# Computer-assisted investigation of structure–activity relationships in serotonin-uptake blockers

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*Starting from 15 known serotonin-uptake blockers, a family of seven have been selected for a study of their structural features aimed at identifying a 'minimum requirement' for such activity. It was found that all of these molecules could adopt low-energy conformers, where an aliphatic nitrogen was at a fixed position in space relative to an aromatic moiety (4.9–5.2 Å away and roughly in the same plane). The apparent order of activity could be rationalized within this framework. In one case (Indalpine), these studies have led to a suggestion that the mode of action of this molecule differs from that of the other members of the family despite biochemical similarities.*

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The role of the neurotransmitter serotonin (5HT) in a variety of physiological and pharmacological actions has long been recognized. Two major receptor systems have been identified which are affected by 5HT. These have been classified as $5HT_1$ and $5HT_2$. Besides these receptor systems, there exist mechanisms for the reuptake of 5HT into the presynaptic neuron after it has fired. These may be governed by receptor subtypes which are different again. This study is aimed at discovering the structural similarities that exist between a group of compounds which inhibit this reuptake of 5HT in a similar manner.

Agents that affect the level of this neurotransmitter have shown a variety of pharmacological activities[1–3]. This may be taken as an indication of the great importance of this neurotransmitter in man. For example, the tricyclic antidepressants such as clomipramine (compound (1)) (see Figure 1) have been shown to inhibit the reuptake of 5HT once it has been released into the synaptic cleft. Other compounds for which similar claims have been made include quipazine (compound (2))[4], diclofensine (RO-8-4650) (compound (3))[4], Zimelidine (compound (4))[5], Femoxetine (compound (5))[6], Fluoxetine (compound (6))[7] and fluvoxamine (compound (7))[7]. Unfortunately, most of these compounds do not display a great deal of selectivity for 5HT receptors as opposed to receptors for other neurotransmitters such as dopamine and epinephrine. Even those which do display some selectivity for 5HT receptors are not highly specific for one or other type of 5HT receptor (such as $5HT_1$ or $5HT_2$, etc.).

In recent years, a role for serotonin in obesity has been suggested. It has been hypothesized that cerebral 5HT levels have a marked influence on the desire to consume carbohydrates, high levels being indirectly associated with a reduced 'carbohydrate urge'[8,9].

These roles of 5HT have provided an impetus to the search for molecules which would selectively affect 5HT without interfering with other neurotransmitters directly, and which specifically inhibit the reuptake of 5HT rather than affecting other 5HT receptor systems.

In principle, there are several possible pathways by which an active transport mechanism such as the reuptake of a neurotransmitter may be blocked. These may include such events as allosteric control or competitive, noncompetitive or uncompetitive inhibition of an enzyme. Though a detailed kinetic study of the purified enzyme system would shed light on the similarities or differences in the modes of action at the molecular level of different compounds on such a system, this is often impossible for practical reasons. An accepted method of classifying compounds in such instances is to group together those compounds with parallel dose–response curves[10]. However, while it is well known that nonparallel dose–response curves indicate differences in the mechanism of action, parallel curves do not necessarily imply similarity of mechanism.

The present paper describes the study of the structural similarities of several known 5HT-uptake blockers. The results provide a good example of how a combination of computer graphics and biochemical pharmacology may provide a more powerful tool than dose–response curves alone for the classification of compounds with similar modes of action at the molecular level when detailed kinetic data is not available.

