-0.057
C-B	1.6	-0.113	C≡C	1.2	-0.101
H-H	0.7	-0.030	C≡N	1.2	-0.079
N-H	1.0	-0.038	C=C(aromatic)	1.4	-0.086
N-N	1.4	-0.050	Branching for saturated bond		
N-O	1.4	-0.042	except bonding with H		-0.050
N-S	1.6	-0.061			

* Taken from reference 28.

van der Waals radii are greater than covalent radii, a correction for shpere overlapping due to covalent bonding between atoms was needed for the calculation of Vw of polyatomic molecules. The calculated bond lengths and correction values are tabulated in Table 2.2. along with the correction for branching.

2.2.(b) Results and Discussion:

The fifteen LSD analogs for which anti-5HT activity was found by Siva Sankar and Kumbar[21] to be related with E_T are listed in Table 2.3. Similarly those for which hallucinogenic (H) activity was found[20] to be related with E_T are listed in Table 2.4. The Vw has been calculated for individual substituents. With the use of data as given in Tables 2.3 and 2.4, the multiple regression analysis revealed the following equations correlating anti-5HT and H activities with Vw's of substituents.

$$\log (\text{anti-5HT}) = 2.457\ Vw.NR_1R_2 + 1.211\ Vw.R_3 + 0.283\ Vw.R_4 - 0.051$$

$$n = 15,\ r = 0.917,\ s = 0.294,\ F_{3,11} = 19.28 \qquad (2.2.3)$$

$$\log (\text{anti-5HT}) = 2.536\ Vw.NR_1R_2 + 1.120\ Vw.R_3 - 0.056$$

$$n = 15,\ r = 0.916,\ s = 0.283,\ F_{2,12} = 31.17 \qquad (2.2.4)$$

TABLE 2.3: Vw AND OBSERVED AND CALCULATED ANTI-5HT ACTIVITIES OF LYSERGAMIDES (III).

Compd. No.	NR_1R_2	R_3	R_4	Vw(10^2 $\AA^3$)			Log (anti-5HT)		
				NR_1R_2	R_3	R_4	Obsd[a]	Calcd.eq. (2.2.3)	Calcd eq. (2.2.4)
1	NH_2	H	H	0.177	0.056	0.056	0.63	0.47	0.46
2	$NHCH_3$	H	H	0.339	0.056	0.056	0.80	0.87	0.87
3	$N(CH_3)_2$	H	H	0.501	0.056	0.056	1.37	1.26	1.28
4	NHC_2H_5	H	H	0.493	0.056	0.056	1.08	1.24	1.26
5	NHC_2H_5	$COCH_3$	H	0.493	0.420	0.056	1.59	1.68	1.66
6	$NH(iC_3H_7)$	H	H	0.597	0.056	0.056	1.35	1.50	1.52
7	$NHCH(C_2H_5)CH_2OH$	CH_3	H	0.819	0.245	0.056	2.60	2.27	2.30
8	$N(C_2H_5)_2$	H	H	0.809	0.056	0.056	2.00	2.02	2.06
9	$N(C_2H_5)_2$	CH_3	H	0.809	0.245	0.056	2.57	2.25	2.27
10	$N(C_2H_5)_2$	OCH_3	H	0.809	0.304	0.056	1.77	2.32	2.34
11	$N(C_2H_5)_2$	$COCH_3$	H	0.809	0.420	0.056	2.32	2.46	2.47
12	$N(C_2H_5)_2$	H	Br	0.809	0.056	0.287	2.18	2.09	2.06
13	$N(C_2H_5)_2$	H	I	0.809	0.056	0.388	1.76	2.11	2.06
14	$N(C_2H_5)_2$	CH_3	Br	0.809	0.245	0.287	2.73	2.31	2.27
15	$N(-C_4H_8-)$	CH_3	H	0.705	0.245	0.056	2.11	1.99	2.01

[a] Taken from reference 20.

TABLE 2:4 Vw AND OBSERVED AND CALCULATED HALLUCINOGENIC ACTIVITIES OF LYSERGAMIDES (III).

R_4 = H.

Compd. No.	NR_1R_2	R_3	Vw(10^2 Å^3)		Log H	
			NR_1R_2	R_3	Obsd[a]	Calcd.eq. (2.2.7)
1	$N(CH_3)_2$	H	0.501	0.056	1.00	0.59
2	$NH(C_2H_5)$	H	0.493	0.056	0.53	0.57
3	$NH(C_2H_5)$	CH_3	0.493	0.245	0.70	0.81
4	$NH(C_2H_5)$	$COCH_3$	0.493	0.420	0.85	1.04
5	$N(C_2H_5)_2$	CH_3	0.809	0.245	1.60	1.60
6	$N(C_2H_5)_2$	OCH_3	0.809	0.304	1.82	1.67
7	$N(C_2H_5)_2$	$COCH_3$	0.809	0.420	1.96	1.82
8	$N(-CH_2-CH=CH-CH_2-)$	H	0.663	0.056	1.00	1.00
9	$N(-C_4H_8-)$	H	0.705	0.056	1.00	1.10
10	$N(-C_2H_4-O-C_2H_4-)$	H	0.786	0.056	1.04	1.30

[a] Taken from reference 20.

$$\log (\text{anti-5HT}) = 2.793\ Vw.NR_1R_2 - 0.032$$

$$n = 15,\ r = 0.888,\ s = 0.311,\ F_{1,13} = 48.49 \qquad (2.2.5)$$

$$\log (\text{anti-5HT}) = 1.784 \Sigma Vw + 0.126$$

$$n = 15,\ r = 0.887,\ s = 0.312,\ F_{1,13} = 48.14 \qquad (2.2.6)$$

$$\log H = 2.474\ Vw.NR_1R_2 + 1.267\ Vw.R_3 - 0.716$$

$$n = 10,\ r = 0.916,\ s = 0.218,\ F_{2,7} = 18.14 \qquad (2.2.7)$$

$$\log H = 2.686\ Vw.NR_1R_2 - 0.612$$

$$n = 10,\ r = 0.822,\ s = 0.289,\ F_{1,8} = 16.65 \qquad (2.2.8)$$

$$\log H = 1.843 \Sigma Vw - 0.515$$

$$n = 10,\ r = 0.882,\ s = 0.239,\ F_{1,8} = 28.01 \qquad (2.2.9)$$

In these equations the various symbols have the following meanings:

$Vw.NR_1R_2$ - Vw for side chain substituent.

$Vw.R_3$ and $Vw.R_4$ - Vw for ring substituents R_3 and R_4 respectively.

ΣVw - sum of Vw's of substituents at all positions.

F - F-ratio between the variances of calculated and observed activities.

Now equations (2.2.3 - 2.2.9) all exhibit significant correlations between activities and Vw. The F - value in all of them is significant at 99% level ($F_{3,11}$ (0.01) = 6.22; $F_{2,12}$ (0.01) = 6.93; $F_{1,13}$ (0.01) = 9.07;

$F_{2,7}$ (0.01) = 9.55; $F_{1,8}$ (0.01) = 11.26). However, a comparison among them shows that both anti-5HT and hallucinogenic activities will largely depend upon the size of side chain substituent (eqs. 2.2.5 and 2.2.8). Eq. (2.2.5) accounts for 79% (r^2 = 0.79) of the variance in anti-5HT activity and eq. (2.2.8) about 68% of that in hallucinogenic activity due to variation in $Vw.NR_1R_2$ alone. Inclusion of $Vw.R_3$ makes a slight improvement in the correlations expressed by eqs.(2.2.5) and (2.2.8) (compare them with eqs. 2.2.4 and 2.2.7 respectively). Compounds treated for hallucinogenic activity (Table 2.4) have no R_4 substituent, but $Vw.R_4$ is found to play little role in the anti-5HT activity (compare eq. 2.2.3 with 2.2.4). Conclusively, for anti-5HT and hallucinogenic activities both, the substitution in the side chain is more important than that in the ring and, as the relating equations show, the bigger substituent will lead to increased activities.

1978

Now Vw describes the direct structural influence on both the activities of LSD analogs. Such direct structural influence on activities could not be interpreted by the correlations obtained by Dunn and Bederka[24] for only side chain substituted analogs with octanol/water partition coefficient (P) (eq. 2.2.10),

by Glennon and Kier[26] for the same with connectivity indices ($^{\chi}$, refer section 4.1.(a), Chapter IV) (eq. 2.2.11) or by Kumbar and Siva Sankar[20,21] for the present series with E_T (eqs. 2.2.1 and 2.2.2) although they were as significant as the correlations obtained by us.

$$\log\ (\text{anti-5HT}) = -0.54\ (\pm 0.32) - 0.74\ (\pm 0.28)D + 0.84\ (\pm 0.35)\log P - 0.14\ (\pm 0.08)(\log P)^2$$

$$n = 14,\ r = 0.94,\ s = 0.20,\ \log Po = 2.90\ (2.40\text{-}4.32) \qquad (2.2.10)$$

$$\log\ (\text{anti-5HT}) = 24.94\ (\pm 3.9) - 0.835\ (\pm 0.033)\ {}^{2}\chi - 0.917\ (\pm 0.083)\ {}^{6}\chi_P - 1/0.0072\ (\pm 0.004)\ {}^{o}\chi^{v}$$

$$n = 16,\ r = 0.940,\ s = 0.196 \qquad (2.2.11)$$

In eq. (2.2.10), D is a dummy variable used to account for amide nitrogen being enclosed in a ring system. Data within parentheses are 95% confidence interval.

All these authors except Kumbar and Siva Sankar, however, could correlate only antiserotonin activity and not the hallucinogenic activity. Here is a single parameter, Vw, which is correlated simultaneously with both the activities, and being theoretically accessible and much simpler than any other parameter, provides an easier basis to rationalize the process of structural

modification. E_T was also found to be correlated with both the activities[20,21], but it could not provide the picture of structural influence on activities as vividly as Vw.

The dependence of activities on molecular size was indicated by Kumbar and Siva Sankar[20] also, when they correlated anti-5HT activity with the number of carbon atoms (N) in alkyl substituents in a small series of side chain monoalkyl substituted lysergamides (Table 2.5). But it was not conclusive, as the series was very small and comprised of only side chain monoalkyl substituted analogs. However, N could not account for the variation in activity of analogs having isomeric branched chain alkyl substituents. For this reason Kumbar and Siva Sankar[20] had to exclude compound 4 for obtaining any meaningful correlation between anti-5HT and N for the series as listed in Table 2.5. The correlation obtained by them was of the type:

$$\text{Anti-5HT} = 1/(0.01185 + 0.2207\, e^{-N}) \qquad (2.2.12)$$

without any mention of statistical data.

The use of Vw, however, permits to include compound 4 also, as Vw takes into account the branching, too; and thus with the use of all seven data points mentioned

TABLE 2.5: Vw AND OBSERVED AND CALCULATED ANTI-5HT ACTIVITIES OF SIDE CHAIN MONOALKYL-SUBSTITUTED LYSERGAMIDES (III).

$R_3 = H, \quad R_4 = H$

Compd. No.	NR_1R_2	Vw.NR_1R_2 (10^2 $\AA^3$)	Log(anti-5HT)	
			Obsd[a]	Calcd.eq. (2.2.13)
1	NH_2	0.177	0.63	0.60
2	$NHCH_3$	0.339	0.81	0.89
3	NHC_2H_5	0.493	1.08	1.16
4	$NH(iC_3H_7)$	0.597	1.35	1.35
5	NHC_3H_7	0.647	1.60	1.44
6	NHC_4H_9	0.801	1.81	1.71
7	NHC_5H_{11}	0.955	1.87	1.99

[a] Taken from A. Cerletti, and W. Doefner, J. Pharmacol. Exp. Ther., 122, 124 (1958).

in Table 2.5, the regression analysis reveals the following linear equation,

$$\log (\text{anti-5HT}) = 1.789\ Vw.NR_1R_2 + 0.282$$
$$n = 7,\ r = 0.977,\ s = 0.113,\ F_{1,5} = 106.38 \qquad (2.2.13)$$

which is highly significant and accounts for about 95% of the variance in the activity.

These relating equations obtained by us are of high predictive values, and though they do not provide any deeper insight into the mechanism of antiserotonin and hallucinogenic activities, they speak clearly of the structural influence in the action. These equations can be successfully utilized to predict the activities of the LSD analogs before their synthesis or screening. Some such predictions are listed in Table 2.6.

2.3. Pyretogenic and Toxic Effect of LSD Analogs:

In addition to hallucinogenic and antiserotonin activities, the LSD analogs have been found to show pyretogenic and toxic effects also, but these effects are least studied. While making the structure activity studies on hallucinogenic and antiserotonin activities using quantum mechanical parameters, Kumbar and Siva Sankar[20,21] attacked on these two effects also, but

TABLE 2.6: PREDICTED ANTI-5HT AND HALLUCINOGENIC ACTIVITIES OF SOME SIDE CHAIN MONOSUBSTITUTED LYSERGAMIDES (III).

$R_3 = H, \quad R_4 = H.$

NR_1R_2	Vw.NR_1R_2 (10^2 Å^3)	Log(anti-5HT), eq. (2.2.5)	Log H, eq. (2.2.8)
$NH(CH_2OH)$	0.407	1.10	0.48
$NH(C_2H_4OH)$	0.561	1.53	0.90
$NH(C_3H_6OH)$	0.715	1.96	1.31
$NH(C_4H_8OH)$	0.869	2.39	1.72
$NH(C_5H_{10}OH)$	1.023	2.82	2.14
$NHCH(CH_3)CH_2OH$	0.665	1.82	1.17
$NHCH_2CH(CH_3)CH_2OH$	0.819	2.25	1.59
$NHC_2H_4CH(CH_3)CH_2OH$	0.973	2.69	2.00
$NH(C_2H_4Cl)$	0.628	1.72	1.07
$NHCH(C_2H_5)CH_2Cl$	0.886	2.44	1.77

they could not find any electronic parameter to be related with them. However, we analyzed them in relation to Vw and found them to be correlated with the latter. The compounds treated were as shown in Table 2.7 along with Vw values calculated for only substituents in them. By a multiple regression analysis, both the side effects are found to be parabolically correlated with Vw as shown by eqs. (2.3.1) and (2.3.2).

$$0.996\ (\log T)^2 - 2.262 \log T + Vw + 0.044 = 0$$
$$n = 9,\ r = 0.901,\ s = 0.103,,\ F_{2,6} = 12.95 \qquad (2.3.1)$$

$$0.960\ (\log Py)^2 - 2.784 \log Py - Vw + 2.628 = 0$$
$$n = 7,\ r = 0.973,\ s = 0.060,\ F_{2,4} = 36.23 \qquad (2.3.2)$$

In eqs. (2.3.1) and (2.3.2), T and Py represent relative toxic and pyretogenic effects respectively. Both the equations are significant at 99% level ($F_{2,6}(0.01) = 10.92$; $F_{2,4}(0.01) = 18.00$), and account for about 81 and 95% of the variance respectively (r^2 being 0.81 and 0.95 respectively).

As already mentioned, the toxic and pyretogenic effects of LSD and its analogs are least studied; hence no biochemical mechanism of these activities are known. The toxicity of LSD in animals leads to respiratory failure and hence to death. In man, death attributable

TABLE 2.7: Vw AND OBSERVED AND CALCULATED LOG T AND LOG P VALUES OF LYSERGAMIDES (III).

Compd. No.	NR_1R_2	R_3	R_4	Vw_o (10^2 A^3)	log T		log P	
					Obsd[a]	Cald,[b] eq. (2.3.1)	Obsd[a]	Cald,[b] eq. (2.3.2)
1	$N(-C_4H_8-)$	H	H	0.817	1.863	1.787 (0.484)	1.000	0.985 (1.915)
2	$N(-C_2H_4-O-C_2H_4-)$	H	H	0.898	1.634	1.722 (0.549)	1.000	0.902 (1.998)
3	$N(CH_3)_2$	H	H	0.613	1.892	1.929 (0.342)	1.634	1.510 (1.390)
4	NHC_2H_5	H	H	0.605		1.934 0.337	1.230	1.381 (1.519)
5	NHC_2H_5	CH_3	H	0.794	0.505	0.466 (1.805)		1.888, 1.012
6	NHC_2H_5	$COCH_3$	H	0.969	0.778	0.614 (1.657)		0.838 2.062
7	$N(-C_4H_8-)$	CH_3	H	1.006	0.602	0.651 (1.621)		2.093, 0.807
8	$N(C_2H_5)_2$	H	Br	1.152	0.699	0.838 (1.433)	0.699	0.698 (2.202)
9	$N(C_2H_5)_2$	H	H	0.921		1.702 0.569	2.000	2.019 (0.881)
10	$N(C_2H_5)_2$	CH_3	H	1.110	0.748	0.774 (1.497)	0.699	0.728 (2.172)
11	$N(C_2H_5)_2$	CH_3	Br	1.341		0.818 1.454		2.323, 0.577
12	$N(C_2H_5)_2$	$COCH_3$	H	1.285	1.279	1.347 (0.924)		0.611 2.289

[a] Taken from reference 20. [b] Out of two values obtained for a single value of Vw, the one that does not agree with available experimental one is put within bracket.

to direct drug effects are not known, but fatal accidents and suicides during states of LSD intoxications have been reported[31]. The pyretogenic effect of LSD and its analogs leads to rise in body temperature.

However, since hallucinogens are supposed to elicit their activity by blocking serotonin receptor through hydrophobic interaction by mimicing the structure of serotonin[31], and since both the toxic and pyretogenic effects are found to be significantly correlated with Vw which has been shown to be related with hydrophobic character of molecules, it may be assumed that these two effects may be a consequence of strong and prolonged hydrophobic binding of lysergamides with the receptor. Whatsoever, eqs. (2.3.1) and (2.3.2) describe the direct structural influence on these two effects, although both the equations show that for a given value of Vw, there would be two values of each activity. But this is not difficult to interpret. A single substituent at two different positions may of course lead to two different values of an activity, while its size will remain the same; and a polar substituent may lead to a different activity value than a non-polar substituent of the same size at the same position.

2.4. Serotonin Receptor Binding Affinities of Phenylalkylamines:

As already pointed out in section 2.1, hallucinogens might act in the brain as antimetabolites of serotonin, therefore serotonin receptor binding affinity (pA_2, negative log of the concentration of drug that produces a response exactly half the size of the maximum one attainable), was also studied for these hallucinogens. Recently Glennon et al[32]. reported pA_2 values for a series of phenylalkylamines (Table 2.8). Since hallucinogenic and antiserotonin activities of psychotomimetics were found to be related with Vw, we analyzed pA_2 also in relation to this parameter. The Vw values were calculated for phenyl ring and side chain substituents separately. When regression analysis was performed, pA_2, like antiserotonin and hallucinogenic activities of LSD analogs, was also found to have certain meaningful correlations with Vw, as shown by eqs. (2.4.1 to 2.4.4),

$$pA_2 = 2.162 \Sigma Vw.ring - 0.277\ Vw.X + 5.337$$

$$n = 8,\ r = 0.889,\ s = 0.306,\ F_{2,5} = 9.45$$

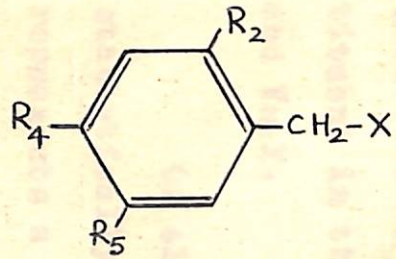
$$F_{2,5}(0.05) = 5.79 \quad (2.4.1)$$

$$pA_2 = 2.103 \Sigma Vw.ring + 5.229$$

$$n = 8,\ r = 0.886,\ s = 0.283,\ F_{1,6} = 21.93$$

$$F_{1,6}(0.01) = 13.74 \quad (2.4.2)$$

TABLE 2.8: Vw AND OBSERVED AND CALCULATED pA_2 VALUES OF SOME PHENYLALKYLAMINES.



