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# Multiconformational composite molecular potential fields in the analysis of drug action. I. Methodology and first evaluation using 5-HT and histamine action as examples

J.G. Vinter* and K.I. Trollope

*Cambridge Centre for Molecular Recognition, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, U.K.*

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## Summary

The quality of molecular electrostatic maps generated by non-quantum mechanical methods has been improved using extended electron distributions. Further simplification has been achieved by distilling these maps down to their energy extrema. A new means of defining surface interaction has been added and the resulting composite map has been plotted for a limited number of low-lying conformers of a series of agonists and antagonists of the $H_2$ and $H_3$ receptors and 5-$HT_{1A}$ and 5-$HT_{1D}$ receptors. The results from the cross-comparison of these maps indicate their ability to distinguish the specific receptor. Interesting consequences of the method are that structural overlay is irrelevant, that several conformations may contribute to the overall binding pattern and that lesser pharmacological activities may be deduced from the results.

## Introduction

The computational comparison of molecular properties based on atom positions alone, usually calculated on a single conformer of an isolated molecule, has never yielded sustained and convincing results that were consistent with biological findings. Literature arguments and explanations of mechanisms and correlations, based on atomic properties and positions, continue to be unsatisfactory. Why is amthamine (Fig. 1, structure **1**) a histamine $H_2$ agonist yet it is not tautomeric across the ring? How is a structure like methiothepin (structure **2**), so different from 5-HT, active at the 5-$HT_1$ receptor and how do such diverse structures as CI930 (structure **3**), anagrelide (structure **4**) and ORG-30029 (structure **5**) all interact with the phosphodiesterase III isoenzyme?

It is well understood that molecules recognise each other by the induced fields that they generate as they approach each other, so it is these fields we should be concerned with rather than the molecular structure, which only serves as a 'skeleton' on which the fields are formed. The molecular field is not only dependent upon the molecule's structure, but it is also modified by the approaching molecular structure. Furthermore, such fields can change drastically with conformation. Field correspondence rather than structure correspondence should be the guide to molecular similarity. These may differ appreciably; for example, the major electrostatic field feature of a carbonyl group consists of two negative energy minima, 2.29 Å from the oxygen atom, approximately where lone pairs would be expected. Structural comparison of two carbonyl groups on different molecules would force us to align them along their bonds. Field alignment results in a large range of possibilities with either one or both 'lone pairs' (Fig. 2).

Attempts to define molecular fields in terms of electrostatic and steric parameters continue to be successful for certain systems (for example CoMFA [2]). These methods are either restricted in the number of conformations they can handle, are bound to fixed grids and ignore interstices, or use energy calculations parameterised for isolated molecules. They often need computationally de-

*To whom correspondence should be addressed.

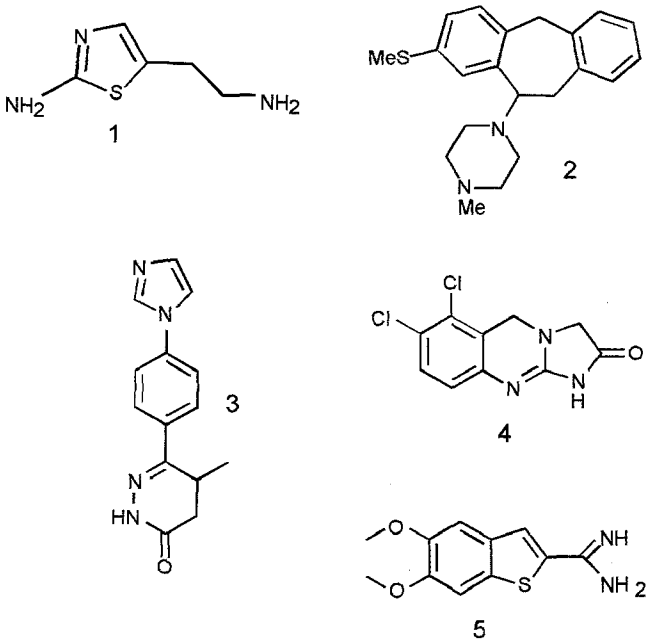


Fig. 1. Some diverse structures of active compounds (see text).

manding wave functions for good electrostatic potentials and charge distributions and, most importantly, they cannot account for the diverse biological observations so familiar to pharmacologists. All the present methods ultimately rely on the comparison of structure for their analyses. It is therefore desirable to be able to treat many conformations of a molecule, take account of the effects of another molecule in its vicinity and (as CoMFA does) find new criteria other than structure to define molecular recognition. It is fully realised that although this may be achieved, the final duty of a model is to feed back to the medicinal chemist some kind of structure which is synthetically feasible. For this reason, we need only to start with structure and finish with structure.