## MATERIALS AND METHODS

### Materials

Male Sprague-Dawley rats weighing 190–200 g (Canadian Breeding Laboratories, St Constant, Quebec, Canada) were used. [$^3$H] hydroxytryptamine binoxalate ([$^3$H]5HT, 15–30 Ci mmol$^{-1}$) was purchased from New England Nuclear. Clomipramine hydrochloride was a gift from Ciba Pharmaceutical Company. Quipazine maleate, 8-hydroxy-(compound (8)) (see Figure 1) and 8-methoxy-DPAT (compound (9)) were synthesized in

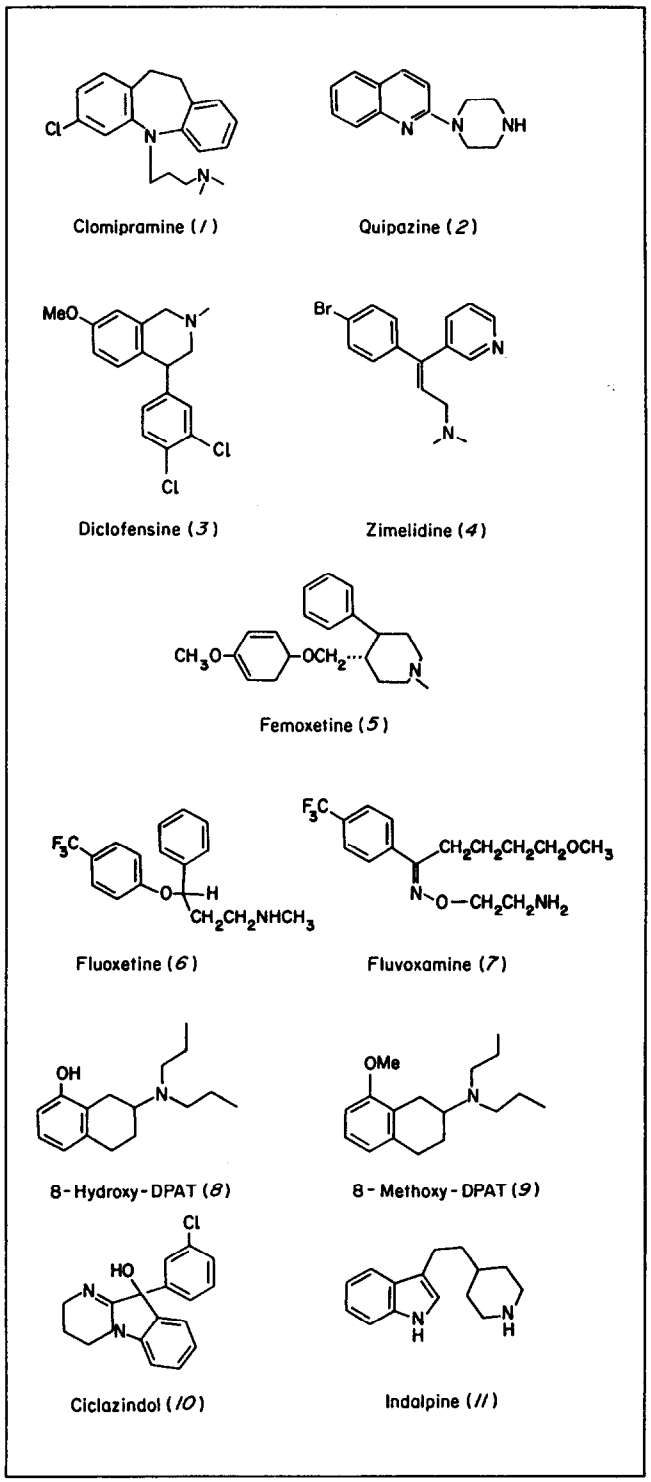


*Figure 1. Compounds affecting the level of serotonin*

our laboratories according to published procedures. Zimelidine and Femoxetine were gifts from Astra, Lakemedel-Sodertalje, Sweden, and Ferrosan, Copenhagen, Denmark, respectively. Indalpine was a gift from Pharmuka, Villeneuve la Garenne, France.

All modelling referred to below was performed using the Sybyl software package provided by Tripos Associates together with some minor modifications and extensions developed by us. The host computer was a Gould/SEL 32/77. Hard copies of structures were produced on a Hewlett-Packard 7221C plotter.