Compd. No.	X	R_2	R_4	R_5	Vw. X (10^2 $\mathring{A}^3$)	Vw.ring (10^2 $\mathring{A}^3$)	pA_2		
							Obsd[a]	Calcd.eq. (2.4.1)	Calcd eq. (2.4.2)
1	CH_2NH_2	H	H	H	0.344	0.168	5.26	5.61	5.58
2	CH_2NH_2	OCH_3	H	OCH_3	0.344	0.664	6.85	6.68	6.63
3	$CHCH_3NH_2$	H	- OCH_2O -		0.498	0.406	6.45	6.08	6.08
4	$CHCH_3NH_2$	OCH_3	H	OCH_3	0.498	0.664	6.83	6.64	6.63
5	$CHCH_3NH_2$	OCH_3	CH_3	OCH_3	0.498	0.853	7.12	7.04	7.02
6	$CHCH_3NH_2$	OCH_3	OCH_3	OCH_3	0.498	0.912	6.81	7.17	7.15
7	$CH_2N(CH_3)_2$	OCH_3	H	OCH_3	0.688	0.664	6.52	6.58	6.63
8	$CHCH_3N(CH_3)_2$	OCH_3	H	OCH_3	0.822	0.664	6.50	6.55	6.63

[a] Taken from reference 27.

$$pA_2 = 8.153 \Sigma Vw - 3.383 (\Sigma Vw)^2 + 1.955$$

$n = 8$, $r = 0.946$, $s = 0.217$, $F_{2,5} = 21.29$

$F_{2,5}(0.01) = 13.27$ (2.4.3)

$$pA_2 = 1.305 \Sigma Vw + 5.047$$

$n = 8$, $r = 0.751$, $s = 0.403$, $F_{1,6} = 7.78$

$F_{1,6}(0.05) = 5.99$ (2.4.4)

Where Σ Vw·ring represents the sum of Vw values of all substituents in the phenyl ring; Vw·X, the Vw of the substituent in the side chain; and Σ Vw, the sum of Σ Vw·ring and Vw.X.

Of all the equations, eq. (2.4.3) exhibits, statistically the most significant correlation; but it represents a parabolic correlation and it would be risky to accept a parabolic correlation for a small number of points and which are found to be unusually scattered on the plot (Fig. 2.1). Seven of the eight points are rather closely scattered with an activity range of only 0.67 units and only 0.58 units in Vw. The only other point is lying low. Of the rest, eq. (2.4.2) expresses the best correlation, accounting about 79 percent of total variance. This equation involves only one variable i.e. the overall size of the ring substituent, or in effect the size of the ring. Inclusion of Vw of side chain substituent in

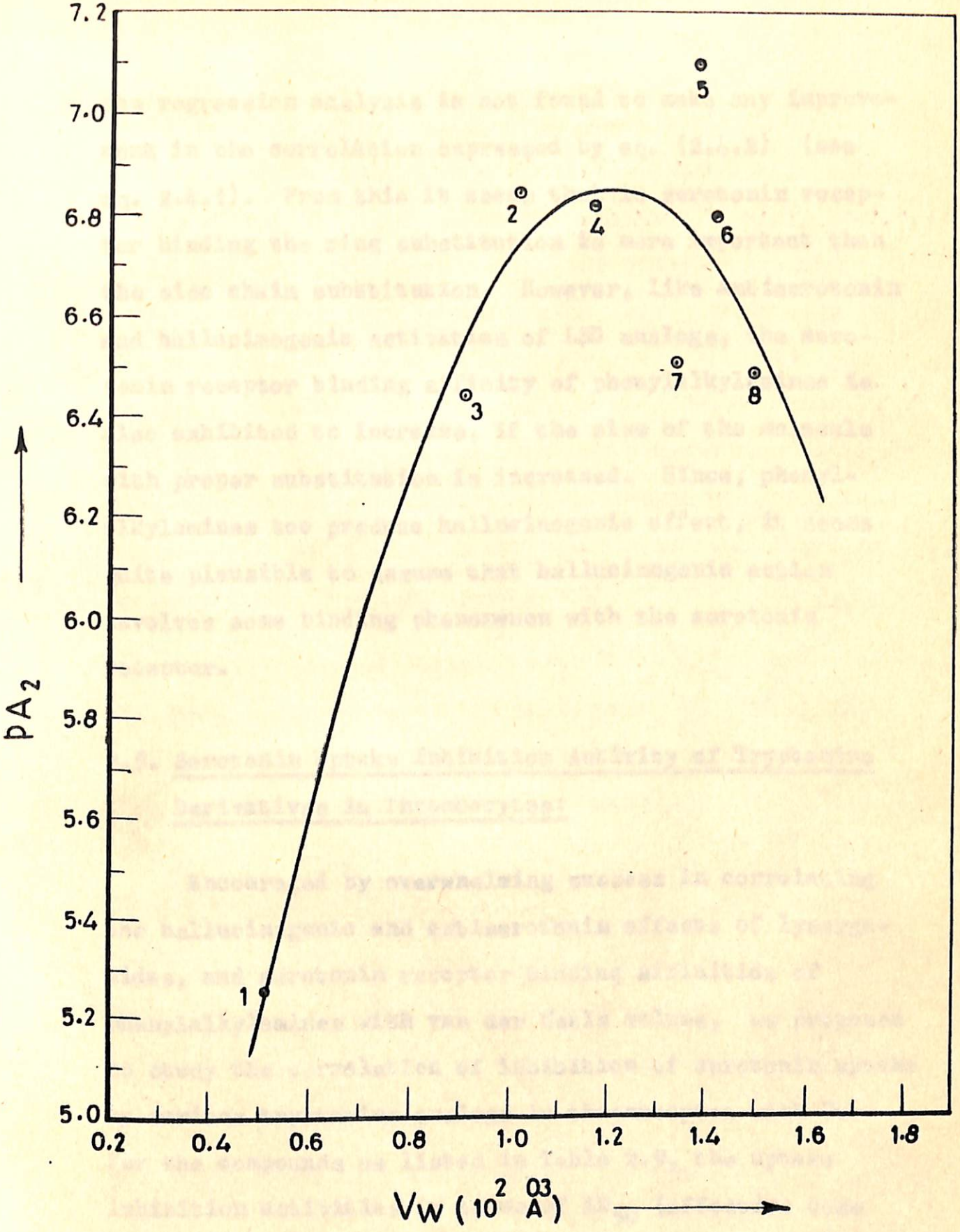


Fig. 2.1. A plot of serotonin receptor binding affinity of phenylalkylamines versus Vw. Numbers refer to the compounds in Table 2.8.

the regression analysis is not found to make any improvement in the correlation expressed by eq. (2.4.2) (see eq. 2.4.1). From this it seems that in serotonin receptor binding the ring substitution is more important than the side chain substitution. However, like antiserotonin and hallucinogenic activities of LSD analogs, the serotonin receptor binding affinity of phenylalkylamines is also exhibited to increase, if the size of the molecule with proper substitution is increased. Since, phenylalkylamines too produce hallucinogenic effect, it seems quite plausible to assume that hallucinogenic action involves some binding phenomenon with the serotonin receptor.

2.5. Serotonin Uptake Inhibition Activity of Tryptamine Derivatives in Thrombocytes:

Encouraged by overwhelming success in correlating the hallucinogenic and antiserotonin effects of lysergamides, and serotonin receptor binding affinities of phenylalkylamines with van der Waals volume, we proposed to study the correlation of inhibition of serotonin uptake by various tryptamine analogs in thrombocytes with Vw. For the compounds as listed in Table 2.9, the uptake inhibition activities in terms of ED_{50} (effective dose producing 50% inhibition) were recently reported by

Kumbar et al[33]. These authors had found the inhibition activities to be related with the total Hückel molecular orbital energy (E_T) and the hydrophobic character of the molecules. In their correlation, the compounds had fallen in two distinct groups : upper group comprising of compounds 1 to 12, and lower group comprising of compounds 13 to 23. On the basis of this, they postulated the involvement of two receptor sites that were sterically and electronically dissimilar in nature. However, they had found the hydrophobicity to be important only in one group-the lower group.

Correlation of activity with Vw will provide an additional information to the involvement of steric factors in the drug-receptor interaction, as Vw determines the molecular size of the molecules.

Results and Discussion:

With the use of data of Table 2.9, the regression analysis revealed the following two equations correlating log ED_{50} with Vw, for upper and lower groups, respectively.

$$\log ED_{50} = 0.928\ Vw + 0.826$$
$$n = 12,\ r = 0.791,\ s = 0.164,\ F_{1,10} = 16.73 \qquad (2.5.1)$$

TABLE 2.9: A SERIES OF TRYPTAMINE DERIVATIVES (I) ALONG WITH THEIR Vw AND SEROTONIN UPTAKE INHIBITION ACTIVITY VALUES.

Compd. No.[a]	NR_1R_2	R'				Vw (10^2 A^3)	Log ED_{50}	
		R_4	R_5	R_6	R_7		Obsd.[b]	Calcd.[c]
1	NH_2	OH	H	H	H	0.482	1.079	1.273
2	NH_2	H	H	H	OH	0.482	1.255	1.273
3	NH_2	H	H	OH	H	0.482	1.279	1.273
4	NH_2	NH_2	H	H	H	0.522	1.097	1.310
5	NH_2	H	OH	OH	H	0.563	1.352	1.348
6	NH_2	H	OH	H	OH	0.563	1.686	1.348
7	NH_2	H	OCH_3	H	H	0.649	1.556	1.428
8	$NHCH_3$	NH_2	H	H	H	0.684	1.549	1.461
9	$N(CH_3)_2$	H	H	H	OH	0.806	1.663	1.574
10	$N(CH_3)_2$	NH_2	H	H	H	0.846	1.415	1.611
11	$N(CH_3)_2$	H	OH	H	OH	0.887	1.628	1.649
12	$N(C_2H_5)_2$	H	OH	H	OH	1.195	1.924	1.935

contd....

TABLE 2.9: CONTD.

Compd. No.[a]	NR_1R_2	R'				Vw (10^2 A^3)	Log ED_{50}	
		R_4	R_5	R_6	R_7		Obsd.[b]	Calcd.[c]
13	$NHCH_3$	H	OH	H	H	0.644	0.279	0.460
14	NHC_2H_5	H	H	H	OH	0.798	0.778	0.628
15	$NHCH_3$	H	H	H	OCH_3	0.811	0.544	0.642
16	NH_2	H	OCH_3	H	OCH_3	0.897	0.978	0.736
17	NHC_2H_5	H	H	H	OCH_3	0.965	0.954	0.811
18	$NHCH_3$	H	OCH_3	H	OCH_3	1.059	0.903	0.914
19	$N(C_2H_5)_2$	NH_2	H	H	H	1.154	0.875	1.017
20	NHC_2H_5	H	OCH_3	H	OCH_3	1.213	0.954	1.082
21	$N(CH_3)_2$	H	OCH_3	H	OCH_3	1.221	0.968	1.091
22	$N(C_2H_5)_2$	H	H	H	OCH_3	1.281	1.255	1.156
23	$N(C_2H_5)_2$	H	OCH_3	H	OCH_3	1.529	1.477	1.427

[a] These numbers are not same as in reference 33; compounds have been listed in each group separately in increasing order of Vw; [b] taken from reference 33; [c] for upper group from eq. (2.5.1) and for lower group from eq. (2.5.2).

$$\log ED_{50} = 1.093\ Vw - 0.244$$

$$n = 11,\ r = 0.890,\ s = 0.152,\ F_{1,9} = 34.46 \qquad (2.5.2)$$

F values in both these equations are significant at 99% level ($F_{1,10}(0.01) = 10.04$; $F_{1,9}(0.01) = 10.56$). Both these eqs. exhibit significant correlations which are comparable to those obtained by Kumbar et al[33] between log ED_{50} and log E_T for upper and lower groups as shown by eqs. (2.5.3) and (2.5.4) respectively.

$$\log ED_{50} = -2.952 + 2.908 \log E_T$$

$$n = 12,\ r = 0.851,\ s = 0.134 \qquad (2.5.3)$$

$$\log ED_{50} = -5.539 + 3.954 \log E_T$$

$$n = 11,\ r = 0.893,\ s = 0.142 \qquad (2.5.4)$$

With hydrophobicity factor (log P), this activity was found by the same authors to have some satisfactory correlation in case of lower group (eq. 2.5.5). For upper group, the correlation between log ED_{50} and log P was very poor (eq. 2.5.6).

$$\log ED_{50} = 7.889 \times 10^{-2} + 0.361 \log P$$

$$n = 11,\ r = 0.801,\ s = 0.189 \qquad (2.5.5)$$

$$\log ED_{50} = 1.215 - 0.223 \log P$$

$$n = 12,\ r = 0.650,\ s = 0.190 \qquad (2.5.6)$$

In our analysis also the two groups remain separated (Fig. 2.2). No correlation is found, if both the groups are treated together. This clearly supports the assumption that two receptor sites of sterically dissimilar nature are involved. Additionally, since steric influence in inhibition is more clearly manifested in our studies (eqs. (2.5.1) and (2.5.2) both show that as the size of the molecule would increase, the activity will decrease), it might be assumed that to act as serotonin uptake inhibitors, the compounds must possess some desired conformation. As it is generally accepted that antiserotonin activity is the result of blocking of serotonin by drug molecules at its receptor sites, it might be said that molecules should achieve a conformation similar to that of serotonin at least while complexing with receptor sites. For this they should have conformational flexibility and this is in conformity with our finding. The larger substituent will decrease the conformational flexibility and hence decrease the chance of complexing with receptor sites leading to decrease in inhibition activity and that is what depicted by eqs. (2.5.1) and (2.5.2).

The separation of compounds into two distinct groups as found by us and Kumbar et al[33] as well, is

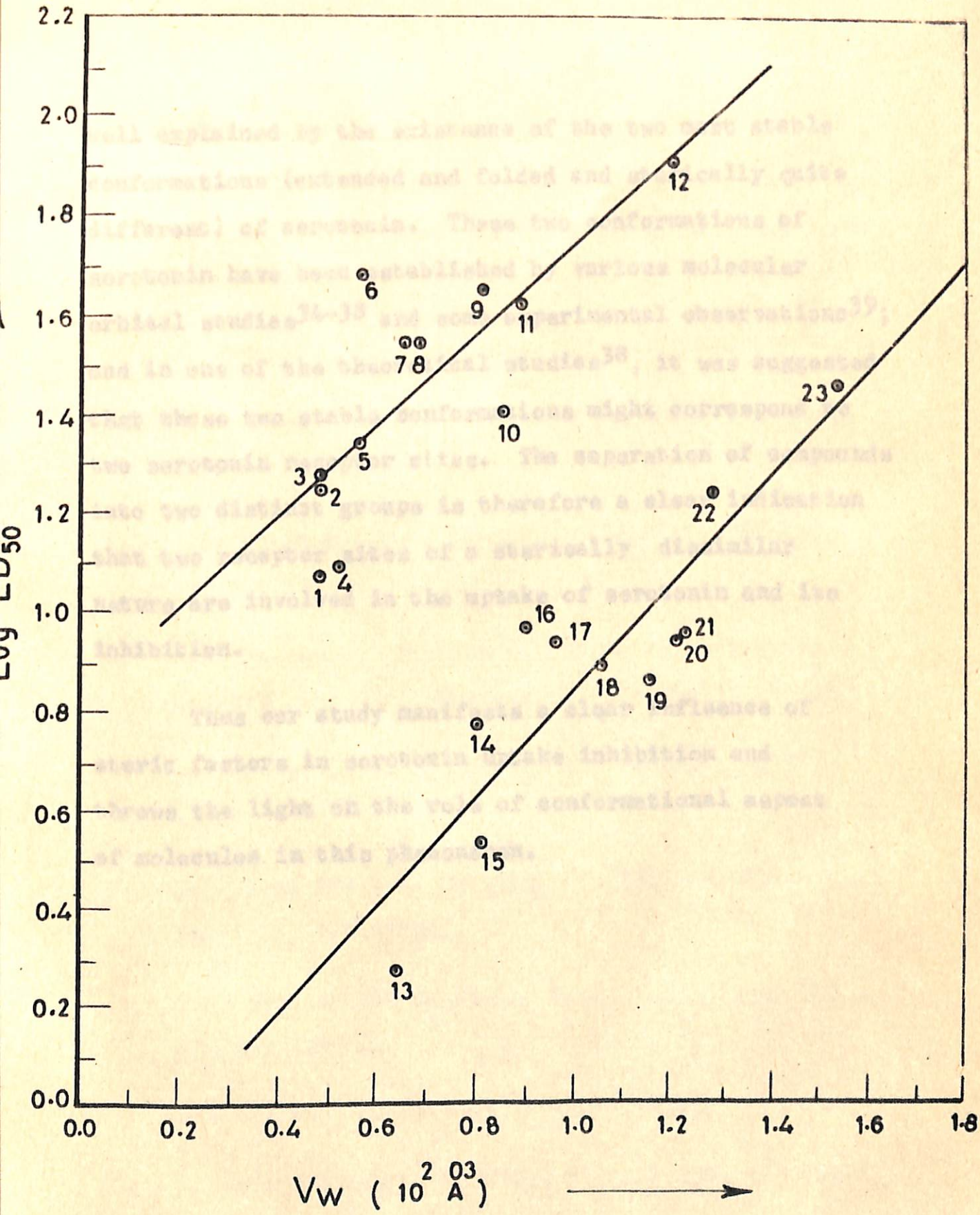


Fig.2.2. A plot of serotonin uptake inhibiton activity of tryptamine derivatives in thrombocytes versus Vw. Numbers refer to the compounds in Table 2.9.

well explained by the existence of the two most stable conformations (extended and folded and sterically quite different) of serotonin. These two conformations of serotonin have been established by various molecular orbital studies[34-38] and some experimental observations[39]; and in one of the theoretical studies[38], it was suggested that these two stable conformations might correspond to two serotonin receptor sites. The separation of compounds into two distinct groups is therefore a clear indication that two receptor sites of a sterically dissimilar nature are involved in the uptake of serotonin and its inhibition.

Thus our study manifests a clear influence of steric factors in serotonin uptake inhibition and throws the light on the role of conformational aspect of molecules in this phenomenon.

REFERENCES:

1. D.F. Downing, in 'Psychopharmacological Agents', M. Gordon, ed.,(Academic Press London and New York), 1964, p. 555.

2. H. Osmond, Ann. N.Y. Acad. Sci., 66, 418 (1957).

3. H. Leuner, Die Experimentelle Psychose (Springer, Berlin), 1962.

4. A.K. Busch and W.C. Johnson, Dis. Nerv. System, 11, 241 (1950).

5. H.A. Abramson, The Use of LSD in Psychotherapy (Josiah Macy, Jr., Foundation, New York), 1959.

6. A. Hoffer, Clin. Pharmacol. and Therap., 6, 183 (1965).

7. E.C. Kast and V.J. Collins, J. Int. Anesth. Res. Soc., 43, 285 (1964).

8. D.W. Woolley and E. Shaw, Proc. Natl. Acad. Sci., 40, 228 (1954).

9. J.H. Gaddum, J. Physiol. 121, 15 (1953).

10. A. Cerletti and E. Rothlin, Nature, 176, 785 (1955).

11. J.H. Gaddum, Ann. N.Y. Acad. Sci., 66, 643 (1957).

12. G. Karreman, I. Isenberg, and A. Szent-Györgyi, Science, 130, 1191 (1959).

13. S.H. Synder and C.R. Merril, Proc. Natl. Acad. Sci., 54, 258 (1965).

14. A. Shulgin, J. Sargent, and C. Naranjo, Nature, 221, 537 (1969).

15. S. Kang and J.P. Green, Nature, 226, 645 (1970).

16. C.F. Barfknecht, D.E. Nichols and W.J. Dunn III, J. Med. Chem., 18, 208 (1975).

17. L.B. Kier and L.H. Hall, J. Med. Chem., 20, 1631 (1977).

18. J.P. Green, in 'Molecular Basis of Some Aspects on Mental Activity', O. Walass, ed., (Academic Press, New York), 1967, p. 95.