We have made the assumption that three distinct interactions are at work as one molecule approaches the other. The first two consist of positive and negative long-range electrostatics and account for overall molecular response, followed by closer ion-pairing, hydrogen-bonding and $\pi$–$\pi$ interactions. The third is a short-range surface attraction made up of induced electrostatics and the van der Waals dispersive force. This attraction increases with surface complementarity only where polar interactions are absent. In order to anticipate the approach of one molecule by another, it has been necessary to develop a new way of describing electron distribution at an atom [1].

Three properties are calculated, i.e., positive and negative electrostatic interactions and surface potential. The properties are described as points in three-dimensional space, each having a fourth energy dimension. These points are derived by hunting for extrema (maxima and minima) within high-resolution energy grids created by three specific probes. Unlike many other published methods, this procedure is sufficiently fast to allow a reasonable set of conformations to be considered for any drug-sized molecule.

Many drug discovery modelling programmes have to be conducted without any useful knowledge of the macromolecular target structure. Even when X-ray data exist for the target receptor, they rarely yield the details of change and movement associated with binding of a hormone or drug. Therefore, in the absence of target structure information (receptor or enzyme), the aim of our long-term project is to define the state and conformation of an active hormone (natural or synthetic, agonist or antagonist) and to be able to create a template onto which new structures can be mapped. The key assumptions are that:

(1) hormone and receptor are not static;

(2) agonists and antagonists affect the same receptor site;

(3) binding is controlled by (i) Coulombic forces (including H-bonds) at long and short range, and (ii) van der Waals surface interactions at short range;

(4) interactions within receptors occur in a low dielectric medium;

(5) species with full charges are often paired during receptor binding;

(6) active conformations of hormone and drug are, where possible, within 3 kcal/mol of their global minimum conformation.

Ideally, we need to be able to generate a manageable number of points around a molecule, describing Coulombic and surface attractions, which can be calculated fast enough to cover a reasonable conformational space. With the present computer technology, we are restricted to

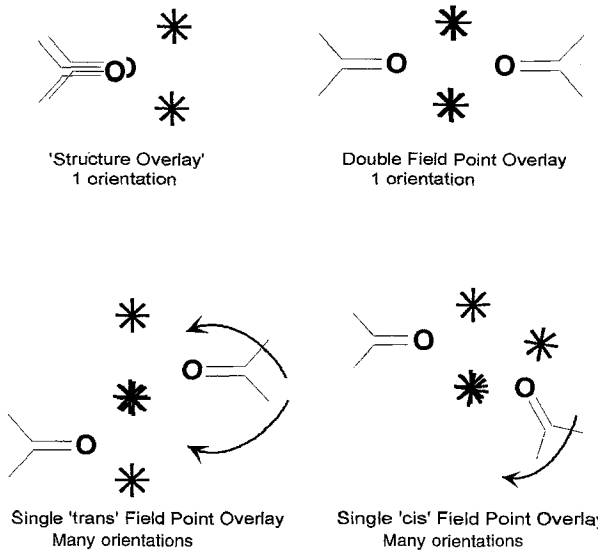


Fig. 2. Possible structure and field alignments for two carbonyl groups.

molecular mechanics if many conformations need to be studied. It was therefore necessary to develop adequate methods to generate electrostatic fields and surface properties and to find ways of comparing them across a large conformational space.

The use of electrostatic fields in defining some of the factors by which molecules recognise each other is well established [4]. As contour maps or 'surface potential areas', their use is cumbersome if comparisons are attempted. Early success with classical molecular electrostatic extrema (electrostatic energy minima and maxima) [5], and lately its validation and extension using high-level quantum wave functions and distributed multipole analysis (DMA) [6], have stimulated us to extend the classical approach to include surface interactions and to apply the resulting composite extrema maps to a representative number of conformations of a series of interest. Other comparison techniques do not cope well with conformational variability and we have found this variability to be of fundamental importance in characterising a specific hormone or substrate behaviour.