## Method

The effect of drugs on the serotonin uptake was determined *in vitro* by using a crude synaptosomal fraction from the hypothalamus dissected from a rat brain as described by Glowinski and Iversen[11] (see Figure 2). The tissue was weighed and homogenized in 20 volumes of 0.32 M sucrose using a glass homogenizer with a Teflon pestle until a uniform suspension was obtained. The homogenate was centrifuged at 1000*g* for 10 min at 4°C. The supernatant was transferred and given a 30 s burst with a Polytron at setting #6 and vortexed before use. A 0.1 ml aliquot of the suspension (corresponding to about 5 mg of original tissue) was added to test tubes containing 1.7 ml Krebs-bicarbonate buffer with the following composition (g $l^{-1}$): NaCl, 6.92; KCl, 0.354; $MgSO_4 \cdot 7H_2O$, 0.294; $CaCl_2$, 0.143; $KH_2PO_4$, 0.162; $NaHCO_3$, 2.1; glucose, 2.0; pargyline, 0.03; ascorbic acid, 0.02; the buffer was gassed with 95%$O_2$–5%$CO_2$ for at least 15 min before use. Drugs or vehicle were added in a volume of 0.1 ml to give the desired final concentrations. After 10 min preincubation in a Dubnoff metabolic shaker at 37°C under an atmosphere of 95%$O_2$–5%$CO_2$with shaking, 0.1 ml of the [$^3$H]5HT was added to give a final concentration of $2 \times 10^{-8}$ M. Incubation was continued for a further 5 min. The reaction was then stopped by adding 5 ml of ice-cold saline and the tubes were placed in an ice bath for 10 min.

The contents of the tubes were filtered under vacuum through a membrane filter (Millipore, 24 mm diameter, size 0.45 $\mu$m). Each filter was then rinsed with $2 \times 5$ ml of ice-cold saline to remove any unbound [$^3$H]5HT adhering to the filter. The filters were removed and placed in counting vials, and 15 ml Aquasol scintillation fluid was added. A diffusional entry blank was carried out at 4°C incubation and the amount of radioactivity accumulated in this blank was subtracted from all experimental samples. It is known that, under these conditions, 85% or more of the synaptosomal content of [$^3$H]5HT is not metabolized[12,13].

## Statistical analyses

The effects of drugs on the synaptosomal uptake of [$^3$H]5HT is expressed as percentage inhibition as compared with control. The percentage inhibition and dosages were transformed initially into logit and log scales respectively so that the sigmoidal dose–response curves became linear. This transformed data was then analysed for linearity and parallelism using the appropriate method of analysis of variance (ANOVA)[14]. When obvious differences were observed between the slopes of the individual lines (i.e. lack of parallelism), selected dose–response lines with apparently similar slopes were grouped, and ANOVA was repeated for each group. This operation was repeated until all dose–response lines within a group showed no significant deviation from linearity and parallelism. It was further shown that the common slopes of each group of dose–response lines are significantly different from each other.

# RESULTS AND DISCUSSION

## Molecular modelling

The starting point of our modelling efforts was the assumption that only those molecules which possessed