19. J.P. Green and S. Kang, in 'Molecular Orbital Studies in Chemical Pharmacology', L.B. Kier, ed., (Springer Verlag, New York), 1970, p. 105.

20. M. Kumbar and D.V. Siva Sankar, Res. Comm. Chem. Path. Pharmacol., 6, 65 (1973).

21. D.V. Siva Sankar and M. Kumbar, Res. Comm. Chem. Pathol. Pharmacol., 7, 259 (1974).

22. J.P. Green, C.L. Johnson and S. Kang, Ann. Rev. Pharmacol., 14, 319 (1974).

23. R.A. Glennon, Res. Comm. Chem. Pathol. Pharmacol., 9, 185 (1974).

24. W.J. Dunn III and J.P. Bederka, Jr., Res. Comm. Chem. Pathol, Pharmacol., 7, 275 (1974).

25. R.A. Glennon, L.B. Kier and A.T. Shulgin, J. Pharm. Sci., 68, 906 (1979).

26. R.A. Glennon and L.B. Kier, Eur. J. Med. Chem., 13, 219 (1978).

27. L.B. Kier and R.A. Glennon, in 'QuaSAR Research Monograph 22', G. Barnett, M. Trsic and R.E.Willette, eds., (Department of Health, Education and Welfare Publication), 1978, p. 159.

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

29. I. Moriguchi and Y. Kanada, Chem. Pharm. Bull. (Tokyo), 25, 926 (1977).

30. A. Bondi, J. Phys. Chem., 68, 444 (1964).

31. Byck Robert, in 'The Pharmacological Basis of Therapeutics', L.S. Goodman and A. Gilman, eds., (MacMillan Publishing Co., New York), 1975, p.152.

32. R.A. Glennon, S.M.Liebowitz and E.C. Mack, J. Med. Chem., 21, 822 (1978).

33. M. Kumbar, V. Cusimano and D.V. Siva Sankar, J. Pharm. Sci., 65, 1014 (1976).

34. L.B. Kier, J. Pharm. Sci., 57, 1188 (1968).

35. S. Kang and M.H. Cho, Theor. Chim. Acta., 22, 176 (1971).

36. S. Kang, C.L. Johnson and J.P. Green, J. Mol. Struct., 15, 453 (1973).

37. C.N.J. Port and B. Pullman, Theor. Chim. Acta., 33, 275 (1974).

38. M. Kumbar and D.V. Siva Sankar, Res. Comm. Chem. Pathol. Pharmacol., 10, 433 (1975).

39. R.R. Ison, P. Partington, and G.C.K. Menon, J. Pharm. Pharmacol., 24, 82 (1972).

III. QSAR STUDIES ON ENZYME INHIBITORS.

3.1. Introduction:

Living cells are amazing chemical factories that can synthesize the huge variety of chemicals needed for energy, cell function, and cell division. These reactions are catalyzed by enzymes, of which some hundreds are known. Cells may differ in which enzymes they can synthesize, depending in part on cell function. Those cells that can not make an essential chemical for growth must be supplied with the chemical to live, function, or divide; such a chemical is called an essential metabolite. For example, primitive bacterial cells such as Escherichia coli can grow on relatively simple media and synthesize all their amino acids and vitamin requirements. In contrast, mammals can not synthesize vitamins or certain essential amino acids; these essential growth factors must be supplied in the diet. Any dietary deficiency can lead to death of the cell. Similarly, any loss of an essential enzyme or its function can cause loss of function or death of the cell because of lack of formation of an essential metabolite. Therefore blockade of the function of an essential enzyme for growth or cell division has been a favoured target for anticancer and antiparasitic chemotherapy. The partial or total blockade of a mammalian enzyme for control of the function of a specialized type cell in the mammals, such as nerve or brain cells, has also been a target for the medicinal chemist.

The mechanisms by which enzymes catalyze reactions are only beginning to be elucidated. Investigations of complexes between the enzyme and substrates or substrate analogs have yielded a significant amount of information concerning the specificity and catalytic mechanisms of enzymes. It is now recognized, although not yet fully understood, that mechanisms of enzymic or receptor systems obey the laws of chemistry. A general mechanism by which enzymes function,can be depicted in the following manner[1]:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E....S \xrightarrow{k_2} E + P$$

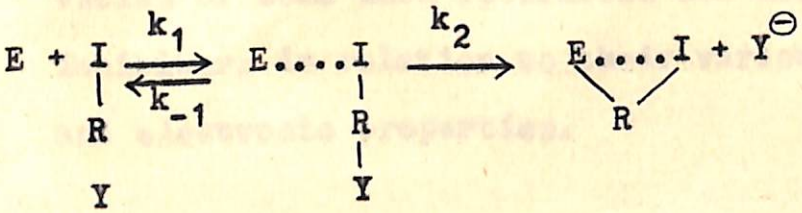
where E is the free enzyme; S,the substrate; E.....S, the initial enzyme - substrate complex; and P, the product. Attractive forces exist between the enzyme and the substrate which control the formation of the initial reversible ES complex. The specificity of enzymic reactions is initially governed by the formation of the ES complex. The second phase of enzyme specificity is controlled when reactive functional groups on the enzyme and the substrate are properly juxtapositioned in the ES complex to allow reaction to occur. In the reaction step, covalent bonds may or may not be formed between the enzyme and the substrate. However, in the formation of the initial ES complex non-covalent interactions between the enzyme and the substrate are involved. The types of bonds that may be involved

in the initial ES complex are charge - charge interactions, hydrogen bonding, protein - water interactions, vander Waals interaction and hydrophobic bonding[2]. When an enzyme and a molecule form a reversible complex which does not yield a product (P); i,e., $k_2 = 0$, inhibition of the enzymic reaction results[3-5]. This equilbrium can be depicted as:

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI$$

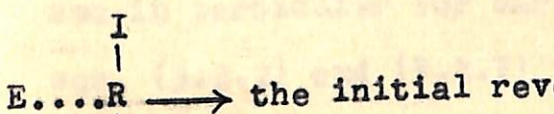
where I is an enzyme inhibitor. In the simplest case, reversible inhibitors can be classified into two main types: (i) competitive and (ii) non-competitive. Competitive inhibitors react reversibly with the free enzyme to form an EI complex which prevents the enzyme from combining with the substrate. Non-competitive enzyme inhibitors do not prevent the formation of the ES complex, but function by inhibiting the breakdown of the ES complex into products. In case, a single substrate is used by several different enzymes, it is highly probable that the active site, or more specifically the site required for binding the substrate to the various enzymes, will be either identical or atleast extremely similar. Therefore, the chances of designing a reversible inhibitor of only one of these enzymes are small. As an approach to this

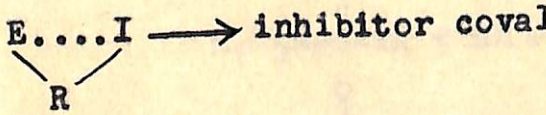
problem, it has been suggested that an extra dimension of biological specificity could be obtained by the preparation of inhibitors which, after the initial formation of a reversible enzyme - inhibitor complex, irreversibly inactivate the enzyme by means of a covalent bond between the enzyme and the inhibitor. Such inhibitors are known as active site - directed irreversible inhibitors[6,7]. The mechanism for active site - directed irreversible inhibition can be depicted in the following manner:



where

E ⟶ Free enzyme





Compared to a reversible inhibitor, the extra specificity of active site - directed irreversible inhibitor is dependent upon the ability of the reversibly bound inhibitor to bridge to and form a covalent bond with a nucleophilic group on the enzyme surface and upon the nucleophilicity of the enzymic group being covalently linked.[8]

The present chapter aims at analysing the activities of some such reversible and irreversible enzyme inhibitors in relation to their various physico-chemical and electronic properties.

3.2. Cholinesterase Inhibitors: Carbamates, Phosphates and Amides:

It is now generally believed that cholinesterase inhibitors, such as carbamates, phosphates etc. exert their inhibitory effect upon the enzyme through the enzymic hydrolysis[9-11] as shown in general by eq.(3.2.1) and in particular for carbamates and phosphates by eqs. (3.2.2) and (3.2.3) respectively.

$$AX + EH \rightleftharpoons AX.EH \longrightarrow AE + XH \qquad (3.2.1)$$

$$R_1R_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}OR + EH \rightleftharpoons R_1R_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}OR.EH \longrightarrow$$

$$R_1R_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}E + ROH \qquad (3.2.2)$$

$$(RO)_2\overset{O}{\overset{\|}{P}}\text{-OR'} + EH \rightleftharpoons (RO)_2\overset{O}{\overset{\|}{P}}\text{-OR'.EH} \longrightarrow$$

$$(RO)_2\overset{O}{\overset{\|}{P}}\text{-E} + R'OH \qquad (3.2.3)$$

where the first step involves the attack of the enzyme (EH) on a particular atom or bond of the inhibitors and the second step is the rate determining step. The reactions are analogous to the alkali hydrolysis of diethylaryl phosphates to give corresponding phenols and diethyl phosphates.

Molecular orbital calculations have shown[12-26] that enzyme hydrolysis and hence the inhibitory activity of the inhibitors depend upon the electronic indices, such as charge density, superdelocalizibility, frontier orbital density, etc. of the site of the enzymic attack in the molecules. For example, Ban and Nagata[19] on the basis of Hückel molecular orbital (HMO) calculation on a series of substituted phenyl N-methylcarbamates showed that the inhibitory power of the inhibitors against cholinesterase (fly brain) was related to the π-charges on both the oxygen atoms of the ester group, and that the ease of hydrolysis roughly paralleled with the nucleophilic superdelocalizibility index, S^N; while Neely[15] in his studies using HMO approximation only on the phenol portion of the carbamates found that the pKa was related

with the dipositive character of the C-O bond (a bond is said to be dipositive if its both ends possess formal positive charges). Similarly, Pullman and Valdemoro[20] using HMO calculation on some organophosphates showed that the enzyme inhibitory power of these compounds was parallel to the value of the positive charge on their phosphorous atom. Earlier Pullman and Pullman[27] had proposed that practically all the fundamental type of biochemical substrates undergoing enzymic hydrolysis have the common feature of undergoing this reaction on a π-electron deficient bond (π-electron deficiency of a bond is measured by its dipositivity). Later Fukui et al[12] in their study on a series of diethyl substituted phenyl phosphates showed a rough parallelism between the inhibitory power of the compounds and the S^N of their phosphorous atom. Likewise Gupta et al[25] noted that the authelmintic activity of organophosphates, which depended upon the ability of the compounds to undergo enzymic hydrolysis, was related to S^N of the phosphorous atom and the dipositive character of the P-O bond.

Inouye et al[13] and later Cammarata[22] studied the case of nicotinic acid derivatives as cholinesterase inhibitors and found that their inhibitory power was related with the frontier orbital charge density at the carbonyl carbon. Similarly, studies made on a series of

N-alkyl-substituted amides[17,18,22,24] showed the inhibitory power to have some connection with the charges at amide nitrogen, carbonyl carbon and carbonyl oxygen. Further, in a study on a series of 3-hydroxyphenyltrimethylammonium derivatives, it was noted[21] that their cholinesterase inhibition activity was dependent upon the hydrogen bonding ability of their 3-hydroxy group. However, only a very few of these structure-activity relationship studies were tested statistically. So far QSAR studies have been made only on nicotinic acid derivatives[23], N-alkyl-substituted amides[23] and 3-hydroxyphenyltrimethylammonium derivatives[21]. Nonetheless, QSAR studies made on N-alkylsubstituted amides can not be said to be complete. The QSAR studies on cholinesterase inhibition by carbamates and phosphates have not been made so far. We therefore proposed to supplement this study by making QSAR studies on remaining series of inhibitors. The present investigations together with those made in past may provide a deeper insight into the mechanism of cholinesterase inhibition.

In the present QSAR studies on carbamates, phosphates and N-alkylsubstituted amides, electronic structure as obtained by previous workers has been used. In the regression analysis only those electronic indices have been used which were shown qualitatively to have some relation with the activity.

Results and Discussion:

Carbamates: As already mentioned, Neely[15] had calculated the electronic structure only of the phenolic moiety of carbamates and pointed out a relationship between pKa (-log acidity constant) and the charges at the ends of C-O bond. With the use of data in Table 3.1 for the same, we however found Q_c (charge at the carbon) and Q_o (charge at the oxygen) to be quantitatively correlated with pKa as shown by eqs. 3.2.4 - 3.2.6.

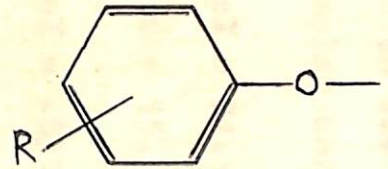
$$pKa = -79.343\ Q_c + 14.056$$
$$n = 9,\ r = 0.962,\ s = 0.579,\ F_{1,7} = 86.85 \qquad (3.2.4)$$

$$pKa = -395.088\ Q_o - 39.686$$
$$n = 9,\ r = 0.971,\ s = 0.503,\ F_{1,7} = 117.23 \qquad (3.2.5)$$

$$pKa = -32.081\ Q_c - 243.970\ Q_o + 30.011$$
$$n = 9,\ r = 0.978,\ s = 0.475,\ F_{2,6} = 66.58 \qquad (3.2.6)$$

In eqs. (3.2.4 - 3.2.6), r values exhibit highly significant correlations between pKa and electronic indices, and F values in all these equations are significant at 99% level ($F_{1,7}(0.01) = 12.25$, $F_{2,6}(0.01) = 10.92$). However, Q_c and Q_o together are found to lead to a better correlation (eq. 3.2.6) than they do so individually. Thus Pullmans' observation that enzymic hydrolysis will

TABLE 3.1: ELECTRONIC PARAMETERS* AND pKa VALUES* FOR PHENOL PORTION OF CARBAMATE INHIBITORS.



S.No.	R	Q_C^a	Q_O^b	pKa	
				Obsd.	Cald.Eq.(3.2.6)
1	3-Cl	0.055	0.076	9.2	9.7
2	2-Cl	0.076	0.079	8.4	8.3
3	2,4-di-Cl	0.087	0.081	7.7	7.5
4	2,4,5-tri-Cl	0.086	0.080	7.2	7.7
5	$2\text{-}CH_3$	0.039	0.075	10.2	10.5
6	$3\text{-}CH_3$	0.057	0.076	10.1	9.6
7	$2\text{-}NO_2$	0.090	0.084	7.2	6.6
8	$2,4\text{-di-}NO_2$	0.117	0.090	4.0	4.3
9	H	0.056	0.076	9.9	9.7

* Reference 15.

a Charge at the carbon atom attached to oxygen.

b Charge at the carbonyl oxygen.

depend upon the dipositivity of the bond is supported quantitatively also.

Phosphates: In the case of a series of diethyl substituted phenyl phosphates, the inhibition activity was shown[12] to be related to the nucleophilic superdelocalizibility index of the phosphorus atom, S_P^N, and its charge, Q_P. However, the regression analysis with the use of data as given in Table 3.2 correlates S_P^N and Q_P with the activity in terms of pI (-log median inhibitory molar concentration) as:

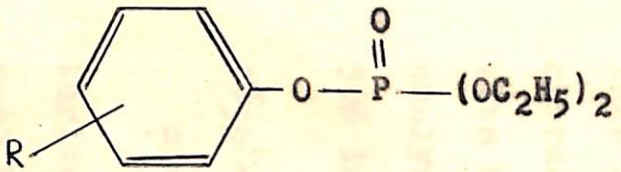
$$pI = 91.115\ S_P^N - 94.415$$
$$n = 15,\ r = 0.542,\ s = 1.629,\ F_{1,13} = 5.41 \qquad (3.2.7)$$

$$pI = -832.608\ Q_P + 239.404$$
$$n = 15,\ r = 0.472,\ s = 1.709,\ F_{1,13} = 3.74 \qquad (3.2.8)$$

$$pI = 95.478\ S_P^N + 51.787\ Q_P - 113.738$$
$$n = 15,\ r = 0.542,\ s = 1.695,\ F_{2,12} = 2.50 \qquad (3.2.9)$$

Equations (3.2.7 - 3.2.9) do not show any significant correlation of activity with S_P^N or/and Q_P. However, a better correlation with S_P^N than that with Q_P leads to assume that after the attack on the phosphorus atom by the enzyme the formation of intermediate complex involves the charge transfer phenomenon, as S_P^N incorporates the charge transfer concept.

TABLE 3.2: ELECTRONIC PARAMETERS* AND CHOLINESTERASE INHIBITION ACTIVITY* OF DIETHYL SUBSTITUTED PHENYL PHOSPHATES.



S.No.	R	S_P^N	Q_P	pI	
				Obsd.	Cald.Eq.(3.2.7)
1	2,4-di-NO_2	1.132	.278	8.523	8.727
2	o-NO_2	1.120	.279	7.301	7.633
3	p-NO_2	1.119	.279	7.585	7.542
4	p-CHO	1.106	.279	6.824	6.358
5	p-CN	1.106	.280	6.886	6.358
6	2,4,5-tri-Cl	1.100	.280	8.222	5.811
7	2,4-di-Cl	1.100	.280	6.301	5.811
8	o-Cl	1.099	.280	4.690	5.720
9	p-Cl	1.099	.280	4.523	5.720
10	H	1.097	.281	8.000	5.538
11	m-NO_2	1.097	.281	7.301	5.538
12	p-CH_3	1.097	.281	3.000	5.538
13	m-OCH_3	1.096	.281	3.886	5.447
14	p-OCH_3	1.096	.281	3.000	5.447
15	p-$N(CH_3)_2$	1.094	.282	6.398	5.264

* Reference 12.

N-alkyl-substituted amides: Considering only the π-electron delocalization in this series of inhibitors, Purcell[17] first observed the activity to be related to the charge at the amide nitrogen contrary to the view[28] that inhibition should be related to the electrophilic character of the carbonyl carbon in the amide group. With the use of data in Table 3.3, the regression analysis correlates pI_{50}(-log molar concentration causing 50% inhibition) with Q_N as,

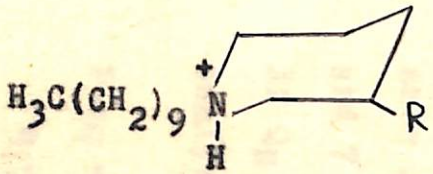
$$pI_{50} = 5.348\ Q_N + 3.512$$
$$n = 5,\ r = 0.738,\ s = 0.318,\ F_{1,3} = 3.59 \qquad (3.2.10)$$

Earlier Martin[23] had also attempted the correlation between these two variables (eq. 3.2.11),

$$pI_{50} = 8.116\ Q_N + 3.04$$
$$n = 6,\ r = 0.696,\ F_{1,4} = 3.77 \qquad (3.2.11)$$

but eq. (3.2.11) shows a little less satisfactory correlation than eq. (3.2.10) (Martin had used six data points and values of Q_N as given within bracket rounded up to three figures of decimal, while we used Q_N values up to four figures and did not include the compound 5 and 7 in the regression analysis, as the data for them were incomplete). In Martin's study several other discrepancies were found which are listed in the footnotes of Table 3.3.