## Methodology

*The generation of molecular electrostatic potential extrema (MEPEs)*

It is accepted that molecular electrostatic potential maps are poorly reproduced by classical means (i.e., applying Coulomb's law to a charged probe scanning over a structure which has been allocated atom-centred partial charges). Lone-pair effects are distorted, aromatic π interactions are missed and positive probes return exaggerated maps with little meaning. Since reliable quantum calculations are too slow to study many conformations, we have developed an alternative way of describing electron charge distribution [1] which has been shown to reproduce electrostatic extrema generated by high-level quantum-mechanical distributed multipole analysis [7]. These extended electron distribution points (or XEDs) are added to the target molecule before field addition is commenced.

MEPEs are added in the following way. A grid of points is set up at 3 Å above the molecular van der Waals surface. The interval between points is adjusted so that the whole molecular surface is covered using a maximum of 250 points (this varies according to the compromise between the interval and the area of the molecule). Each grid point is given a point (unit) charge (+1 as a positive probe and −1 as a negative probe). The energy of interaction of the probe is calculated using Eq. 1.

More precisely, for each grid point p, 3 Å above the heavy atoms on the molecular surface, the energy of a point charge $q_p$ at p is calculated by Eq. 1 for each charged point i (an atom or XED) at a distance r from p. The dielectric (D) is left to choice.

$$E_c = \sum_{i}^{i} \frac{q_p q_i}{4\pi e_o} \cdot \frac{1}{r_{ip}} \cdot \frac{1}{D} \qquad (1)$$

(in kcal/mol), where $q_i$ are all charges on atom i. The summation is over all partial charges i (atoms and XEDs) on the molecule. The probe charge is constant.

The van der Waals energy between each atom and the probe is given by Eq. 2 (a Morse potential as defined in Ref. 8), with the probe set at the van der Waals radius of an oxygen atom:

$$E_{vdW} = \sum_{1}^{j} -E_{pj}\left[z^2 - 2z\right] \qquad (2)$$

where

$$z = e^{-b_{pj}\left[1-\left(r_{pj}/r_{pj}^0\right)\right]} \qquad (3)$$

$E_{pj}$ and $b_{pj}$ are constants, r is the distance between the atom/probe pair and $r_{pj}^0$ is the sum of the vdW radii (values in Refs. 1 and 8). The total energy at a grid point p is:

$$E_p = E_c + E_{vdW} \qquad (4)$$

After allocating an interaction energy to every grid point as described above, each grid point is allowed to find its own local extremum (maximum or minimum, whichever is appropriate) using a three-dimensional Simplex algorithm. In other words, the energy of interaction of the grid point with the molecule is minimised with respect to the position of the grid point (now no longer confined to the grid itself) in space, using the same potentials as above. Each 'grid' point is minimised in this way and the result is that many minimised positions eventually coincide. After filtering out coincident extrema, the final, uniquely placed MEPEs are stored with their type (+ve, −ve probe), position (co-ordinates) and energy (well depth) information.

Two sets of MEPEs are plotted on the same grid, the first using a positive probe and the second using a negative one. The dielectric, D, is fixed at 3 for these electrostatic probes to conform with the SIPs (see below) protocol.

*The generation of surface interaction points (SIPs)*

Hydrophobic interactions are a complex composite of enthalpy and entropy terms, involving van der Waals forces, surface contact areas, translation and rotation changes, water/solute H-bond exchanges and other unanticipated processes. Many of these terms are undefinable, especially in the absence of target receptor information. We have decided to define a 'surface potential' between a neutral probe and a ligand as follows. The total energy at a grid point p is:

$$E_s = E_I + E_{vdW} \qquad (5)$$

where

$$E_I = 0.5 \cdot \alpha_p \cdot \sum_{1}^{j} \frac{q_j^2}{D r_{pj}^4} \qquad (6)$$

(in kcal/mol) and $\alpha$ is the atom polarisability [9].

This equation defines dispersive and inductive energy only (vdW and I). Firstly, the surface extrema (minima) are calculated in the same way as the MEPEs. For MEPEs, a single grid is used to calculate both negative and positive points, because they are complementary and never occupy the same space. SIPs are calculated on a separate grid. They are then minimised and coincidences are removed as for MEPEs.