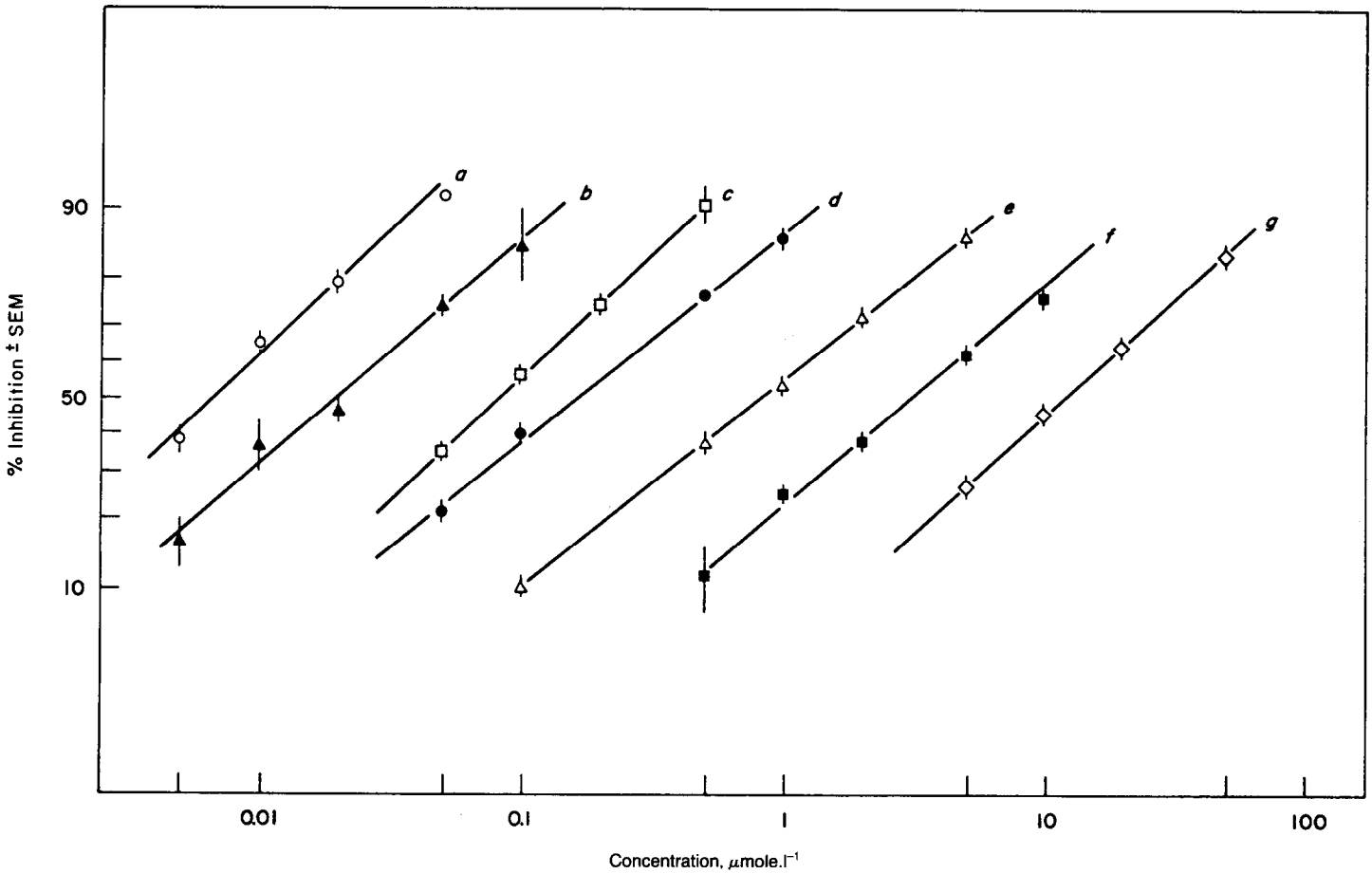


*Figure 2. Effect of various compounds on serotonin uptake;* (a) *indalpine,* (b) *Femoxetine,* (c) *quipazine,* (d) *Zimelidine,* (e) *8-hydroxy-DPAT,* (f) *8-methoxy-DPAT,* (g) *cyclazindol*

parallel dose–response curves as defined above were worthy of comparison structurally, since nonparallel dose–response curves are generally accepted[15] to imply nonsimilarity of the mechanism of action. It was felt that a successful discovery of the common features that all of these molecules shared could shed light on the necessary stuctural elements for this type of activity. On the other hand, structural features which only some members have in common may be used to rationalize degree of activity within the family as measured by the $IC_{50}$ values.

Many of the above molecules have been shown to have activities other than 5HT-uptake blockade. These other activities, though extremely important pharmacologically and medicinally, are of no direct consequence to an analysis of their structural similarities which has as its purpose the discovery of the structural feature(s) which endow(s) them all with 5HT blocking activity.