TABLE 3.3: ELECTRONIC PARAMETERS* AND CHOLINESTERASE INHIBITION ACTIVITY* OF N-SUBSTITUTED 1-DECYL-3 (CARBAMOYL) PIPERIDINES.



S.No.	R	Q_N^a	μg^b (D)	pI_{50} Obsd.	pI_{50} Cald. Eq.(3.2.12)
1	$CONH_2$	0.1375 (0.137)	3.77	4.206	4.283
2	$CONHCH_3$	0.2126 (0.212)	4.21	4.458	4.559
3	$CONHC_2H_5$	0.2088 (0.209)	4.23	4.863	4.571
4	$CON(CH_3)_2$	0.2764 (0.276)	4.87	4.664	4.971
5	$CONCH_3C_2H_5$	0.2733 (-)	-	-	-
6	$CON(C_2H_5)_2$	0.2702 (0.273)[c]	5.05	5.278	5.084
7	$CON(C_3H_7)_2$	- (0.270)[d]	5.12	5.979	5.125

* References 17, 18 and 22.

a π-charge at amide nitrogen; data within bracket are those used by Martin[23] in QSAR studies.

b The measured dipole moment of the amide groups in Debye unit taken from Ref.18.

c This datum seems to be wrong as rounded figure upto 3 decimal point should be 0.270 and not 0.273.

d Martin has claimed to have taken this datum also from Ref. 17 but no data for charge is given for compound 7 in this reference.

In a successive paper, Purcell et al[18], showed the inhibition activity of these amides to be related with the experimental values of the amide group moment μg (Table 3.3). Quantitatively, the μg is found to be correlated with pI_{50} as,

$$pI_{50} = 0.625\ \mu g + 1.925$$
$$n = 5,\ r = 0.805,\ s = 0.279,\ F_{1,3} = 5.53 \qquad (3.2.12)$$

From eq. (3.2.12), we find that activity is better correlated with μg than with Q_N (eq. 3.2.10). For the sake of comparison only, we left out the compounds 5 and 7 in eq. (3.2.12) also as we did in eq. (3.2.10). If Q_N and μg together are included in the regression analysis, we get eq. (3.2.13),

$$pI_{50} = -4.463\ Q_N + 1.088\ \mu g + 0.865$$
$$n = 5,\ r = 0.820,\ s = 0.330,\ F_{2,2} = 2.06 \qquad (3.2.13)$$

which does not show any better correlation than eq. (3.2.12), as the value of s is significantly greater in eq. (3.2.13) than in eq. (3.2.12), though r value is slightly improved.

Millner and Purcell[24] re-attempted the problem by calculating both σ - and π - charges and examined the relation between the total charges at amide nitrogen,

carbonyl carbon and carbonyl oxygen, Q_N^T, Q_C^T and Q_O^T respectively, and the inhibition activity, but were unable to find any apparent relation between them (Table 3.4). Thus they could not draw any conclusion regarding the role of the electronic character of the amide group but speculated that the hydrophobic character of alkyl group on amide was controlling factor in the enzyme inhibition.

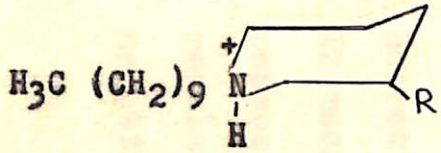
However, when the problem is studied quantitatively, a highly significant correlation is obtained as shown by eq. (3.2.14), between the electronic character of the amide group and the activity.

$$pI_{50} = -51.877\ Q_C^T - 35.993\ Q_N^T - 3576.355\ Q_O^T - 1124.571$$
$$n = 7,\ r = 0.983,\ s = 0.101,\ F_{3,3} = 28.24 \qquad (3.2.14)$$

Thus a QSAR study establishes the role of the electronic character of the amide group in the enzyme inhibition, and a further analysis reveals that out of Q_N^T, Q_C^T and Q_O^T delineating the electronic character of the amide group, Q_C^T plays a better role (eq. 3.2.15) than Q_N^T (eq. 3.2.16) or Q_O^T (eq. 3.2.17).

$$pI_{50} = -73.945\ Q_C^T + 25.131$$
$$n = 7,\ r = 0.793,\ s = 0.256\ F_{1,5} = 8.49 \qquad (3.2.15)$$

TABLE 3.4: ELECTRONIC PARAMETERS* AND CHOLINESTERASE (BUTYRYL) INHIBITION ACTIVITY* OF N-SUBSTITUTED 1-DECYL-3 (CARBAMOYL) PIPERIDINES.



S.No.	R	Q_N^{Ta}	Q_C^{Tb}	Q_O^{Tc}	pI_{50}	
					Obsd.	Cald.Eq.(3.2.14)
1	$CON(C_2H_5)_2$	-0.1235	0.2726	-0.3186	5.276	5.281
2	$CONHCH_3$	-0.2747	0.2807	-0.3170	4.458	4.425
3	$CON(CH_3)_2$	-0.1185	0.2731	-0.3185	4.664	4.840
4	$CON(CH_3)(C_2H_5)$	-0.1210	0.2728	-0.3186	5.009	4.847
5	CO-N-pyrrolidyl	-0.1240	0.2725	-0.3186	5.114	4.877
6	CO-N-piperidyl	-0.1240	0.2665	-0.3186	5.495	5.477
7	CO-N-morpholinyl	-0.1200	0.2729	-0.3185	4.590	4.860

* Reference 24.

a Net $(\sigma+\pi)$ charge on amide nitrogen.

b Net $(\sigma+\pi)$ charge on carbonyl carbon.

c Net $(\sigma+\pi)$ charge on carbonyl oxygen.

$$pI_{50} = 3.516\ Q_N^T + 5.449$$

$$n = 7,\ r = 0.529,\ s = 0.357,\ F_{1,5} = 1.94 \qquad (3.2.16)$$

$$pI_{50} = -396.573\ Q_O^T - 121.303$$

$$n = 7,\ r = 0.613,\ s = 0.333,\ F_{1,5} = 3.01 \qquad (3.2.17)$$

However, contrary to the expectation that inhibitory power should increase as the positive charge at carbonyl carbon increases, eqs. (3.2.14) and (3.2.15) both show that as the positive charge at this atom increases, pI_{50} decreases implying that the inhibition activity decreases. It means that the attacking site of the enzyme also possesses the net positive charge so as to experience the repulsion from the carbonyl carbon and attack at the negatively charged carbonyl oxygen.

With all these conflicting conclusions, it appears to be safe to assume that in these amide inhibitors, the polarity of the bonds of $CONH_2$ group, which can be estimated by the group moment, is responsible for the enzyme inhibition.

Conclusion:

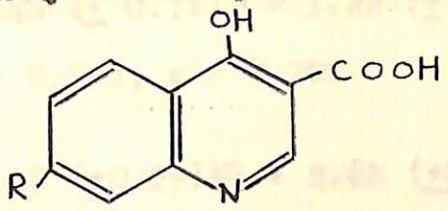
From these QSAR studies and those made in the past, we find that in cholinesterase inhibition, there is, in general, a nucleophilic attack by a particular site of enzyme at a particular site of the inhibitors. In the

inhibitors either a single atom or a complete group of atoms may be involved in the enzyme inhibition. If a single atom is involved, then its charge may be the controlling factor, otherwise, if a group is involved, the group moment may be responsible for the inhibition. In all types of cholinesterase inhibition reactions, a particular bond of the inhibitor is broken and new one formed between the inhibitor and the enzyme as shown by eq. (3.2.1). Further, the formation of intermediate complex in this process may involve sometimes a charge transfer phenomenon.

3.3. Malate Dehydrogenase (MDH) Inhibitors: 7-substituted 4-hydroxyquinoline-3-carboxylic Acids:

Recently Shah and Coats[29] synthesised a series of 7-substituted 4-hydroxyquinoline-3-carboxylic acids (Table 3.5) as inhibitors of cellular respiration and evaluated their ability to inhibit the respiration of Ehrlich ascites cells as a whole cell model and malate-dehydrogenase (MDH) as an intracellular target enzyme model. These authors then tried to correlate the biological data with physico-chemical parameters such as π, MR, σ, σ^+, σ^- etc. Every meaningful combination of these parameters with squared and cross product terms

TABLE 3.5: VAN DER WAALS VOLUME AND CELLULAR RESPIRATION INIHIBITION ACTIVITIES OF 7-SUBSTITUTED 4-HYDROXYQUINOLINE-3-CARBOXYLIC ACIDS.



Compd. No.	R	V_w (10^2 A^3)	pI_{50}(Ascites)		pI_{50}(MDH)	
			Obsd.[a]	Cald.Eq. (3.3.5)	Obsd.[a]	Cald Eq. (3.3.6)
1	H	1.486	2.98	3.28	b	2.28
2	Cl	1.651	3.84	3.01	2.44	2.67
3	F	1.532	3.30	3.19	1.98	2.39
4	OCH_3	1.721	3.28	2.93	b	2.83
5	$COCH_3$	1.815	3.10	2.87	3.04	3.05
6	$N(CH_3)_2$	1.909	3.33	2.86	3.32	3.27
7	$OCH_2C_6H_5$	2.415	4.41	4.42	4.49	4.44
8	$OCH_2C_6H_3$-3,4-Cl_2	2.745	4.82	4.80	5.32	5.21
9	NO_2	1.673	3.24	2.98	2.72	2.72
10	$CONH_2$	1.760	2.24	2.90	3.13	2.92
11	COOH	1.729	2.24	2.92	2.97	2.85
12	SO_2CH_3	1.946	2.75	2.87	3.18	3.35
13	OH	1.554	3.04	3.15	3.31	2.44
14	SO_2NH_2	1.883	2.47	2.86	3.02	3.21
15	SO_3^-	1.825	2.88	2.87	2.67	3.07

[a] taken from ref. 29

[b] omitted from the correlations due to the lack of estimate.

were examined with eqs. (3.3.1 - 3.3.4) surfacing as the most significant correlations.

$$pI_{50}\ (\text{ascites}) = 0.46\ (\pm\ 0.11)\pi + 3.22\ (\pm\ 0.16)$$
$$n = 14,\ r = 0.933,\ s = 0.280 \qquad (3.3.1)$$

$$pI_{50}\ (\text{ascites}) = 0.45\ (\pm 0.29)MR + 2.68\ (\pm 0.47)$$
$$n = 14,\ r = 0.699,\ s = 0.554 \qquad (3.3.2)$$

$$pI_{50}\ (\text{MDH}) = 0.26\ (\pm 0.22)\pi + 3.31\ (\pm 0.45)$$
$$n = 13,\ r = 0.604,\ s = 0.716 \qquad (3.3.3)$$

$$pI_{50}\ (\text{MDH}) = 0.70\ (\pm 0.17)MR + 2.29\ (\pm 0.30)$$
$$n = 13,\ r = 0.939,\ s = 0.315 \qquad (3.3.4)$$

In these equations, I_{50} is the concentration of the compound causing 50 percent inhibition.

Among these four equations, eqs. (3.3.1) and (3.3.4) are the most significant. Thus Shah and Coats found the Ascites cell inhibition activity to be significantly correlated with the hydrophobic constant, π, and the MDH inhibition to be significantly correlated with the molar refractivity, MR. Thus two mutually related inhibition activities, both equally responsible for cellular respiration, are found to be correlated with two entirely different parameters. Therefore, the correlations obtained by Shah and Coats (eqs. 3.3.1 and 3.3.4) can not be

successfully utilized for the drug design as it is not necessary that a substituent which will increase the molar refractivity of the compound will also increase its hydrophobicity. Hence, it becomes necessary to find a single parameter with which both inhibition activities may be significantly correlated.

As the van der Waals volume (Vw), which determines the molecular size of the compounds, has been found to be significantly correlated with the hydrophobic behaviour of drug molecules and various biological activities of drugs (section 2.2(a)), attempts were made to analyse the correlation between the respiratory inhibition and Vw.

The regression analysis with the use of data as given in Table 3.5 is found to afford significant correlations between the inhibition activities of respiratory inhibitors and Vw. A parabolic correlation, as shown by eq. (3.3.5), is found in case of ascites cell inhibition and a linear correlation, as shown by eq. (3.3.6), in case of MDH inhibition.

$$pI_{50}\ (\text{ascites}) = 2.644\ Vw^2 - 9.983\ Vw + 12.280$$
$$n = 15,\ r = 0.824,\ s = 0.442,\ F_{2,12} = 12.67 \qquad (3.3.5)$$

$$pI_{50}\ (\text{MDH}) = 2.32\ Vw - 1.171$$
$$n = 13,\ r = 0.925,\ s = 0.341,\ F_{1,11} = 65.55 \qquad (3.3.6)$$

In both these equations, the F values are significant at 99% level ($F_{2,12}$ (0.01) = 6.93; $F_{1,11}$ (0.01) = 9.65). Further, the inhibition activity values reproduced from these equations are found to be in good agreement with the experimental ones (Table 3.5). Thus both the inhibition activities are found to be significantly correlated, though in different manners, with a single parameter, Vw.

From eqs. (3.3.5) and (3.3.6), it is evident that molecular size plays an important role in the inhibition of cell respiration, and ascites cell as well as MDH inhibitions both would depend upon this. The correlation of both inhibition activities with a single parameter, Vw, is quite relevant as in addition to the hydrophobic behaviour of the molecules being shown to be correlated with Vw, the molar refractivity[30,31] has also been pointed out to be parallel to Vw.

3.4. Carbonic Anhydrase Inhibitors: Sulfonamides:

The carbonic anhydrase (CA) is an extremely efficient catalyst of reversible hydration of carbon-dioxide and plays an important role in the respiration as well as in other physiological and pharmacological processes such as diuresis[32-34]. Aromatic sulfonamides

are specific inhibitors of this enzyme and unsubstituted $-SO_2NH_2$ group is required for inhibition. Any N-sulfamyl substitution leads to a greatly reduced or abolished inhibitory power. This class of inhibitors is of immense importance in studies related to physiological and pharmacological functions of the enzyme. A large number of sulfonamides have been studied and their inhibition activity reported[35]. A few structure-activity studies[36-37] have shown that the inhibition activity of sulfonamides will depend upon the electronic properties of the sulfamyl group, as it was found to be correlated with various electronic parameters, such as the Hammett σ value, NMR chemical shift of the sulfamyl protons, pKa[36], and nitrogen - 14 nuclear quadrupole resonance (NQR) data[37].

Spectroscopic studies[38] provide evidence that anionic form ($-SO_2NH^-$) of sulfamyl group is bound to the Zn^{2+} ion of the enzyme. On this basis it has been suggested that sulfonamides mimic bicarbonate ion in the transition state (Fig. 3.1) and hence are potent inhibitors. All these studies have been mainly concerned with the $-SO_2NH_2$ and Zn^{2+} ion interactions and throw very little light on the rest of the molecular structure and its relation to the binding mechanism. The X-ray studies[32], however, provide evidence for other secondary

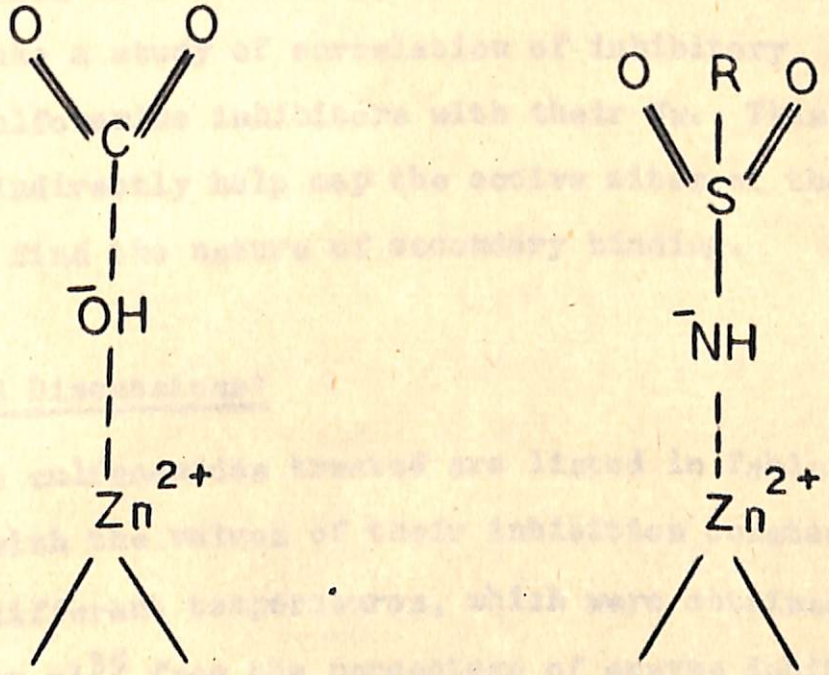


Fig. 3.1. Schematic diagrammes of binding of HCO_3^- and SO_2NH^- with Zn^{2+} of carbonic anhydrase.

binding sites in the sulfonamides - CA complexes. But it remains to be established what is the nature of, and the active site in enzyme for, the secondary binding. Since the size of the molecules is supposed to play an important role in the binding, we considered it worthwhile to make a study of correlation of inhibitory power of sulfonamide inhibitors with their Vw. This study may indirectly help map the active sites of the enzyme and find the nature of secondary binding.

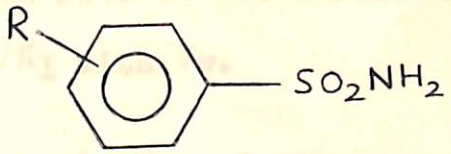
Results and Discussions:

The sulfonamides treated are listed in Table 3.6 along with the values of their inhibition constants K_I at two different temperatures, which were obtained by Kakeya et al[35] from the percentage of enzyme inhibition (i) measured by them, using the formula[39,40],

$$\frac{I_o}{i} = K_I \frac{1}{1-I} + iE \qquad (3.4.1)$$

where I_o and E are the concentrations of inhibitor and enzyme respectively. The enzyme used was purchased from Sigma Chemical Co.[35] The calculated Vw values listed in Table 3.6 are only for the substituents in the sulfonamides.

TABLE 3.6: CARBONIC ANHYDRASE INHIBITION CONSTANTS AND V_w VALUES FOR SULFONAMIDES.



	R	V_w (10^2 Å^3)	π [a]	Log $1/K_I$ [b]	
				0.2°C	15°C
1	H	0.056	0.000	0.215	0.124
2	p-NH_2	0.177	-1.137	-0.363	-0.398
3	p-Cl	0.244	0.531	0.721	0.959
4	p-CH_3	0.245	0.505	0.420	0.496
5	p-CN	0.268	-0.083	0.959	1.187
6	p-Br	0.287	1.053	0.921	0.959
7	p-NO_2	0.287	0.328	1.046	1.260
8	p-CH_3O	0.304	0.163	0.347	0.301
9	p-CH_3NH	0.339	-0.231	-0.176	-0.046
10	p-CH_3CO	0.420	-0.105	0.959	0.886
11	m,p-diCl	0.488	1.134	1.400	1.522
12	m-NO_2-p-Cl	0.531	1.603	1.769	1.602
13	m-CF_3-p-NO_2	0.670	1.420	1.854	1.658
14	m-Cl	0.244	0.981	0.638	0.921
15	m-CH_3	0.245	0.540	0.301	0.223
16	m-NO_2	0.287	0.242	0.886	1.125

[a] taken from ref. 36.

[b] K_I is in unit of 10^{-5}M; taken from ref. 36.

The regression analysis with the use of all sixteen data points reveals the following equations correlating log $1/K_I$ with Vw.

$$\log 1/K_I\ (0.2^o) = 3.261\ Vw - 0.294$$
$$n = 16,\ r = 0.774,\ s = 0.407,\ F_{1,14} = 20.86 \qquad (3.4.2)$$

$$\log 1/K_I\ (15^o) = 2.880\ Vw - 0.118$$
$$n = 16,\ r = 0.686,\ s = 0.465,\ F_{1,14} = 12.46 \qquad (3.4.3)$$

Though eqs. (3.4.2) and (3.4.3) do not show any significant correlation, the effect of substituents' size on CA inhibition is manifested. A more fruitful discussion will follow when we treat para - and meta - substituted analogs separately.