Possible probes include: CH ($D=1$, $\alpha=1$), $H_2O$ ($D=78$, $\alpha=2$), $C_6H_6$ ($D=2$, $\alpha=10$), a protein/water interface probe [10] of water size ($D=8$, $\alpha=2$) and an intra-protein probe of water size ($D=3$, $\alpha=3$). This last probe was used as the default in all the studies reported here.

After locating the minima (these are always attractive), $E_s$ is further scaled by (i) the carbon atom surface area as 'seen' by the probe at its minimum point and (ii) the extent of electron distribution associated with the carbon atoms that it sees. The use of 'visible area' is an attempt to spread the vdW minimum point across the fraction of the molecular surface that it can affect. A high carbon 'electron distribution' is more conducive to hydrophobic forces than a low one and is estimated from a rough calculation of the $^{13}C$ NMR shift of each carbon atom. For a discussion on the calculation of NMR shifts from electron density, see Ref. 11. After multiplying in these factors, the whole is divided by the square of the distance between probe and molecular carbon atom to retain the dimensions of energy; SIPs = $\{E_s *$ visible CARBON atom surface area $*$ nuclear shielding $(240 - {}^{13}C_{NMR}$ shifts$)\} / r_{pi}^2$ (240 is the shift associated with the least shielded carbon atom on our scale).

The resulting data file contains all the input atomic data (with XEDs), plus the points defining the MEPEs and SIPs. These last points are collectively called the composite molecular potential field (CPF).

*Preparing for CPF comparisons*

To illustrate the general method, we will use selected data from the example of 5-HT expanded upon later in this paper. The aim of the experiment is to compare the fields of some representative 5-HT-active compounds with that of 5-HT itself, considering as many accessible conformations of each species as possible.

5-HT and each molecule in the list of agonists and antagonists to be studied is built, relaxed (all-atom minimisation) and submitted to a conformation hunter [12]. In our example, all conformers within 3 kcal/mol [13] of the global minimum (defined in this context as the lowest energy conformer found in the hunting process) or a convenient number of conformers are kept (typically the lowest 50 of a flexible system are held, to keep computing time in the next stage tractable). XEDs [1] are added to each conformer in the pack, followed by the calculation of a CPF for each conformer. Adding the CPFs may take maximally 30 min for 50 conformers of a molecule the size of 5-HT on an SGI Indy-4000. The whole process is repeated for the complete series of drugs to be studied. Figure 3 outlines the process.

*CPF comparisons*

Because the field points are essentially electrostatic in nature, their relationship to any complementary points in a receptor site will be inversely dependent upon their distance from those points (undefined of course). It would seem sensible, therefore, to look for field similarity in ligands by reversing the polarity of one of the pair and using a simple Coulombic term to find their best mutual overlay. It would further seem sensible, as a first approximation, to use the natural hormone or substrate of the chosen series as the reference with which to compare others acting on that same site. Accordingly, the natural ligand is always one of the pair in the first analysis. Later, cross reactions can be done to check on inactive species.