Compounds (8) and (9) differ only slightly from one another. Thus it was felt that in the initial search it would suffice to consider only one of them. Large differences in their activities could be explained if it was postulated that the change from hydroxy in compound (8) to methoxy in compound (9) resulted in a major change in the capacity to interact with an important secondary binding site. This hypothesis would receive circumstantial support only if it could be shown that other molecules also had functionality which interacted with this region. Furthermore, the extremely low activity of cyclazindol (compound (10)) ($IC_{50}$ four times that of the next compound in Table 1) tended to suggest that use of this compound for the discovery of the essential structural feature may be misleading. The retrospective rationalization of its low activity would, however, be necessary, and is deferred to a later part of this paper. Seven compounds in the original set were thus reduced to five for initial consideration.

The choice of a reference molecule is rather arbitrary and merely functions as a frame of comparison. It is

**Table 1. Inhibition of synaptosomal uptake of 5HT by various drugs *in vitro***

| Drug | $IC_{50}$, μM* | Relative potency | Individual slope† |
|---|---|---|---|
| Indalpine | 0.007 | 12.22 | 2.75 |
| Femoxetine | 0.022 | 3.83 | 2.64 |
| Quipazine | 0.084 | 1.00 | 2.83 |
| Zimelidine | 0.180 | 0.45 | 2.38 |
| 8-Hydroxy-DPAT | 0.840 | 0.09 | 2.34 |
| 8-Methoxy-DPAT | 3.570 | 0.02 | 2.52 |
| Cyclazindol | 12.300 | 0.01 | 2.63 |

* Concentration of drug which gives 50% inhibition

† These are the slopes of the linear dose–response equations expressing the logit (percentage inhibition) *versus* log (dose) relationship. Statistical analysis showed one common slope of 2.52 share among all seven equations.
$N \geq 3$ for all determinations.

**Table 2. Distances in quipazine between $sp^3$ N atoms and aryl rings**

| | Distance, Å | |
|---|---|---|
| | Ring A | Ring B |
| $N^1$ | 5.010 | 2.810 |
| $N^2$ | 7.756 | 5.549 |

convenient to choose the most rigid member since all relevant distances are well defined and are not easily changeable without a considerable expenditure of energy. Quipazine (compound (2)) has only one acylic rotatable bond, namely that joining the piperazine to the quinoline. Furthermore, rotation about this bond does not change the disposition in space between the proximal piperazine nitrogen and the aromatic system. The distance between the centre of either aromatic ring and the distal piperazine nitrogen also remains relatively constant, with this atom sweeping out a circle at the end of a cone whose apex is the centre under consideration. The various other ring conformations of the piperazine ring, such as twist-boat, boat etc., could change this distance relationship. These were briefly considered but were discarded on the basis of energy considerations. Throughout this study the underlying criterion has been to search for conformers which are relevant within the framework of certain constraints, and then independently to evaluate the significance of the found conformers by comparing their internal energy with that of the conformer with the lowest found internal energy.

The conformation of quipazine (compound 2)) with the lowest found internal energy is shown in Colour plate 1. Distances between the centre of each aromatic ring and each aliphatic nitrogen are shown in Table 2.

The next molecule for which structural constraints were expected to be rather stringent was compound (8). This molecule has a number of rotatable bonds in the side chain as well as several possible conformations of the reduced ring. The latter could seriously affect the spatial relationship between the centre of the aryl ring and the nitrogen atom, while the former could have a bearing on the internal energy of the particular conformer under consideration. Since it was impossible, within the framework of our program package, to change dihedral angles within a ring, the following device was used to generate the extreme conformations of the reduced ring. One of the ring bonds was removed, the others were defined as rotatable, and all sterically allowed conformations which brought the two separated ends of the removed bond within bonding distance were sought by stepping each dihedral angle through a complete circle in increments of 10°. This resulted in two groups of ring conformers from which typical members have been extracted in Colour plate 2. It can be seen that one of these conformers has the carbon attached to the nitrogen atom above the adjacent nonbenzylic ring carbon, and the other conformer has it below this carbon. These two conformers were compared energetically for a given conformation of the *N*-alkyl groups and were found to be closely similar ($\pm$2kcal $mol^{-1}$). They were thus both included in further considerations.