In Kakeya et al's studies[36], the other property that was found, in addition to electronic characteristic of sulfamyl group, to influence the binding of sulfonamides with CA, was the hydrophobic character of molecules. The hydrophobicity constant was found to be correlated with log $1/K_I$ as shown by eqs. (3.4.4 - 3.4.7).

$$\log 1/K_I\ (0.2^o) = 0.509\ \pi_c + 0.324$$
$$n = 16,\ r = 0.462,\ s = 0.581 \qquad (3.4.4)$$

$$\log 1/K_I\ (0.2^o) = 0.702\ \pi + 0.439$$
$$n = 16,\ r = 0.789,\ s = 0.435 \qquad (3.4.5)$$

$$\log 1/K_I\ (15^\circ) = 0.494\ \pi_c + 0.329$$
$$n = 16,\ r = 0.450,\ s = 0.621 \qquad (3.4.6)$$

$$\log 1/K_I\ (15^\circ) = 0.670\ \pi + 0.508$$
$$n = 16,\ r = 0.758,\ s = 0.462 \qquad (3.4.7)$$

where π and π_c represent hydrophobicities measured from the partition coefficients determined in n-octanol-water and chloroform-water systems respectively. Only π was found to have some correlation but not π_c . However, if it is assumed that it is the hydrophobicity of the molecules that is responsible for the secondary binding of sulfonamide with CA, one can argue that molecular size effects the binding through the change in this property of the compounds. Hydrophobicity of a series of a polar molecules, in fact, has been shown[41] to be related with Vw, but for polar molecules this relation is not certain, as in their case solute-solvent interactions also play very important roles. In the very present case, the π is not found to be related with Vw at all (eq. 3.4.8).

$$\pi = 2.896\ Vw - 0.488$$
$$n = 16,\ r = 0.610,\ s = 0.573,\ F_{1,14} = 8.31 \qquad (3.4.8)$$

Therefore, some independent role of molecular size in sulfonamide - CA interaction must be expected. Further, informations are gathered when para - and meta - substituted analogs are analysed separately.

For the para - substituted sulfonamides including the p, m-disubstituted analogs and the parent one (1-13), the correlations obtained are as shown by eqs. (3.4.9) and (3.4.10), and for meta substituted derivatives which again include the p, m - disubstituted analogs and the parent one (1, 11 - 16), they are as shown by eqs. (3.4.11) and (3.4.12).

$$\log 1/K_I\ (0.2^\circ) = 3.278\ Vw - 0.313$$
$$n = 13,\ r = 0.737,\ s = 0.447,\ F_{1,11} = 16.72 \qquad (3.4.9)$$

$$\log 1/K_I\ (15^\circ) = 1.203\ Vw + 0.254$$
$$n = 13,\ r = 0.350,\ s = 0.541,\ F_{1,11} = 1.54 \qquad (3.4.10)$$

$$\log 1/K_I\ (0.2^\circ) = 3.069\ Vw - 0.096$$
$$n = 7,\ r = 0.958,\ s = 0.211,\ F_{1,5} = 56.34 \qquad (3.4.11)$$

$$\log 1/K_I\ (15^\circ) = 2.741\ Vw + 0.038$$
$$n = 7,\ r = 0.902,\ s = 0.302,\ F_{1,5} = 21.85 \qquad (3.4.12)$$

Now as obvious, the correlation between inhibition activities and Vw for para substituted analogs are of little significance, but there exist highly significant correlations between them for meta substituted ones. However, π is found to bear no correlation here in any case with the activity (eqs. 3.4.13 - 3.4.16).

$$\log 1/K_I\ (0.2^\circ) = 0.978\,\pi + 0.686$$
$$n = 13,\ r = 0.656,\ s = 0.535,\ F_{1,5} = 8.32 \qquad (3.4.13)$$

$$\log 1/K_I\ (15^\circ) = 0.647\,\pi + 0.595$$
$$n = 13,\ r = 0.533,\ s = 0.488,\ F_{1,15} = 4.37 \qquad (3.4.14)$$

$$\log 1/K_I\ (0.2^\circ) = 0.137\,\pi + 0.952$$
$$n = 7,\ r = 0.067,\ s = 0.737,\ F_{1,5} = 0.02 \qquad (3.4.15)$$

$$\log K_I\ (15^\circ) = 0.260\,\pi + 0.917$$
$$n = 7,\ r = 0.134,\ s = 0.695,\ F_{1,5} = 0.09 \qquad (3.4.16)$$

And also there exists practically no correlation between π and Vw in any case (eqs. 3.4.17 and 3.4.18).

$$\pi = 1.099\ Vw - 0.274$$
$$n = 13,\ r = 0.388,\ s = 0.438,\ F_{1,11} = 1.95 \qquad (3.4.17)$$

$$\pi = 0.212\ Vw + 0.34$$
$$n = 7,\ r = 0.136,\ s = 0.358,\ F_{1,15} = 0.09 \qquad (3.4.18)$$

The picture that emerges from the above analysis is that meta substituted sulfonamides have a highly complimentary binding site and that their interaction with secondary binding site of CA will depend upon the size of the meta substituent. As shown in Fig. 3.2, the primary active site of the enzyme molecule consists of a Zn^{2+} ion with three ligands from protein, all of which are

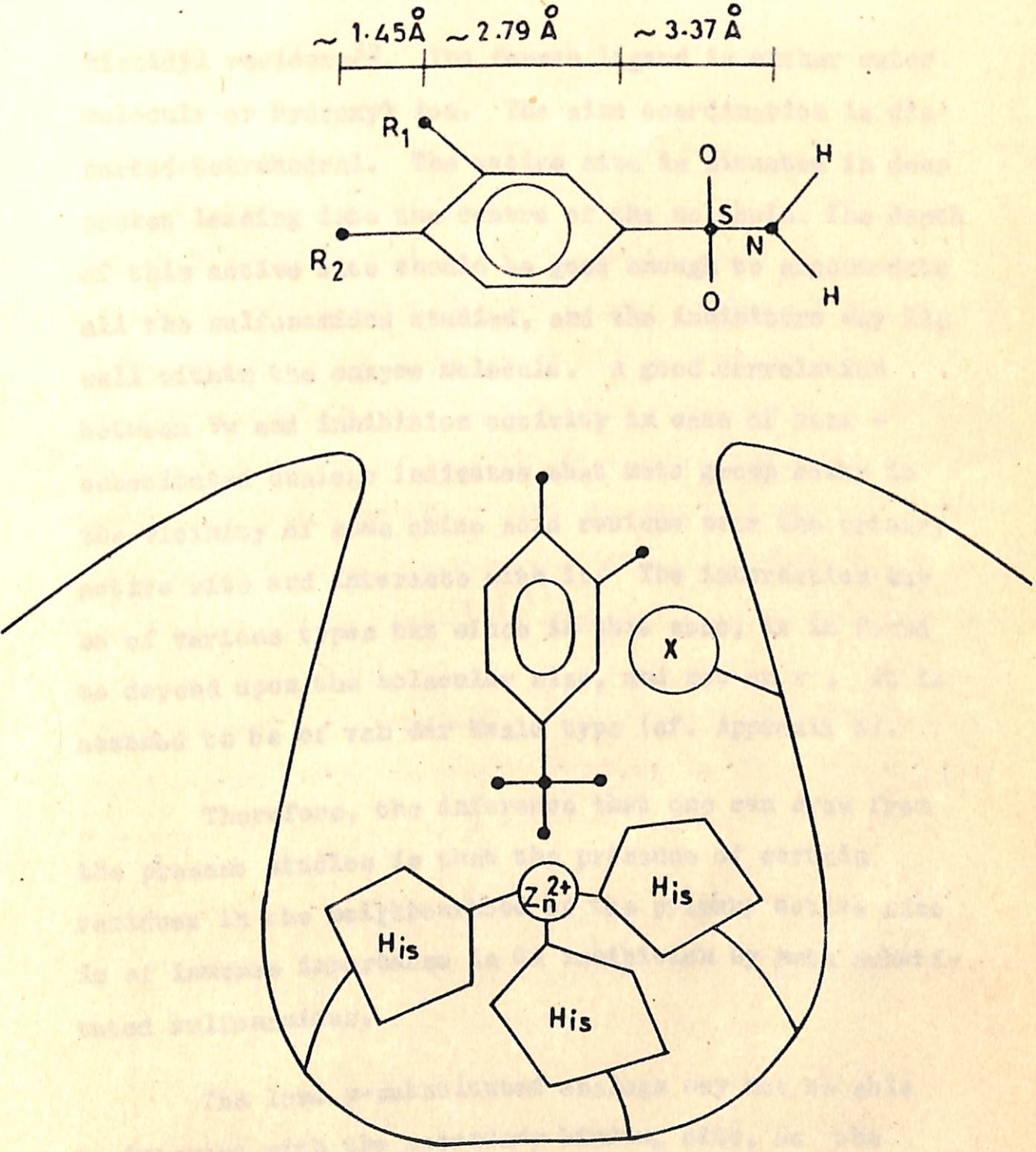


Fig. 3.2. A representative diagram of sulfonamide and its binding to carbonic anhydrase. X denotes the amino acid residue in enzyme interacting with meta substituent of sulfonamide.

histidyl residues[32]. The fourth ligand is either water molecule or hydroxyl ion. The zinc coordination is distorted-tetrahedral. The active site is situated in deep pocket leading into the centre of the molecule. The depth of this active site should be good enough to accommodate all the sulfonamides studied, and the inhibitors may lie well within the enzyme molecule. A good correlation between Vw and inhibition activity in case of meta - substituted analogs indicates that meta group comes in the vicinity of some amino acid residue near the primary active site and interacts with it. The interaction may be of various types but since in this case, it is found to depend upon the molecular size, and not on π , it is assumed to be of van der Waals type (cf. Appendix A).

Therefore, the inference that one can draw from the present studies is that the presence of certain residues in the neighbourhood of the primary active site is of immense importance in CA inhibition by meta substituted sulfonamides.

The lone p-substituted analogs may not be able to interact with the secondary binding site, as the substituents in them may not be able to orient themselves towards that site (Fig. 3.2). They therefore would be exerting their inhibitory effect through only the electronic influence of the substituents.

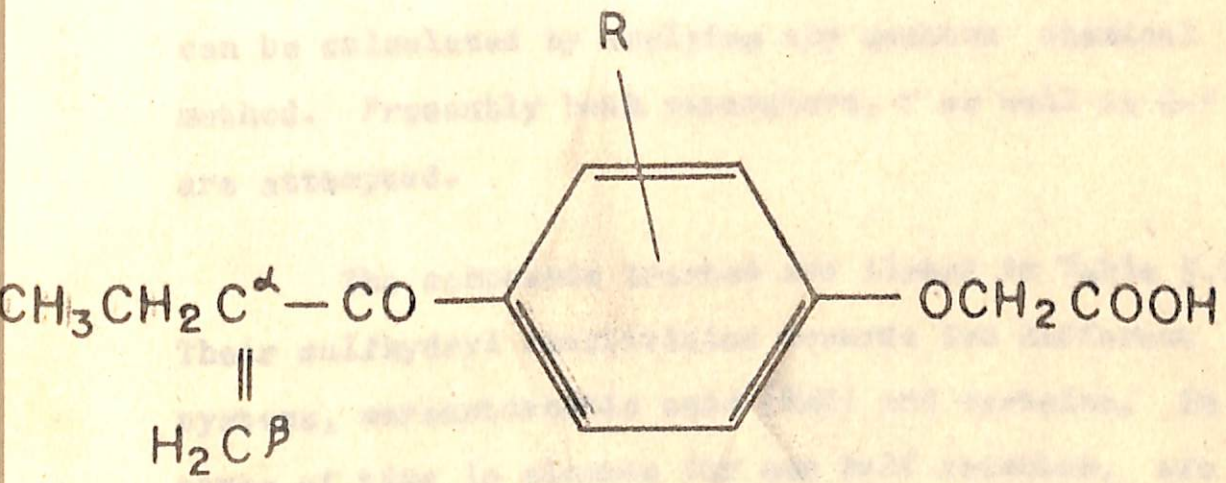
3.5. ATPase Inhibitors: Unsaturated Acylphenoxyacetic Acids:

Unsaturated acylphenoxyacetic acids (APAAs) of general structure (I) have been found to act as diuretics[42-45]. The diuretic activity of these acids is said to be associated with the presence of an α,β-unsaturated ketonic moiety ($-\overset{|}{C}=\overset{|}{C}-\overset{O}{\overset{\|}{C}}-$) in these molecules. In has been proposed that unsaturated APAAs act, like organic mercurials, by binding with renal sulfhydryl containing enzyme (Adenosine triphosphatase, ATPase, of the tubular membrane) through olefinic bond present in the unsaturated ketonic moiety[42,46]. However sufficient ATPase inhibition data for these compounds are not available. But ATPase inhibition leading to diuretic action would be a function of the ability of APAAs to react with the enzyme's sulfhydryl group or for that matter with any compound containing a sulfhydryl group in 'in vitro' system and therefore should be related with the parameter with which sulfhydryl reactivity of APAAs, in general, is found to be related. An unsaturated APAA will react with any compound containing sulfhydryl group as:

$$CH_3CH_2\overset{H_2C}{\overset{\|}{C}} - \overset{O}{\overset{\|}{C}} - Ar - OCH_2\cdot COOH + RSH \longrightarrow \quad (3.5.1)$$

$$CH_3CH_2\underset{CH_2SR}{\overset{|}{CH}} - \overset{O}{\overset{\|}{C}} - Ar - O - CH_2\cdot COOH$$

Since the group RS^- is a nucleophile, this chemical reaction (eq. 3.5.1) will depend upon the electrophilic nature



(I)

of the C-β atom which is influenced by the electronic character of substituents in the phenyl ring. Since the latter can be measured by the Hammett constant σ, the sulfhydryl reactivity can be expected to be related with σ, otherwise, this can be directly correlated with the net positive charge on C-β atom at which the nucleophile RS^- will attack. The charge at C-β, Q-β, can be calculated by applying any quantum chemical method. Presently both parameters, σ as well as Q-β are attempted.

The compounds treated are listed in Table 3.7. Their sulfhydryl reactivities towards two different systems, mercaptoacetic acid (MAC) and cysteine, in terms of time in minutes for one half reaction, are also listed alongwith them. Table 3.7 also lists σ and Q-β. Q-β was obtained by calculating sigma - and pi-charges separately using Del Re[47] and HMO[48] methods respectively. The parameters used were those as given in literatures[46,47]. The source for σ is given below Table 3.7.

Results and Discussion:

The sulfhydryl reactivities of APAAs towards MAC and cysteine both are found to be satisfactorily related with σ and Q-β as shown by eqs. (3.5.2 - 3.5.5).

TABLE 3.7: ELECTRONIC PARAMETERS AND SULFHYDRYL REACTIVITY OF α,β-UNSATURATED ACYLPHENOXY ACETIC ACIDS (I).

Compd. No.	R	$\Sigma\sigma$[a]	Q-β	Sulfhydryl reactivity log $T_{1/2}$ MAC[b]			Cysteine[c]		
				Obsd.[d]	Cald.eq. (3.5.2)	Cald.eq. (3.5.3)	Obsd.[d]	Cald.eq. (3.5.4)	Cald.eq. (3.5.5)
1	H	0.00	0.1205	1.954	1.602	1.517	1.255	0.744	0.676
2	3-Cl	0.23	0.1226	0.845	1.357	1.148	0.176	0.427	0.247
3	3-CH_3	-0.07	0.1195	1.681	1.676	1.692	0.845	0.840	0.880
4	2-Cl	0.37	0.1205	1.431	1.208	1.517	-	0.235	0.676
5	2-CH_3	-0.07	0.1205	1.954	1.676	1.517	1.041	0.840	0.676
6	2,3-diCl	0.60	0.1226	0.778	0.962	1.148	-0.097	-0.082	0.247
7	2,3-diCH_3	-0.14	0.1195	1.653	1.751	1.692	0.740	0.937	0.880
8	3,6-diCl	0.60	0.1226	0.778	0.962	1.148	-	-0.082	0.248
9	2,6-diCl	0.74	0.1215	1.342	0.813	1.341	-	-0.275	0.472
10	3,5-diCl	0.46	0.1266	0.778	1.112	0.446	-	0.111	-0.569
11	3,5-diCH_3	-0.14	0.1185	-	1.751	1.868	0.954	0.937	1.085
12	3,6-diCH_3	-0.14	0.1195	1.763	1.751	1.692	0.875	0.937	0.880
13	2,3,5-triCH_3	-0.21	0.1185	1.863	1.826	1.868	0.875	1.033	1.085
14	2,3,5,6-tetraCH_3	-0.28	0.1185	1.778	1.900	1.868	1.079	1.130	1.085

[a] with respect to position 4, taken from C. Hansch and E.W. Deutsch, Biochim. Biophys. Acta., 126, 117 (1966); [b] equimolar MAC in pH 6.5 phosphate buffer at room temperature[45]; [c] two molar equivalents of cysteine in phosphate buffer at pH 7.1 at room temperature[45]; [d] taken from reference 45.

$$\log T_{\frac{1}{2}}(MAC) = 1.602 - 1.066\sigma$$

$$n = 13,\ r = 0.795,\ s = 0.301,\ F_{1,11} = 18.84 \qquad (3.5.2)$$

$$\log T_{\frac{1}{2}}(MAC) = 22.664 - 175.496\ Q\text{-}\beta$$

$$n = 13,\ r = 0.821,\ s = 0.283,\ F_{1,11} = 22.81 \qquad (3.5.3)$$

$$\log T_{\frac{1}{2}}(cysteine) = 0.744 - 1.377\sigma$$

$$n = 10.\ r = 0.850,\ s = 0.293,\ F_{1,8} = 22.88 \qquad (3.5.4)$$

$$\log T_{\frac{1}{2}}(cysteine) = 25.282 - 204.195\ Q\text{-}\beta$$

$$n = 10,\ r = 0.756,\ s = 0.290,\ F_{1,8} = 10.65 \qquad (3.5.5)$$

In eqs. (3.5.2 - 3.5.4), F is significant at 99% level ($F_{1,11}(0.01) = 9.65$; $F_{1,8}(0.01) = 11.26$), but in eq. (3.5.5) it is significant only at 95% level ($F_{1,8}(0.05) = 5.32$).

As discussed earlier, APAAs are supposed to exert their diuretic action through selective reaction with functionally important sulfhydryl groups and these unsaturated compounds will add sulfhydryl at the double bond. In fact ethacrynic acid has been shown to react with sulfhydryl group in vivo[49,50]. Our quantum chemical studies thus quantitatively support the proposed mechanism that APAAs act by binding with renal sulfhydryl containing enzyme through olefinic bond present in the unsaturated ketonic moiety and that the activating substituents present in the aromatic nucleus markedly influence the activity.