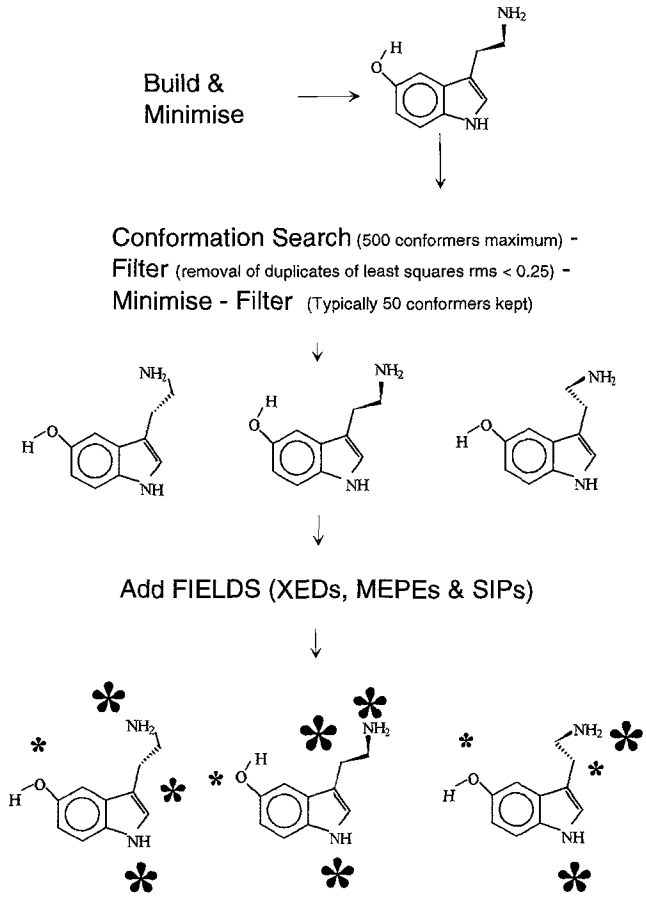


Fig. 3. Schematic of the preparation of a multiconformational CPF file.

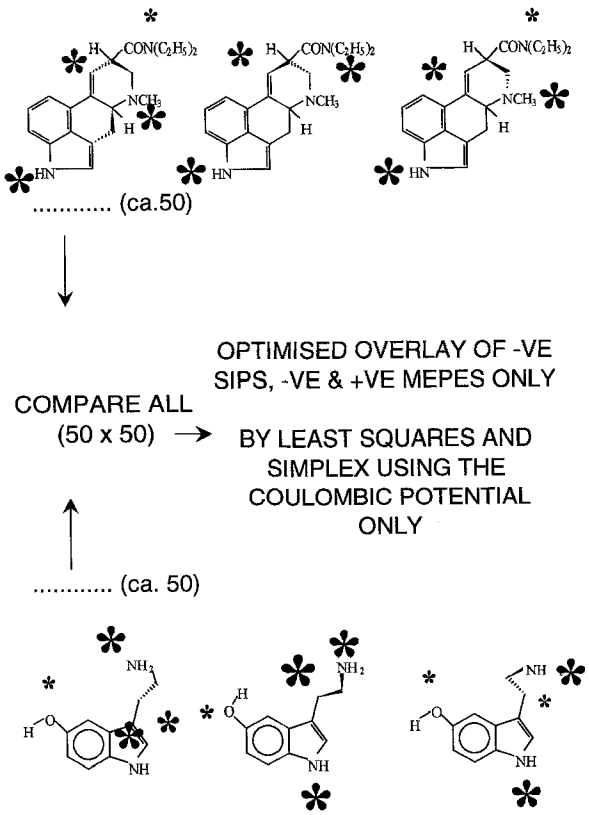


Fig. 4. Schematic of the comparison of a multiconformational CPF file.

Before summation, the electrostatic points (MEPEs) are calculated as one interaction and the surface points (SIPs) as another, to avoid mixing of the two potentials. The structural features of the pair are no longer relevant in this process, although the essentials of shape are reflected in the SIPs.

Figure 4 shows the procedure for comparing the fields of two multiconformation field files. We have tried several ways to optimise field overlay, including extensive grid searches, but the most successful method to date (that which results in the lowest Coulombic energy overlay) is by permutatively overlaying points of the same type (–ve MEPEs, +ve MEPEs, SIPs) by least-squares distance before allowing the whole field descriptor (all field points) to be moved to optimisation by a 6D Simplex. Plainly, there will be N×M pair overlays, where N is the number of conformers for one structure and M is the number of conformers for the other. The comparison of 20 conformers of 5-HT with 20 of histamine would be expected to take 2 h on the SGI Indy-4000, whereas the comparison of 50 conformers each of famotidine and impromidine, having more atoms than the natural hormones, could take two days.

*Analysis of results*

The best 50 overlays, judged purely by Coulombic overlay energy, are kept. These final 50 can also be subjected to a weighting procedure and are ordered accordingly [14]. A simplified results table is reproduced in Table 1. All pair entries of the set of 50 were sorted and a diagram of the lowest lying links was constructed.

The representative data from Table 1 are shown pictorially in Fig. 5. Each pair from the comparison of NAN190 (a 5-$HT_1$ antagonist) with the natural hormone was linked. Data from another comparison output (methysergide–5-$HT_1$ active) have been added to clarify the next step, which was to colour code common links. This process was continued for all pair links over the best half-dozen or so comparisons. Where no unique links were found, colour was allocated on the basis of the strongest link (marked with a small black dot) or the largest number of common links. Occasionally a conformer had to be allocated as 'mixed' when equal weights occurred.