Each rotatable bond in the side chains was now driven through a complete circle independently, and the energy profile was plotted as a function of angle. Each generated a profile containing some valleys. All of the combinations of these valley angles were independently set up and made the starting point of an energy minimization (force field). Altogether 24 conformations were studied for each of the two ring conformers. The lowest-energy conformer found by this method is conformer A in Colour plate 2. (Internal energy of $-1.624$ kcal $mol^{-1}$ was calculated by the Simplex method defined in the Tripos package.) It should be pointed out that this method of sampling the conformation–energy space of a given molecule is not exhaustive, and it is possible that the lowest found energy does not coincide with the global minimum energy. However, it is believed that this method is at least as good as other currently used methods based on random sampling of this conformational space[16] or on substructure searches of the Cambridge crystallographic database.

In conformer A of compound (8), the distance between the nitrogen atom and the centre of the aromatic ring was 5.210 Å, and the nitrogen was 0.18 Å above the plane of this ring.

A similar search of conformational space was repeated using only the constraint obtained with quipazine that the nitrogen atom should be 4.9–5.1 Å from the aryl centroid and in the plane of this ring. The other distances in Table 2 were also used but no sterically allowed conformations were found for them. In the former case, 93 such valid conformers were found. One of these, when minimized to completion, afforded a distance of 5.197 Å and a height of 0.1 Å with an internal energy of $-1.611$ kcal $mol^{-1}$. In other words, the lowest-energy conformer found using purely energy considerations coincided with one of the required conformations for superposition between ring A of quipazine and the aryl ring of compound (8) on the one hand, and N1 of quipazine and the aliphatic nitrogen of compound (8) on the other.

In principle, there are several ways in which these two molecules may be placed on top of each other. An alternative for example, would be ring B–N2 of quipazine on the aryl ring–N atom of compound (8). The advantage of the present method is that it effectively allowed a rational choice of one of these alternatives.

Each subsequent molecule was subjected to the combined constraints of all previously studied molecules. Thus the distance requirements became increasingly more stringent as further molecules were examined. This process is extremely efficient since, if an erroneous assumption is made about the required spatial relationships of different groups, a situation is rapidly reached where no conformers of a given molecule are allowed. This would signal that another choice of required groups in space must be made and the whole process repeated.

A similar analysis to that mentioned above for compound (8) was repeated for Zimelidine (compound (4)). Sixteen conformers of the molecule were used as 'seed points' for a sampling of its conformation—energy space. The lowest-energy conformation here had an internal energy of 3.418 kcal $mol^{-1}$. The search for valid conformers was restricted to those where the

critical relationship between either aryl group and the aliphatic nitrogen coincided (within ±0.05 Å) with an allowed conformation of compound (8) as defined above. In all, 72 valid conformations were recorded. However, all of these could be grouped into four families. Each family was separately minimized. one family had an internal energy of 3.968 kcal $mol^{-1}$ (i.e. only 0.55 kcal $mol^{-1}$ above the lowest-energy conformer found). The critical distance between the nitrogen atom and the pyridine ring in the isomer was 4.851 Å after it had been minimized and the height was 0.65 Å. No valid conformations were found where the distance constraints applied between the phenyl ring and the N atom.

The next molecule to be scrutinized was Femoxetine (compound (5)). Again, the distance constraints applied were those obtained from Zimelidine. Although the requirements of coincidence (within ± 0.05 Å) in the case of the substituted phenyl ring did not result in any valid conformations, it was possible to find seven conformers of Femoxetine where the requirement was met between the piperidine nitrogen and the phenyl ring which was directly attached. Two of these conformers were found to have energies of 2.636 kcal $mol^{-1}$ and 2.583 kcal $mol^{-1}$ as compared to a lowest independently found value of 2.884 kcal $mol^{-1}$ using 24 starting conformers. (Note: the energies of conformers found through a constrained search are somewhat, though not significantly, below this value.)