REFERENCES:

1. L. Michaelis and M.L. Menton, Biochem. Z. 49, 333 (1913).

2. C.G. Hammes, Accounts Chem. Res., 1, 321 (1968).

3. M. Dixon and E.C. Webb, Enzymes (Academic Press, New York), 1958.

4. J.L. Webb, Enzyme and Metabolic Inhibitors, Vols.1, 2 and 3 (Academic Press, New York), 1963, 1965, 1966.

5. R.M. Hochster and J.H. Quastel, eds. Metabolic Inhibitors, Vols. 1 and 2 (Academic Press, New York), 1963.

6. B.R. Baker, J. Pharm. Sci., 53, 347 (1964).

7. B.R. Baker, Design of Active-Site-Directed Irreversible Enzyme Inhibitors (Wiley, New York), 1967.

8. J.M. Reiner, Behaviour of Enzyme Systems (Burgess, Minneapolis, Minn.), 1959, p. 151.

9. R.D. O'Brien in 'Drug Design', Vol. II, E.J. Ariëns, ed., (Academic Press, New York), 1971, p.162.

10. I.B. Wilson and H.C. Froede, in 'Drug Design', Vol. II, E.J. Ariëns, ed., (Academic Press, New York), 1971, p. 213.

11. R.L. Metcalf, in 'Pesticides in the Environment - Part I', Vol.1, R. White-Steven, ed., (Marcel Dekker, New York), 1971, p. 1.

12. K. Fukui, K. Morokuma, C. Nagata and I. Imamura, Bull. Chem. Soc. Japan, 34, 1224 (1961).

13. A. Inouye, Y. Shinagawa and Y. Takaishi, Arch.Intern. Pharmacodyn., 144, 319 (1963).

14. A. Inouye, Y. Shinagawa and Y. Takaishi, Arch.Intern. Pharmacodyn., 145, 546 (1963).

15. W.B. Neely, Mol. . Pharmacol., 1, 137 (1965).

16. Y. Shinagawa, A. Inouye and T. Ban, J. Physiol.Soc. Japan, 22, 519 (1960).

17. W.P. Purcell, J. Med. Chem., 9, 294 (1966).

18. W.P. Purcell, J.G. Beasley, R.P. Quintana and J.A. Singer, J. Med. Chem., 9, 297 (1966).

19. T. Ban and C. Nagata, Jap. J. Pharmacol., 15, 32 (1965).

20. B. Pullman and C. Valdemoro, Biochem. Biophys. Acta., 43, 548 (1960).

21. A. Cammarata and R.L. Stein, J. Med. Chem., 11, 829 (1968).

22. A. Cammarata, J. Med. Chem., 11, 1111 (1968).

23. Y.C. Martin, J. Med. Chem., 13, 145 (1970).

24. O.E. Millner (Jr.) and W.P. Purcell, J. Med.Chem., 14, 1134 (1971).

25. S.P. Gupta, S.S. Sharma and P. Singh, Indian J. Chem., 15B, 731 (1977).

26. L.B. Kier, Molecular Orbital Theory in Drug Research (Academic Press, New York), 1971.

27. A. Pullman and B. Pullman, Proc. Natl. Acad. Sci. (USA), 45, 1572 (1959).

28. F. Bergmann, I.B. Wilson and D. Nachmansohn, J. Biol. Chem., 186, 693 (1950).

29. K.J. Shah and A. Coats, J. Med. Chem., 20, 1001 (1977).

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

31. M. Yoshimoto, and C. Hansch, J. Org. Chem., 41, 2269 (1976).

32. K.K. Kannan in 'Biomolecular Structure, Conformation, Function and Evolution,' Vol.1, R. Srinivasan, ed., (Pergamon Press, Oxford and New York), 1980, p.165.

33. K.H. Beyer and J.E. Baer, Pharmacol. Rev., 13, 517 (1961).

34. G. deStevens, Diuretics - Chemistry and Pharmacology (Academic Press, New York), 1963.

35. N. Kakeya, N. Yata, A. Kamada, and M. Aoki, Chem. Pharm. Bull.(Tokyo), 17, 1010 (1969).

36. N. Kakeya, N. Yata and A. Kamada, Chem. Pharm.Bull. (Tokyo), 17, 2558 (1969).

37. S.N. Subbarao and P.J. Bray, J. Med. Chem., 22, 111 (1979).

38. K. Kumar, R.W. King, and P.R. Carey, Biochemistry, 15, 2195 (1976).

39. L.H. Easson and E. Stedman, Proc. Roy. Soc.(London), B127, 142 (1936).

40. T.H. Maren, J. Pharmacol. Exptl. Therap., 139, 140 (1963).

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

42. E.M. Schultz, E.J. Cragoe (Jr.), J.B. Bicking, W.A. Bolhofer and J.M. Sprague, J. Med. Chem., 5, 660 (1962).

43. J.E. Baer, J.K. Michaelson, D.N. Mckinstry and K.H. Beyer, Proc. Soc. Exptl. Bio. Med., 115, 87 (1969).

44. K.H. Beyer, J.E. Baer, J.K. Michaelson and H.F. Russo, J. Pharmacol. Exptl. Therap., 147, 1 (1965).

45. J.M. Sprague in 'Topics in Medicinal Chemistry', Vol.2, J.L. Rabinowitz and R.M. Myerson, eds. (Interscience Publishers, New York), 1968, p.1.

46. R. Komorn and E.J. Cafruny, J. Pharmacol. Exptl. Therap. 148, 367 (1965).

47. G. Del Re, J. Chem. Soc.(London), 4031 (1958).

48. A. Streitwieser Jr., Molecular Orbital Theory For Organic Chemists (Wiley, New York), 1961.

49. R.M. Komorn and E.J. Cafruny, Science, 143, 133 (1964).

50. R.Z. Gussin and E.J. Cafruny, J. Pharmacol. Exptl. Therap., 149, 1 (1965); 153, 148 (1965).

IV. QSAR STUDIES ON CARDIOVASCULAR AGENTS AND SOME MISCELLANEOUS DRUGS.

4.1. Cardiovascular Agents:

Cardiovascular diseases occupy first place as causa mortis in civilized countries. This group of diseases is comprised of heart diseases and diseases of blood vessels and lymphatics. In the treatment of heart diseases, the drugs chiefly used are cardiotonics, antiarrhythmics, and antihypertensives including diuretics. For the treatment of diseases of blood vessels and lymphatics, the measures taken are - surgery, and administration of vasodilators, antihypertensive agents and hypocholesterolemic agents. In the present section of this Chapter we have made discussion on some diuretics and vasodilators.

Diuretics are defined often improperly as substances that increase urinary volume. In the clinical sense, these are the drugs which promote the excretion of Na^+ ions which constitute one of the main electrolytes of the extracellular fluid. They are used in the relief of edema and as adjuvant in the management of hypertension. A diuretic action per se produces a lower blood volume, which reduces cardiac output and in turn decreases elevated arterial blood pressure[1]. The sustained antihypertensive effects of the diuretic agents, particularly the thiazides, appear related to a decrease

and translocation of body sodium and extracellular fluid, in addition to diuretic effect[2]. Adaptation of body systems to this sodium depletion results in a decrease of the peripheral vascular resistance[1], although the exact mechanism for this gradual adaptation remains a mystery. Diuretics are used alone with success in mild hypertensive states, but their great utility resides in their ability to potentiate the hypotensive activity of several other antihypertensive agents[3].

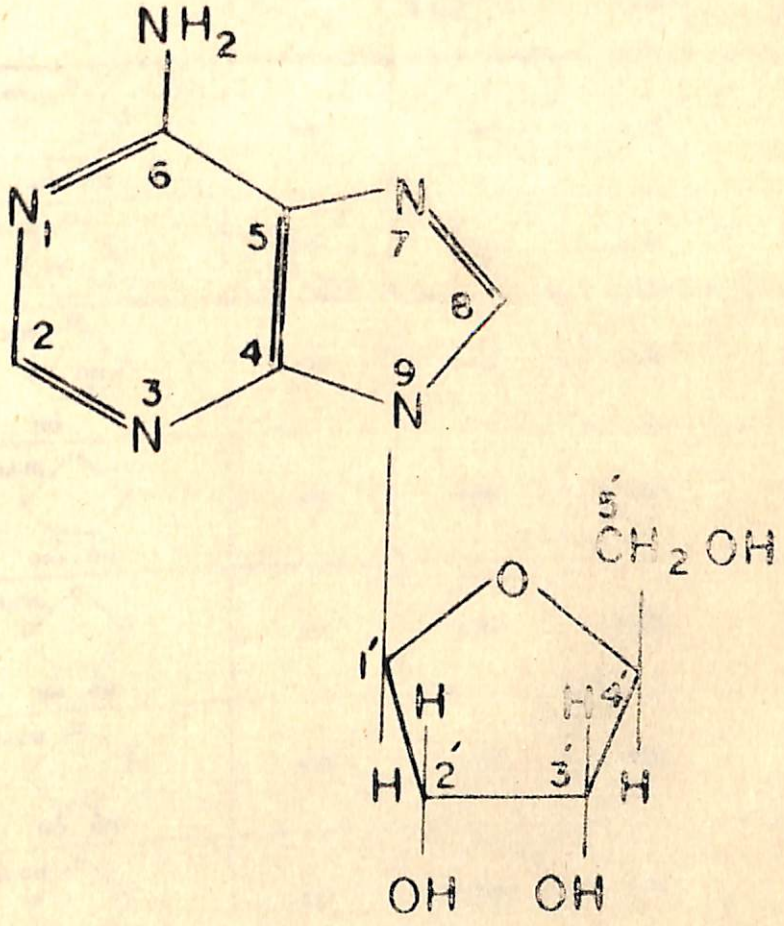
Unsaturated acylphenoxyacetic acids have been shown to act as diuretics through the inhibition of ATPase enzyme of the renal tubular membrane. The inhibition of ATPase by these acids dependsupon their ability to react with sulfhydryl group of the enzyme. This activity of a series of unsaturated acylphenoxyacetic acids has already been analyzed in relation to their electronic structure in Chapter III (sec. 3.5).

Aromatic sulfonamides exhibit their diuretic effect through the inhibition of carbonic anhydrase enzyme. Their inhibition activity has also been analyzed in Chapter III (sec. 3.4) in relation to the molecular size.

Vasodilators are agents that increase blood flow. They are divided into coronary vasodilators and peripheral

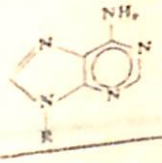
vasodilators. The first ones are used for relief of pain in angina pectoris attacks, while second ones are useful in peripheral vasoconstrictive conditions. The adenosine (I) and its analogs have been recognized for a long time as vasodilators[4] and are supposed to cause the dilation of peripheral vessels[5,6]. Adenosine itself is suggested to be a physiological mediator of vasodilation[7] but its observed effects, in all cases, are of very short duration due to the rapid metabolism of nucleoside to inosine[8] or phosphorylation to the corresponding nucleotide. A series of esters and amides of adenosine -5'-carboxylic acids were recently reported to be potent cardiovascular agents that caused a marked increase in coronary sinus oxygen tension and prolonged hypotension[9,10]. These compounds are supposed to act directly at an adenosine receptor.

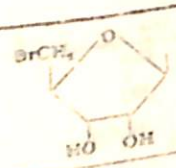
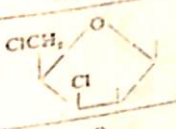
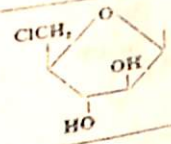
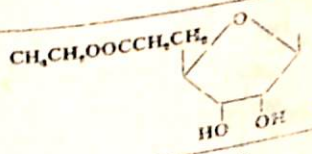
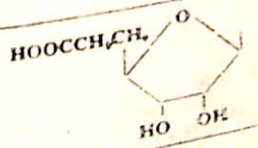
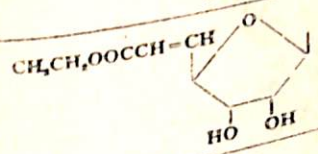
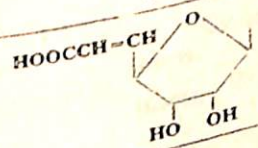
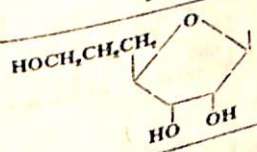
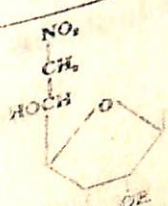
In an attempt to investigate the vasodilator activities of adenosine analogs further, Gangjee et al[11] recently synthesized a diverse series of adenosine analogs (Table 4.1) and studied their peripheral activity in the hindlimb vasculator of the dog. The structure modifications included mono and dihalo substituents in the sugar moiety, the extension of the 5'-position by one and two carbon atoms, an unsaturation in the carbon chain attached to the 5'-position, alterations in the



(I)

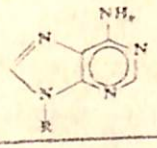
TABLE 4.1: ADENOSINE ANALOGS AND THEIR $^{1}\chi^{v}$, Vw AND VASODILATOR ACTIVITY VALUES.

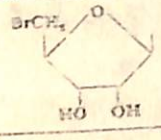
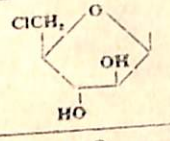
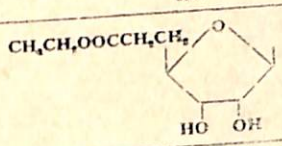
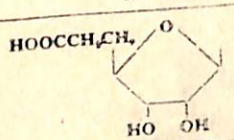
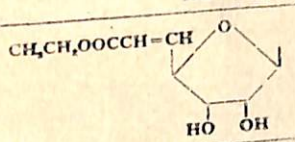
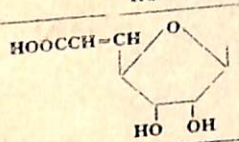
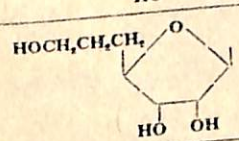
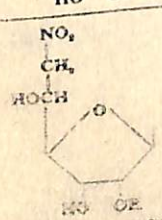


Compd.	R	$^{1}\chi^{v}$	Vw (10^2 Å^3)	Vasodil.activity[a] Obsd.[b]	Cald.Eq. (4.1.6)
I		3.80	1.145	0.360	0.093
II		3.58	1.091	0.160	0.087
III		3.25	1.147	0.008	0.025
IV		4.65	1.692	0.011	0.006
V		3.67	1.371	0.009	0.015
VI		4.35	1.650	0.008	0.006
VII		3.37	1.329	0.003	0.010
VIII		3.71	1.340	0.013	0.019
IX		3.61	1.388	0.006	0.014

contd...

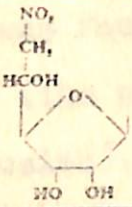
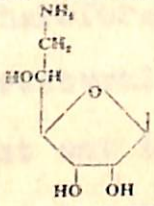
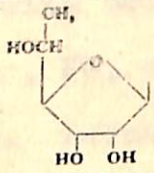
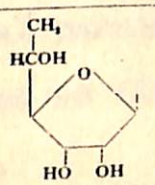
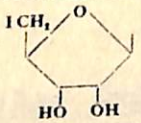
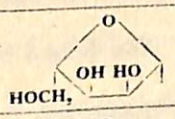
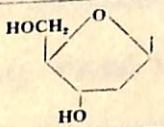
TABLE 4.1: ADENOSINE ANALOGS AND THEIR $^{1}\chi^{v}$, Vw AND VASODILATOR ACTIVITY VALUES.



Compd.	R	$^{4}\chi^{v}$	Vw (10^2 Å^3)	Vasodil. activity[a] Obsd.[b]	Vasodil. activity[a] Calcd. Eq. (4.1.6)
I		3.80	1.145	0.360	0.093
II		3.58	1.091	0.160	0.087
III		3.25	1.147	0.008	0.025
IV		4.65	1.692	0.011	0.006
V		3.67	1.371	0.009	0.015
VI		4.35	1.650	0.008	0.006
VII		3.37	1.329	0.003	0.010
VIII		3.71	1.350	0.012	0.019
IX		3.61	1.368	0.006	0.014

contd...

TABLE 4.1: CONTD.

Compd.	R	χ^v	V_w (10^2 Å^3)	Vasodil.activity[a] Obsd.[b]	Calc.Eq. (4.1.6)
X		3.61	1.368	0.027	0.014
XI		3.40	1.295	0.025	0.014
XII		3.16	1.196	0.038	0.013
XIII		3.16	1.196	0.009	0.013
XIV		4.79	1.253	0.049	0.124
XV		2.71	1.042	0.011	0.006
XVI		2.60	0.974	0.004	0.005

[a] Relative to adenosine; [b] Taken from ref. 11.

sugar moiety, and a modification in the imidazole ring system.

However, none of the compounds synthesized by these authors was found to possess the potency greater than adenosine (the observed potency in Table 4.1 is relative to adenosine). In order to have more potent drug it becomes, therefore, essential to rationalize the process of structural modifications. This can be done in the simplest way by analysing the relationship of the activity with well known physicochemical parameters like π, MR, σ, ER, σ^+ etc., or with molecular parameters like $^{\chi}$ and Vw. Because of the lack of estimate of physicochemical parameters at present for these compounds, it was proposed to find the correlation of the activity with $^{\chi}$ and Vw.

4.1.(a) Molecular Connectivity:

The molecular connectivity index, $^{\chi}$, signifies the degree of branching or connectivity in a molecule and is derived from the numerical extent of branching or connectivity in the molecular skeleton[12]. Kier et al have shown that this index can be correlated with several physicochemical and biological properties of the molecules[12]. The connectivity index has several versions. The simplest as well as extended versions in

it ($^{m}\chi$) all are calculated from a hydrogen suppressed graph of the molecule. The simplest version designated as $^{1}\chi$ and known as first-order term in χ is computed by,[12,13]

$$^{1}\chi = \sum (\delta_i \, \delta_j)^{-\frac{1}{2}} \qquad (4.1.1)$$

where the sum is over all connections or edges in the hydrogen suppressed graphs, and δ_i and δ_j, are number assigned to each atom reflecting the number of atoms adjacent or connected to atoms i and j, which are formally bonded. The nature of the atoms is not considered in the calculation.

To account for the nature and unsaturation of the bonds in χ, Hall and Kier[14,15] proposed the valence molecular connectivity (χ^{v}) where the atom connectivity term, δ^{v}, is defined as

$$\delta_i^{v} = Z_i^{v} - h_i \qquad (4.1.2)$$

in which Z_i^{v} represents the number of valence electrons of atom i, and h_i the number of hydrogen attached to it. Thus the use of δ^{v} permits the calculation of valence term of the first-order, $^{1}\chi^{v}$, by the expression:

$$^{1}\chi^{v} = \sum (\delta_i^{v} \, \delta_j^{v})^{-\frac{1}{2}} \qquad (4.1.3)$$

Using eq. (4.1.2) the δ^v values for oxygen and nitrogen in several variations of bonding can be calculated; thus for oxygen in alcohols and ethers, δ^v values are 5 and 6 respectively, and for nitrogen in primary amines and pyridine, they are 3 and 5 respectively. Since halogen atoms have an identical number of valence electrons, this description yields identical values of δ^v. It was therefore necessary to derive empirical values of δ^v for the halogens by calibrating them to a physical property. The molar refraction was chosen for this assignment[14]. The δ^v values for some heteroatoms including halogens are given in Table 4.2.

An extended term of χ of the order ${}^2\chi$ is computed from:

$$ {}^2\chi = \sum (\delta_i \delta_j \delta_k)^{-\frac{1}{2}} \qquad (4.1.4) $$

where i, j, and k are atoms bonded in sequence or in a path, and the sum is over all distinct sets of two-edge paths. In general, extended terms of χ, ${}^m\chi_p$, are computed for linear paths, p of m bonds by;

$$ {}^m\chi_p = \sum_{s=1}^{n_m} \prod_{i}^{m+1} (\delta_i)_s^{-\frac{1}{2}} \qquad (4.1.5) $$

where n_m is the number of paths with m edges, and s identifies a particular subgraph.

TABLE 4.2: VALENCE 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
$C \equiv N$	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
NH_4^+	1	Cl	0.690
$>N<^+$	6	Br	0.254
$= NH_2^+$	3	I	0.085

* Taken from reference 12.