Thus, in the case of quipazine, DPAT, Zimelidine and Femoxetine, low-energy conformers of each molecule could be found where an aliphatic nitrogen atom was 4.9–5.1 Å (±0.05 Å) away from an aromatic ring and virtually in the plane of that ring (0.7 Å).

Indalpine (compound (11)) was the last remaining molecule requiring consideration. When the appropriate constraints from Femoxetine were applied, 11 conformers of indalpine that satisfied them were found. However, minimization of each of these conformers did not result in a conformation which was sufficiently low in energy (the lowest was about 5 kcal $mol^{-1}$ higher than the lowest-energy conformer found by the independent sampling of conformation–energy space). Furthermore, minimization resulted in considerable movement of the same critical functionalities. Thus the final structure was unacceptable on both energy and distance-constraint grounds.

The possibility that this may be a sign of the breakdown of the original hypothesis about the spatial requirement of N and aryl groups is finite at this point. However, an equally plausible explanation for this result would be that the parallelism of the dose–response curve of indalpine with the others in this series is coincidental. Our testing methods are not sufficiently refined to distinguish between these alternatives. However, the success in finding allowed conformers for all these molecules, despite an increasingly stringent constraint, supports the belief that the nonconformity of indalpine with the others from this structural point of view is an indication that it acts by a different mechanism at the molecular level.

## Structure–activity analysis

The relevant conformers of quipazine, DPAT, Zimelidine and Femoxetine have been superimposed and three orientations of the resulting picture have been drawn in Colour plate 3. All H atoms have not been drawn to simplify the picture. The initial question concerned a possible explanation of cyclazindol's low activity. This molecule contains two aryl rings and two nitrogen atoms. The first striking feature that distinguishes cyclazindol from the others is that neither nitrogen is 'purely' $sp^3$ hybridized (i.e. pyramidal). This may be expected to play an important role in its binding capability or site affinity which would translate into a high $IC_{50}$ value. Of the various different ways of placing this molecule on quipazine as reference, one is shown in Colour plate 4 again in the same three orientations as used in Colour plate 3. The distance between the relevant nitrogen and aromatic ring is 5.094 Å and this nitrogen is 0.43 Å above the plane of the ring. It can be seen that this spatial relationship is well within the bounds of the constraints previously used. Thus cyclazindol does indeed present the postulated pharmacophore for binding to the 5HT-uptake site. However, it can also be seen from Colour plate 4 that it has, in addition, several areas which it does not share with any of the other members. The volumes represented by these areas have been displayed in Colour plate 5. All of these regions represent areas of negative electrostatic potential (see Colour plate 6). Thus not only does the steric picture of this molecule demonstrate a difference from the others, but also the electronic picture of this molecule may be quite different. Any one of these differences — the nature of nitrogen, the extra steric volume, or the electronic differences — may be instrumental in the low affinity (high $IC_{50}$) of cyclazindol.

Regarding the original five molecules, several interesting features emerged in addition to the discovery of the so-called primary similarities. Some of these are mentioned below. All discussion which follows refers to the conformers and orientation shown in Colour plate 5. First, it appears that the pyridine nitrogen atom of Zimelidine and the hydroxy group of 8-hydroxy-DPAT are relatively close to each other and contribute negative electrostatic potential in roughly the same region of space. Second, the p-bromophenyl group of Zimelidine and the p-methoxyphenyl group of Femoxetine, although not superimposed on each other, appear in approximately the same region of space with the plane of one phenyl group at an angle to that of the other (see below). Third, the piperazine ring of quipazine utilizes a unique area which could possibly be another secondary recognition site.

In order to probe these relationships further, the partial charges for each molecule have been computed and visually displayed as electrostatic potential maps (at the −150 level). These have been superimposed in Colour plate 5. All of the molecules share the potential associated with the critical aryl ring and nitrogen. Beyond this, however, each molecule has little in common with all the others. It may be speculated that areas which only some of the molecules share may represent the secondary functional groups which are so important in determining the specificity and/or affinity of a 5HT ligand for one type of 5HT site over another, or even the selectivity for 5HT sites over other neurotransmitter recognition sites.