Terms describing nonlinear arrangements of bonds such as clusters of three bonds, $^{3}\chi_{C}$, and circuits (or chains) of six atoms, $^{6}\chi_{CH}$, are computed in the same way[12,16].

In the present problem the simplest but more meaningful index $^{1}\chi^{v}$ was tried along with Vw.

4.1.(b) Regression Analysis and Discussion:

The $^{1}\chi^{v}$ and Vw values were calculated for only group substituents (R) of adenosine analogs and are listed in Table 4.1. Using these data and those for activity, the regression analyses were performed; and in all the attempts, the most significant correlation that surfaced was as given by eq. (4.1.6).

$$pA = 0.764\ {}^{1}\chi^{v} + 22.033\ \frac{1}{{}^{1}\chi^{v}} + 2.979\ Vw - 11.080$$

$$n = 16,\ r = 0.773,\ s = 0.399,\ F_{3,12} = 5.93 \qquad (4.1.6)$$

The F value in eq. (4.1.6) is significant at 95% level ($F_{3,12}(0.05) = 3.49$), and the activity values reproduced from this equation were found to be in good agreement with the observed ones. However, from the value of correlation coefficient, the correlation expressed by eq.(4.1.6) can not be said to be very significant. Nonetheless, some fruitful conclusions can be drawn from it.

As a matter of fact it does indicate that molecular size and shape play some important role in the vasodilator activity of the molecules. The linear dependence on Vw and the parabolic dependence on $^{1}\chi^{v}$ of the activity show that an increase in either of Vw and $^{1}\chi^{v}$ will lead to an increase in the pA value. Since Vw and χ both have been shown[15,17] to be linearly related with hydrophobicity constant, it would be plausible to assume that the activity should depend upon the hydrophobicity or lipid solubility of the molecules. Hence an estimate of their partition coefficient (P) or hydrophobicity constant (π) may be of great help in rationalizing the process of substituent selection more fruitfully. Presently eq. (4.1.6) can be exploited to have a rough estimate of the activity of a prospective adenosine analog before it is actually synthesized and subjected to screening. Thus, our study provides a ground to rationalize the designing of more potent vasodilators in a series of adenosine analogs.

4.2. General Anaesthetics: Halogenated Hydrocarbons:

The anaesthetic potency of halogenated hydrocarbons has been shown qualitatively to be correlated[18], among others, with boiling point; and in some systems the boiling point has been found to be fairly well correlated with

molecular connectivity (χ)[12,14,19]. In this section we have analyzed the direct correlation of anaesthetic potency with molecular connectivity, and with boiling point. The data for the boiling point were taken from the literature[18] and the connectivity index was calculated by the procedure as described in section 4.1.(a) of this Chapter.

Results and Discussions:

The connectivity index used is $^1\chi^v$, and its calculated values for 45 halogenated hydrocarbons are listed in Table 4.3. Along with them are also listed the anaesthetic potency, AD_{50} (the vapor concentration at which 50% of the mice are in the side or prone position after 30 minutes exposure to the anaesthetic), and boiling point (B.P.) of the compounds. By regression analysis the $\log_e AD_{50}$ is found to be correlated with $^1\chi^v$ as:

$$\log_e AD_{50} = -1.670\ ^1\chi^v + 3.259$$
$$n = 45,\ r = 0.753,\ s = 0.809,\ F_{1,43} = 56.35 \qquad (4.2.1)$$

and with boiling point as:

$$\log_e AD_{50} = -0.035\ B.P. + 2.337$$
$$n = 45,\ r = 0.892,\ s = 0.555,\ F_{1,43} = 168.32 \qquad (4.2.2)$$

while the boiling point itself is found to be correlated

TABLE 4.3: MOLECULAR CONNECTIVITY ($^1\chi^v$), BOILING POINT AND OBSERVED AND CALCULATED ANAESTHETIC POTENCIES OF HALOGENATED HYDROCARBONS.

S. No.	Compound	$^1\chi^v$	B.P.[a] (°C)	$\log_e AD_{50}$		
				Obsd[a]	Cald eq. (4.2.1)	Cald eq. (4.2.2)
1	CH_2Cl_2	1.703	40	0.49	0.42	0.93
2	$CHFCl_2$	1.261	9	1.12	1.15	2.02
3	CHFClBr	1.712	38	0.18	0.40	1.00
4	CHF_2CH_2Cl	1.000	36	0.77	1.59	1.07
5	CHF_2CH_2Br	1.553	57	0.26	0.67	0.34
6	CHFClCHFCl	1.465	60	-0.60	0.81	0.23
7	CHF_2Cl	0.437	-41	3.02	2.53	3.77
8	$CHF_2CF_2CH_2Cl$	1.012	54	0.18	1.57	0.44
9	$CHF_2CF_2CH_2Br$	1.563	74	-0.63	0.65	-0.26
10	$CHF_2CF_2CH_2ClBr$	1.936	99	-1.66	0.03	-1.13
11	CF_2ClCH_2Cl	1.583	47	0.25	0.62	0.69
12	CF_2ClCH_2Br	2.135	68	-0.22	-0.31	-0.05
13	CF_2BrCH_2Cl	1.973	71	-0.29	-0.04	-0.15
14	$CF_2ClCHFCl$	1.233	30	1.10	1.20	1.29
15	CF_3CHCl_2	1.343	24	0.87	1.02	1.50
16	$CF_2ClCHFBr$	1.683	52	0.18	0.45	0.51
17	$CF_2BrCHFCl$	1.623	53	0.11	0.55	0.48
18	$CF_3CHBrCl$	1.794	52	-0.16	0.26	0.51
19	$CF_2BrCHFBr$	2.074	76	-0.43	-0.20	-0.33
20	CF_3CHBr_2	2.244	73	-0.63	-0.49	-0.22

contd...

with $^{1}\chi^{v}$ as:

B.P. = 45.716 $^{1}\chi^{v}$ - 23.275

n = 45, r = 0.810, s = 18.368, $F_{1,43}$ = 81.98 (4.2.3)

Eqs. (4.2.1 - 4.2.3), show fairly good correlations between the variables, and in all of them F value is significant at 99% level ($F_{1,43}(0.01)$ = 7.25).

However from the practical point of view, the correlation between the activity and connectivity is little less satisfactory ($\log_e AD_{50}$ values calculated from eq. (4.2.2) are in better agreement with the observed ones than those calculated from eq. (4.2.1); Table 4.3) and our attempt to improve this correlation by multiple regression analysis using square and inverse of the $^{1}\chi^{v}$ values has been unsuccessful. Further there was no basis to assume that it will be improved by the use of higher terms in χ. As a matter of fact χ has a very limited scope in SAR studies[20,21].

4.3. Insecticides: Dinitrophenols and Alkylthiocyanates:

Dinitrophenols[22,23] and alkylthiocyanates[22,24] have been found to be useful as insecticides. The dinitrophenols exert their activity by inhibiting the oxidative phosphorylation in muscle tissues of insects. Likewise thiocyanates have rapid paralytic action to

insects. The activities of dinitrophenols and thiocyanates both vary as the alkyl chain length varies. (Tables 4.4 and 4.5). However to provide an effective rationalization to drug-design, the quantitative picture of structure-activity relationship is essential. The change in alkyl chain length can be accounted for by the change in the van der Waals volume (V_w) of the molecule. Consideration of V_w, rather than the chain length or the number of carbon atoms as such, is essential as to account for the effect of a cyclic substituent in place of a normal chain with same number of carbon atoms.

V_w values for substituents in dinitrophenol and thiocyanate are calculated and listed in respective Tables. The plots of log LD_{50} of dinitrophenols and log LC_{50} of thiocyanates versus V_w are shown respectively in Figs. 4.1 and 4.2. Almost a straight line is observed upto the first 5 compounds in Fig. 4.1; beyond that, the nature of the trace changes and becomes parabolic. Consequently, the linear correlation (eq. 4.3.1) obtained by the regression analysis is found to be less significant than the parabolic one (eq. 4.3.2):

$$\log LD_{50} = 1.741 - 0.914\ V_w$$
$$n = 10,\ r = 0.836,\ s = 0.204,\ F_{1,8} = 18.55 \qquad (4.3.1)$$

TABLE 4.4: Vw AND TOXICITY* OF DINITROPHENOLS(2,4-)

Compd. No.	Substituent in ring (6 position)	Vw (10^2 Å^3)	log LD_{50} (µg/g)	
			Obsd.	Cald. eq. (4.3.2)
1	CH_3	0.245	1.690	1.768
2	C_2H_5	0.399	1.462	1.428
3	C_3H_7	0.553	1.255	1.158
4	C_4H_9	0.707	0.954	0.959
5	cyclo-C_5H_9	0.757	0.954	0.910
6	C_5H_{11}	0.861	0.903	0.832
7	cyclo-C_6H_{11}	0.911	0.845	0.805
8	C_6H_{13}	0.973	0.602	0.783
9	C_7H_{15}	1.127	0.602	0.778
10	C_8H_{17}	1.281	1.000	0.843

* To 5th instar silk worm Bombyx mori larva[22,23].

TABLE 4.5: Vw AND TOXICITY* OF ALKYL THIOCYANATES.

Compd. No.	Compound	Vw (10^2 Å^3)	log LC_{50}(ppm)	
			Obsd.	Cald.eq. (4.3.4)
1	$C_6H_{13}SCN$	1.395	2.921	2.897
2	$C_8H_{17}SCN$	1.703	2.602	2.658
3	$C_{10}H_{21}SCN$	2.011	2.553	2.526
4	$C_{12}H_{25}SCN$	2.319	2.522	2.501
5	$C_{14}H_{29}SCN$	2.627	2.568	2.582
6	$C_{16}H_{33}SCN$	2.934	2.769	2.769

* To green chrysanthemum aphid[22,24].

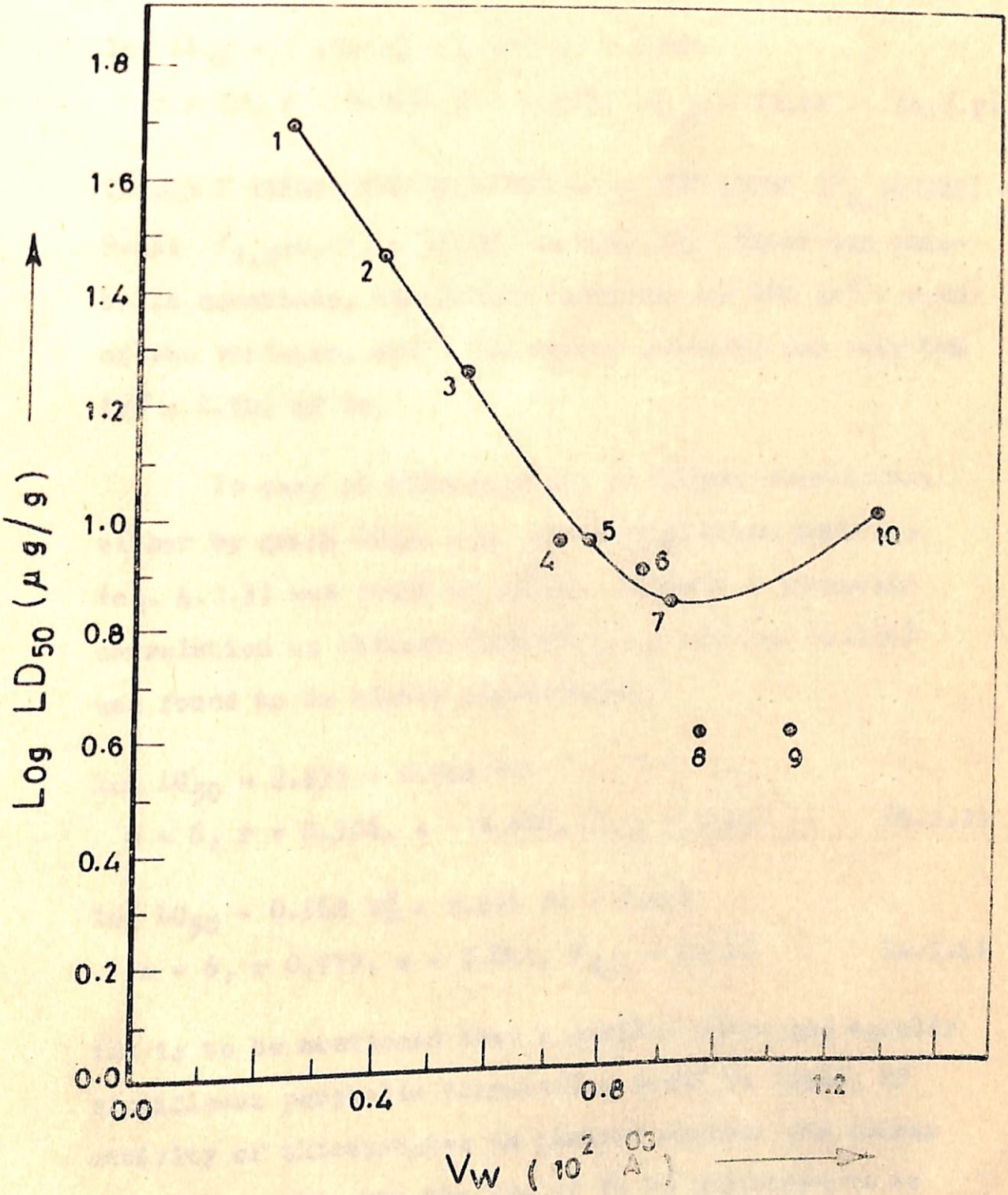


Fig. 4.1. A plot of log LD_{50} of dinitrophenols versus Vw. Numbers refer to compounds in Table 4.4.

$$\log LD_{50} = 1.494\ V_w^2 - 3.173\ Vw + 2.456$$
$$n = 10,\ r = 0.947,\ s = 0.127,\ F_{2,7} = 30.43 \qquad (4.3.2)$$

Though F values are significant at 99% level ($F_{2,7}(0.01)$ = 9.55; $F_{1,8}(0.01)$ = 11.26) in both the linear and parabolic equations, the latter accounts for 90% (r^2 = 0.90) of the variance, while the former accounts for only 70% (r^2 = 0.70) of it.

In case of thiocyanates, no linear correlation either by graph (Fig. 4.2) or by regression analysis (eq. 4.3.3) was found to exist. However a parabolic correlation as obvious from Fig. 4.2 and eq. (4.3.4) was found to be highly significant.

$$\log LC_{50} = 2.835 - 0.083\ Vw$$
$$n = 6,\ r = 0.306,\ s = 0.166,\ F_{1,4} = 0.41 \qquad (4.3.3)$$

$$\log LC_{50} = 0.562\ V_w^2 - 2.516\ Vw + 5.313$$
$$n = 6,\ r\ 0.979,\ s = 0.041,\ F_{2,3} = 34.52 \qquad (4.3.4)$$

(It is to be mentioned that a similar curve and equally significant parabolic correlation would be found, if activity of thiocyanates is plotted against the number of carbon atoms, but the use of Vw is preferred as to make the quantitative correlation to be useful for predicting the activity of the future compounds which might have some other types of the substituents).

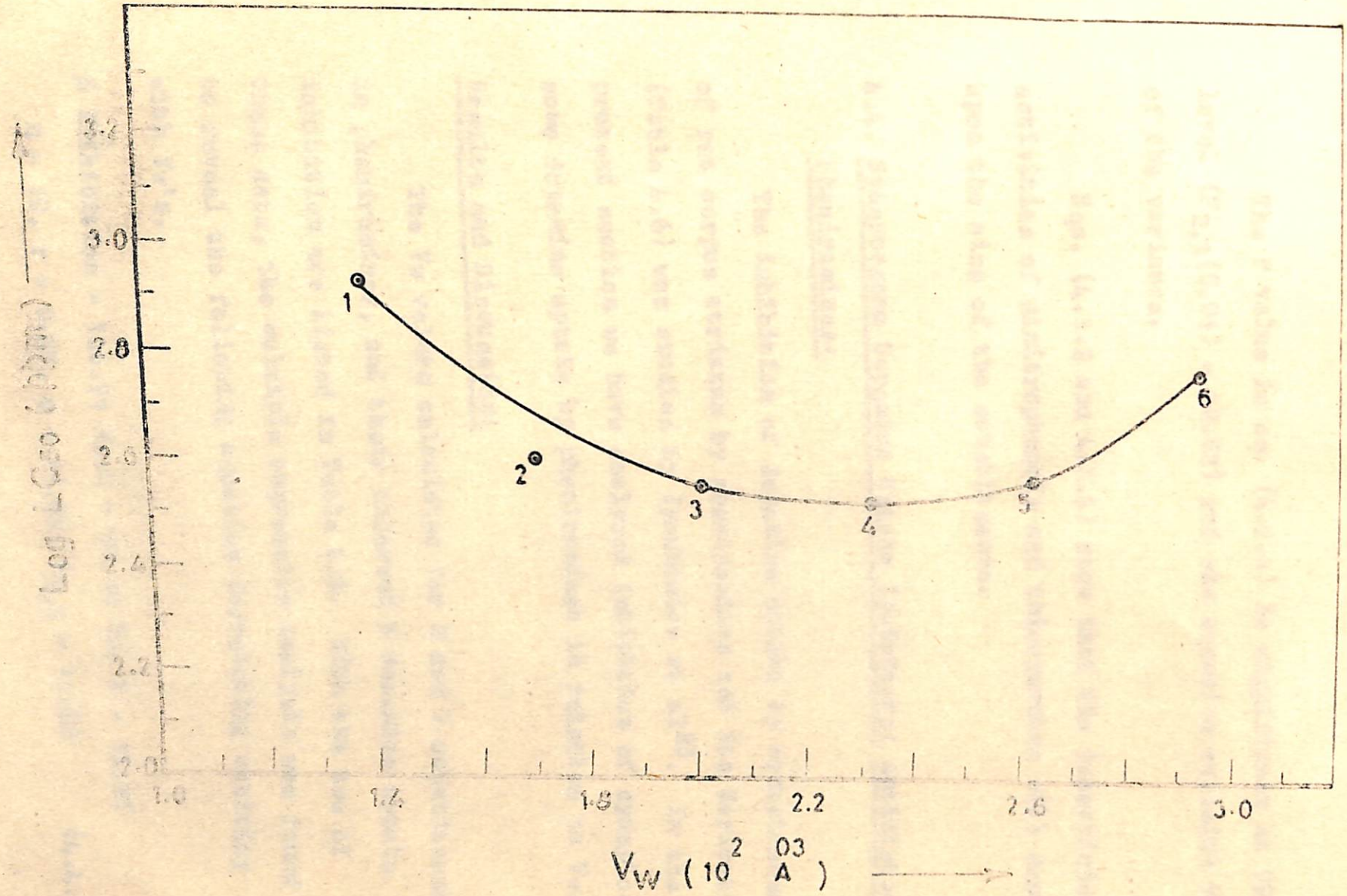


Fig. 4.2. A plot of log LC_{50} of alkylthiocyanates versus Vw. Numbers refer to compounds in Table 4.5.

The F value in eq. (4.3.4) is significant at 99% level ($F_{2,3}(0.01) = 30.82$) and the equation explains 96% of the variance.

Eqs. (4.3.2 and 4.3.4) show that the insecticidal activities of dinitrophenols and thiocyanates will depend upon the size of the substituents.

4.4. Synaptosome Dopamine Uptake Inhibitors: Antihistaminic Pheniramines:

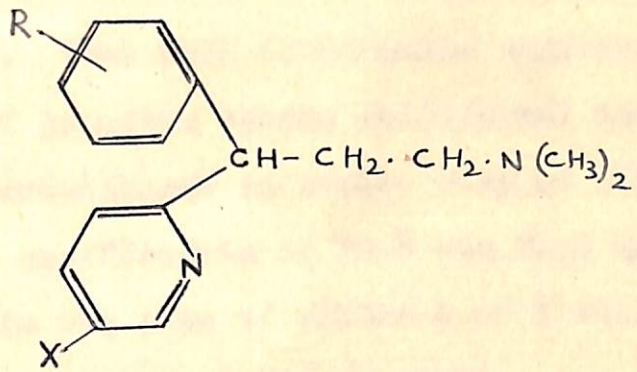
The inhibition of dopamine uptake by synaptosomes of rat corpus striatum by pheniramines and its derivatives (Table 4.6) was studied by Symchowicz et al[25]. In the present section we have analyzed inhibition of synaptosome dopamine uptake by pheniramines in relation to Vw.

Results and Discussion:

The Vw values calculated for R and X substituents in pheniramines, and their observed % dopamine uptake inhibition are listed in Table 4.6. With the use of these data, the multiple regression analysis was found to reveal the following equation correlating activity with Vw's.

$$\% \text{ Inihibition} = 146.91\ Vw.R + 86.10\ Vw.X - 13.95$$
$$n = 10,\ r = 0.87,\ s = 11.65,\ F_{2,7} = 11.08 \qquad (4.4.1)$$

TABLE 4.6: % INHIBITION OF SYNAPTOSOME DOPAMINE UPTAKE BY PHENIRAMINES AND Vw VALUES OF PHENIRAMINES.



	R	X	Vw (10^2 Å^3) R	Vw (10^2 Å^3) X	% Inhibition[a]
1	H	H	0.056	0.056	5.3 ± 6.7
2	4-F	H	0.115	0.056	11.6 ± 1.2
3	4-NO_2	H	0.265	0.056	12.0 ± 2.6
4	4-NH_2	H	0.177	0.056	26.3 ± 1.3
5	4-Br	H	0.287	0.056	31.4 ± 1.1
6	4-Cl	H	0.244	0.056	33.8 ± 0.9
7	3,4-diCl	H	0.488	0.056	73.4 ± 1.1
8	4-Cl	Br	0.244	0.287	47.1 ± 1.6
9	3-Cl	H	0.244	0.056	8.8 ± 4.7
10	H	Br	0.056	0.287	18.5 ± 2.8

[a] At drug concentration of 1 x 10^{-6}M.

In this equation F value is significant at 99% level ($F_{2,7}(0.01) = 9.55$) and the correlation coefficient accounts for about 76% ($r^2 \sim 0.76$) of the variance in the activity. Thus this correlation suggests that inhibition of dopamine uptake will depend upon the size of the substituent in either ring of pheniramines. The positive coefficients of Vw.R and Vw.X show that an increase in the size of either R or X will lead to an increased activity of the molecule.

Kumbar and Siva Sankar[26] had found this activity to be related with E_{HOMO}, but the number of data points used by them was too small to draw any meaningful conclusion from their correlation equation.

The correlation of inhibition activity with Vw leads to suggest that drug-receptor interaction may be of van der Waals type (cf. Appendix A).

REFERENCES:

1. L. Tobian, Ann. Rev. Pharmacol., 7, 399 (1967).

2. M. Nickerson and J. Ruedy in 'Pharmacological basis of Therapeutics', L.S. Goodman and A.Gilman, eds., (MacMillan, New York), 1975, p. 712.

3. A.D. Bender, in 'Topics in Medicinal Chemistry', Vol. 1, J.L. Rabinowitz and R.M. Myerson eds., (Interscience Publishers, New York), 1967, p.177.

4. A.N. Drury, A. Szent-Györgi, J. Physiol. (London), 68, 213 (1929).

5. A. Fleish, I. Sibul, Arch. Physiol. 231, 787 (1933).

6. B. Issekutz, Jr., Arch. Exp. Pathol. Pharmakol., 199, 233 (1942).

7. R.M. Berne, Am. J. Physiol., 204, 317 (1963).

8. H.P. Baer, G.I. Drummond, E.L. Duncan, Mol. Pharmacol., 2, 67 (1966).

9. H.H. Stein, J. Med. Chem., 16, 1306 (1973).

10. H.H. Stein, P. Somani and R.N. Prasad, Ann. N.Y. Acad. Sci., 255, 380 (1975).

11. A. Ganjee, H.P.C. Hogencamp and J.M. Kitzen, J. Pharm. Sci., 67, 121 (1978).

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

13. M. Randić, J. Am. Chem. Soc., 97, 6609 (1975).

14. L.B. Kier, L.H. Hall, J. Pharm. Sci., 65, 1806 (1976).

15. W.J. Murray, L.H. Hall and L.B. Kier, J. Pharm. Sci., 64, 1978 (1975).

16. L.B. Kier, W.J. Murray, M. Randić and L.H. Hall, J. Pharm. Sci., 65, 1226 (1976).

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

18. R.H. Davies, R.D. Bagnall and W.G.M. Jones, Int. J. Quant. Chem. Quantum Biology Symp. No. 1, 201 (1974).

19. L.H. Hall, L.B. Kier and W.J. Murray, J. Pharm. Sci., 64, 1974 (1975).

20. S.P. Gupta and P. Singh, Indian J. Chem., 16(B), 709 (1978).

21. Y.C. Martin in 'Drug Design', Vol. 8, E.J. Ariëns ed., (Academic Press, New York), 1979, p. 1.

22. R.L. Metcalf in 'Pesticides in Environment - Part 1', Vol. 1, R. White-Stevens, ed., (Marcell Dekker, New York), 1971, p. 1.

23. J. Kagy, J. Econ. Entomol., 34, 660 (1941).

24. R.L. Metcalf, Organic Insecticides (Wiley, Interscience, New York), 1955.

25. S. Symchowicz, C.A. Korduba and J. Veals, Life Sci., 10, 35 (1971).

26. M. Kumbar and D.V. Siva Sankar, Res. Comm. Chem. Pathol. Pharmacol., 4, 707 (1972).

Appendix A

The intermolecular interactions of van der Waals type deal with a situation when interacting particles are separated by a distance to exclude overlap of electronic orbitals[1]. The total intermolecular interaction energy between the two molecules or atoms of dipole moments μ_1 and μ_2, ionization potentials I_1 and I_2, polarizabilitien α_1 and α_2, placed at a distance of r in a medium of refractive index η and dielectric constant K is given by[1-3]

$$E = -\frac{1}{r^6}\left[\frac{1}{(4\pi\epsilon_0 K)^2}\cdot\frac{2\mu_1^2\mu_2^2}{3KT} + \frac{\alpha_1\mu_2^2 + \alpha_2\mu_1^2}{(4\pi\epsilon_0 K)^2} + \frac{3}{2}\cdot\frac{1}{(4\pi\epsilon_0)^2}\cdot\frac{1}{\eta^4}\,\frac{(I_1 I_2)(\alpha_1\alpha_2)}{(I_1 + I_2)}\right] \qquad (1)$$

where ϵ_0 is the permitivity of free space, K, the Boltzmann constant and T, the absolute temperature. This relation is valid for $KT \gg (\mu_1\mu_2/4\pi\epsilon_0 K r^3)$ and is true for polar as well as non-polar molecules. For the non-polar molecules, the first two terms, in fact, vanish. The first term represents the dipole-dipole interaction; the second one, the dipole - induced dipole interaction; and the third one, an instantaneous dipole - induced

dipole interaction. The first term decreases as the temperature increases and the second one is independent of the temperature.

In a biological system where water is the medium, the first and second terms become much smaller than the third one[2] due to the high value of the dielectric constant of water ($K \sim 80$). Therefore, for all practical purposes the third term dominates in biological system in aqueous medium whether the interacting molecules are polar or non-polar. And in this term the energy is determined by the polarizability of the molecules. Since the polarizability is the function of the volume of the molecule as shown by eq. (2), the total energy due to van der Waals type of interaction becomes the function of the molecular size, whether the first and second terms are affective or not.

$$\alpha = 3\epsilon_0 (4/3)\pi R^3 = 3\epsilon_0 V \qquad (2)$$

Hence if drug activity is found to depend upon the molecular size, van der Waals type of interaction can be expected between the drug and its receptor.

REFERENCES:

1. D. Langbein, in 'Theory of van der Waals Attraction : Springer Tracts in Modern Physics' Vol.72, G. Hohler ed., (Springer Verlag, Berlin), 1974.

2. R.B. Setlow and E.G. Pollard, Molecular Biophysics (Addison - Wesley Publishing Co., Reading, Mass), 1974.

3. M. Karplus and G. Porter, Atomic and Molecular Physics (Addison - Wesley Publishing Co., Reading Mass), 1970.

Appendix B

1. Simple Linear Regression:

The simple linear regression of a dependent variable y on a single independent variable x reveals an equation of the form:

$$Y = a + bx \tag{1}$$

Eq.(1) represents a straight line and any value of Y, Y_i, corresponding to any value of x, x_i, will be on this straight line, but the actual experimental value of y, y_i, may be different from Y_i. Therefore if we assume E_i to be the deviation of y_i from Y_i, we can write,

$$\begin{aligned} y_i &= Y_i + E_i \\ &= a + bx_i + E_i \end{aligned} \tag{2}$$

Now using the least square method, the values of a and b are so determined that the sum of squares of deviations of the observed values from the straight line (eq.1) is a minimum, i.e.,

$$E_i^2 = (y_i - a - bx_i)^2 = \text{min.} \tag{3}$$

Therefore, by differentiating eq. (3) partially with respect to a and b separately and equating each derivative

to zero we find

$$b = \frac{\sum x_i y_i - \frac{\sum x_i \sum y_i}{n}}{\sum x_i^2 - \frac{(\sum x_i)^2}{n}}$$

$$= \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sum (x_i - \bar{x})^2} \qquad (4)$$

and $a = \bar{y} - b\bar{x}$ (5)

where $\bar{x}$ and $\bar{y}$ are the averages of x's and y's respectively and the summation extends in each case over the entire data points n.

The deviation of the actual observation y_i from the regression line can be expressed as the difference between two deviations : (1) the deviation of the observation y_i from the total average $\bar{y}$, and (2) the deviation of the corresponding point Y_i on the regression line from the total average $\bar{y}$. Thus we have:

$$y_i - Y_i = (y_i - \bar{y}) - (Y_i - \bar{y}) \qquad (6)$$

By squaring and summing we get:

$$(y_i - Y_i)^2 = \sum (y_i - \bar{y})^2 - \sum (Y_i - \bar{y})^2 \qquad (7)$$

That means the sum of squares of y's about regression or the sum of squares of residual error (SSerror) will be

the difference between the sum of squares of y's about mean (SSmean) and the sum of squares due to the regression (SSreg). We can therefore write eq. (7) as:

$$SSerror = SSmean - SSreg \tag{8}$$

The partitioning of corresponding degrees of freedom gives

$$n - 2 = (n - 1) - 1 \tag{9}$$

The standard deviation or standard error about the fitted regression line (s) is then defined as:

$$s = \sqrt{SSerror/\ (n - 2)} \tag{10}$$

2. Significance of the Obtained Relationship:

From eq. (9), we have

$$SSmean = SSreg + SSerror \tag{11}$$

Clearly, as data fit a line better and better, the SSerror becomes smaller and smaller. The r^2 statistic is designed to take advantage of this property; it is defined as:

$$r^2 = \frac{SSreg}{SSmean} = \frac{SSmean - SSerror}{SSmean} \tag{12}$$

Thus as SSerror becomes smaller, r^2 approaches 1.0. It can be seen that r^2 is also the fraction of the total

variance in the data which is explained by the regression. The r is known as correlation coefficient and can assume values between zero and 1.0. r or r^2 is a very important characteristic of a statistical fit. It can be calculated more easily by the equation

$$r^2 = \frac{b\sum(x_i - \bar{x})\,(y_i - \bar{y})}{\sum(y_i - y)^2} \qquad (13)$$

which is derived from eq. (12).

Another statistical parameter to assess the significance of the correlation equation is the F-ratio (F) which is defined in general as:

$$F_{k,n-1-k} = \frac{\text{Mean square among groups } (MS_B)}{\text{Mean square within groups } (MS_W)}$$

$$= \frac{SSreg/k}{SSerror/(n-1-k)} \qquad (14)$$

Where k is the number of independent variables. Eq.(14) also shows that as SSerror becomes smaller and smaller, the F becomes larger and larger. The F-values for various degrees of freedom and at different levels of significance (α) are tabulated[1]. The tabulated F-values enable one to find out if a particular relationship is acceptable or not. If a calculated F-value for a given

relationship exceeds the corresponding tabulated value at a particular level of significance, say 0.01, it signifies that the probability of the relationship being false is only 0.01, which means that the relationship is significant at $[(1-0.01) \times 100]\%$ i.e. 99% level of significance. From eqs. (12) and (14), the F and r are found to be mutually related as:

$$F_{k,n-1-k} = \frac{n-1-k}{k} \frac{r^2}{1-r^2} \qquad (15)$$

The calculation of Student's t value provides a measure to the significance of the calculated regression parameter b. It is defined as:

$$t = \frac{b\left[\sum(x_i - \bar{x})^2\right]^{1/2}}{s} \qquad (16)$$

t-values are also tabulated for different degrees of freedom (n-1-k) and at different confidence levels(α)[1]. If the calculated t-value is greater than the corresponding tabulated oneat required confidence level and degrees of freedom, the slope of the line is said to be significantly different from zero. The standard error of b, s(b), is estimated by:

$$s(b) = \frac{s}{\left[\sum(x_i - \bar{x})^2\right]^{1/2}} \qquad (17)$$

Similarly the standard error of the intercept a, s(a), can also be calculated using the equation:

$$s(a) = s\left[\frac{1}{n} + \frac{\bar{x}^2}{\sum(x_i - \bar{x})^2}\right]^{1/2} \qquad (18)$$

For further assessment of significance of the values of b and a, confidence intervals are calculated. A $100(1-\alpha)\%$ confidence interval for b is,

$$b \pm s(b)\, t_{\alpha/2,n-2} \qquad (19)$$

and for a is,

$$a \pm s(a)\, t_{\alpha/2,n-2} \qquad (20)$$

The tabulated t-values are used for their calculation. Within these confidence intervals are supposed to fall the values of parameters in $100(1-\alpha)$ cases out of 100 samples of equal size. Hence, smaller is the confidence interval, more significant is the value of parameter.

The confidence interval of a predicted value of y, y_i, is often of interest and is calculated by:

$$y_i \pm t_{\alpha/2,n-2}\left[1 + \frac{1}{n} + \frac{\sum(x_i - \bar{x})^2}{SSmean}\right]^{1/2} s \qquad (21)$$

Finally, s, the standard error about the fitted regression line measures how precisely the equation obtained estimates

individual values. Smaller is the value of s, better is the correlation.

3. Multiple Regression:

A multiple regression analysis is performed, when the dependent variable is the function of several independent variables. In this case the linear equation obtained may be of the form

$$Y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + \text{------} b_k x_k \qquad (22)$$

where x_1 , x_2 , x_3 , etc. are different independent variables.

Now by applying the principle of least square and taking partial derivatives with respect to b's and setting them equal to zero, we get the so called reduced normal equations of the form:

$$b_1 X_{11} + b_2 X_{12} + b_3 X_{13} + \text{-------} b_k X_{1k} = X_{1y} \qquad (23.1)$$

$$b_1 X_{21} + b_2 X_{22} + b_3 X_{23} + \text{-------} b_k X_{2k} = X_{2y} \qquad (23.2)$$

$$b_1 X_{k1} + b_2 X_{k2} + b_3 X_{k3} + \text{-------} b_k X_{kk} = X_{ky} \qquad (23.k)$$

where (24)

$$X_{uv} = \sum_i (x_{ui} - \bar{x}_u) (x_{vi} - \bar{x}_v)$$

$$X_{uy} = \sum_{i} (x_{ui} - \bar{x}_u)(y_i - \bar{y}) \qquad (25)$$

Now by solving these equations, b's can be found and then a can be estimated by the equation:

$$a = \bar{y} - \sum_{u=1}^{k} b_u x_u \qquad (26)$$

which is also obtained by applying the least square method and taking the partial derivative with respect to a.

In order to solve the normal equations, the simplest way is to find first the inverse of matrix X. If C be the X^{-1}, b's can be estimated by:

$$b_i = C_{i1} X_{1y} + C_{i2} X_{2y} + \text{-----} C_{ik} X_{ky} \qquad (27)$$

where C_{ij} are elements of matrix C. Now the various statistical parameters that assess the significance of the correlation are obtained by the equations:

Standard error about regression, $s = \left(\frac{SSerror}{n-1-k}\right)^{1/2}$ (28)

Standard error of b_i, $s(b_i) = s\sqrt{C_{ii}}$ (29)

$$t \text{ for } b_i = \frac{|b_i|}{s(b_i)} \qquad (30)$$

$100(1-\alpha)\%$ confidence interval for b_i, $= b_i \pm s(b_i)\, t_{\alpha/2,n-1-k}$ (31)

100(1-α)% confidence interval for $Y_i = Y_i \pm t_{\alpha/2,n-1-k}$

$$\left[1 + \frac{1}{n} + \frac{\sum(\hat{x}_i - \hat{\bar{x}})^2}{SSmean}\right]^{1/2} \qquad (32)$$

$$r^2 = \frac{b_1 X_{1y} + b_2 X_{2y} + b_3 X_{3y} + \text{-----} b_k X_{ky}}{\sum(y_i - \bar{y})^2} \qquad (33)$$

The F can be calculated by using eq. (15). In eq.(32), $\hat{x}_i$, is the vector of physical properties of observation i and $\hat{\bar{x}}$ is the vector of the means of physical properties in the sample.

4. Non-linear Regression:

When observations (x_i, y_i) are plotted, they often fall on a curved line, and theory may call for a non-linear regression. The most commonly occuring situations are:

(1) to fit a specific higher order polynomial

(2) to transform a non-linear equation into a linear one

4.1. Fitting of a Specific High Order Polynomial:

Suppose theory calls for a cubic equation like,

$$y = a + b_1 x + b_2 x^2 + b_3 x^3 \qquad (34)$$

In such a situation, we let $x_1 = x$, $x_2 = x^2$, and $x_3 = x^3$. and then perform regression analysis as discussed in

section 3. The equation obtained would be of the form:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 \qquad (35)$$

which would be equivalent to eq. (35), and the values of all the statistical parameters obtained for eq.(35) would be applicable to eq.(34).

4.2. Transformation of Non-linear Equations into Linear Ones:

If we find that the data would fit adequately to the equation of the type:

$$e^y = a\, x^b \qquad (36)$$

we can simplify the regression by converting it to the equivalent linear equation as:

$$\begin{aligned} y &= \log_e a + b \log_e x \\ &= a' + b \log_e x \end{aligned} \qquad (37)$$

and then by letting $x' = \log_e x$, a' and b can be found as discussed in section 1.

REFERENCES:

1. See for example, H. Grim in 'Biostatistics in Pharmacology', Vol.2, A.L. Delaunois, Ed., (Pergamon Press, Oxford), 1973, p. 771.

In eq. (2.2.1), the value of r was not reported. However, on the basis of these equations, Kumbar and Siva Sankar postulated that some kind of strain in molecules was responsible for these two activities. Among the various factors responsible for this strain in the molecules, one may be the size of the substituents. It was therefore proposed to make a study on the correlation of the hallucinogenic and antiserotonin activities of LSD analogs with van der Waals volume, Vw.

2.2.(a) Calculation of Vw:

The Vw 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. It has been recently shown to be related with hydrophobic behaviour of drug molecules[28] and consequently with various biological activities[29].

To find Vw of molecules, spherical shapes are assumed for all atoms according to Bondi[30] 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