Femoxetine ($IC_{50}$=0.022 $\mu$M) is the most active member. Electronically, it has an area of negative electrostatic potential in its p-methoxyphenyl ring region. If this is the cause of its high affinity, other molecules

which share this binding characteristic must also have high activity. At first glance, it appears that Zimelidine ($IC_{50}$=0.18 $\mu$M) qualifies. However, as shown in Colour plate 3, the orientation of the two rings (the p-bromophenyl of Zimelidine and p-methoxyphenol of Femoxetine respectively) is not the same. Thus the overlap between the two charge clouds is less than optimal. Zimelidine, however, also has negative electrostatic potential around the pyridine nitrogen — an area which it shares with the 8-hydroxy-DPAT molecule. This area in Zimelidine must provide enough attraction for the site to outweigh the poor orientation of the p-bromophenyl group. Such a conclusion receives circumstantial support from the observed difference in activity between the 8-hydroxy ($IC_{50}$=0.930 $\mu$M) and 8-methoxy-DPAT ($IC_{50}$=3.570 $\mu$M) molecules. Presumably the methyl group may interfere sterically at that site to diminish binding in the latter case.

Quipazine, the second most active compound ($IC_{50}$= 0.096 $\mu$M), is extremely interesting. It does not present the aryl region of Femoxetine and Zimelidine nor does it present the hydroxyl-type region of the DPAT molecules. Instead it presents an additional region associated with the second nitrogen of the piperazine ring. As judged from its high activity, this must be an important secondary site.

The natural extension of this discussion is the design of molecules which exploit various combinations of these supposed secondary sites in addition to the 'primary' pharmacophore so as to enhance specificity and selectivity.

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## REFERENCES

1 **Effland, R C,** in **Hess, H J (ed. in chief)** *Annual reports in medicinal chemistry* Vol 17 Academic Press, New York, USA (1982) p 11
2 **Bondinell, W E and Kaiser, C** in **Hess, H J (ed. in chief)** *Annual reports in medicinal chemistry* Vol 17 Academic Press, New York, USA (1982) p 41
3 **Samanin, R,** in *Medicinal chemistry advances* **De Las Heras, F G and Vega, S (eds)** Pergamon, New York, USA (1981) p 271
4 **Heinze. G amd Omer, L M O,** *Curr. Ther. Res., Clin. Exp.* Vol 29 (1981) p 567
5 **Lundström, J, Högberg, T, Gosztonyi, T and de Paulis, T** *Arzneim–Forsch.* Vol 31 (1981) p 486
6 **Larsson, H and Lund, J** *Acta Pharmacol. Toxicol.* Vol 48 (1981) p 425
7 **Wong, D T, Bymaster, F P, Reid, L R and Threlkeld, P G** *Biochem. Pharmacol.* Vol 32 (1983) p 1287
8 **Wurtman, J J and Wurtman, R J** *Science* Vol 198 (1977) p 1178
9 **Wurtman, J J and Wurtman, R J** *Life Science* Vol 24 (1979) p 895
10 **Samanin, P, Bendotti, C, Candelaresi, G and Garattini, S** *Life Science* Vol 21 (1977) p 1259
11 **Glowinski, J and Iverson, L L, J** *J. Neurochem.* Vol 13 (1966) p 655
12 Shaskan, E G and Snyder, S H *J Pharmacol. Exp. Ther.* Vol 175 (1970) p 404
13 **Kannengiesser, M H, Hunt, P and Reynaud, J P** *Biochem. Pharmacol.* Vol 22 (1973) p 73
14 **Finney, D J** *Statistical method in biological assay* 3rd edn MacMillan, New York, USA (1978)
15 *Burger's medicinal chemistry* part 1 Chapter 5 4th edn (1980)
16 See for example the Chemlab program package of Molecular Design Ltd