## Results

We have chosen to use the relationships of (i) 5-HT with a selection of 1A- and 1D-receptor selective compounds; and (ii) histamine with some of its $H_2$ and $H_3$ agonists and antagonists as preliminary evaluation exercises for the method. Because of restricted computer time, only a limited number of conformations was compared using a restriction algorithm (not all pair overlays were investigated), in order to complete the all-pairs comparison in a reasonable time. More extensive comparisons will be published later.

*5-HT*

The structures used are shown in Fig. 6. The biology is set out in Table 2. Out of a total of 54 conformations

TABLE 1
A SECTION OF THE OUTPUT TABLE FROM THE COULOMBIC FIELD POINTS OVERLAY

| Pair number | Coulombic overlay 'energy'[a] | NAN190 conf. number | 5-HT conf. number | Order by Coulombic overlay |
|---|---|---|---|---|
| 5 | –3409 | 7 | 51 | 1 |
| 37 | –3111 | 17 | 51 | 3 |
| 11 | –3202 | 3 | 51 | 2 |
| 42 | –2365 | 1 | 13 | 5 |
| 43 | –2967 | 21 | 9 | 4 |

[a] The unit (energy$^2$/distance) is related to energy.

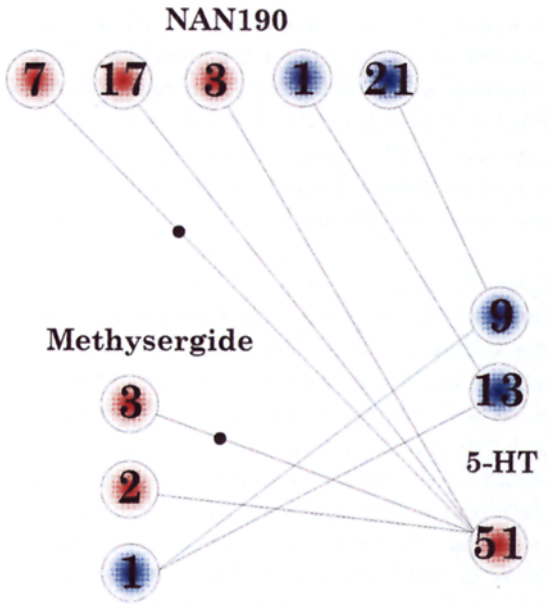


Fig. 5. Pictorial analysis of data from Table 1, coloured according to common links. Methysergide links have been added for clarity.

of 5-HT, only eight associated themselves with the chosen drugs. Figure 7 is a link diagram of the best half-dozen or so CPF overlays of the chosen drugs with 5-HT. Colour allocation is as described above, noting for example that, although conformation 2 of methiothepin overlays conformations 1, 3, 5, 7 and 9 of 5-HT, it is coloured green to match the preponderance of green associated with conformers 1, 3 and 5 of 5-HT. For LSD, however, no criteria could be found for a definite colour allocation and conformer 1 of LSD was designated 'mixed'. At this stage, colour allocations were made without the input from any pharmacological data.

By examining Fig. 7 and applying the pharmacological data in Table 2, the observations could perhaps be made that 5-HT conformations 1, 3 and 5 are associated with 5-$HT_{1D}$ activity and that conformation 51 seems to be solely associated with 5-$HT_{1A}$ recognition. Conformers 7, 9, 13 and 17 make up a separate group, but could be associated with either receptor or represent another of the 5-HT receptors as yet not examined by this method. The striking feature of this analysis is the mixture of pharmacology that is implied across the various drugs and even seen across their enantiomers. Both LSD (for which no pharmacology has been included in Table 2 because of conflicting experimental data) and methysergide are seen to be 'dirty' compounds. 8-OH-DPAT, NAN190 and (*R*)-WAY100135 are untainted by 5-$HT_{1D}$ conformers, whilst the pharmacologically 'mixed' drugs are seen to possess all components. It is interesting to note that (*S*)-WAY100135, described as the first 'silent' 5-$HT_{1A}$ antagonist [19], may be 1D active. It is not known whether this is linked to the discrimination of pre- and postsynaptic activity. At this stage in the development of the analysis, no conclusion can be drawn as to the correctness of these

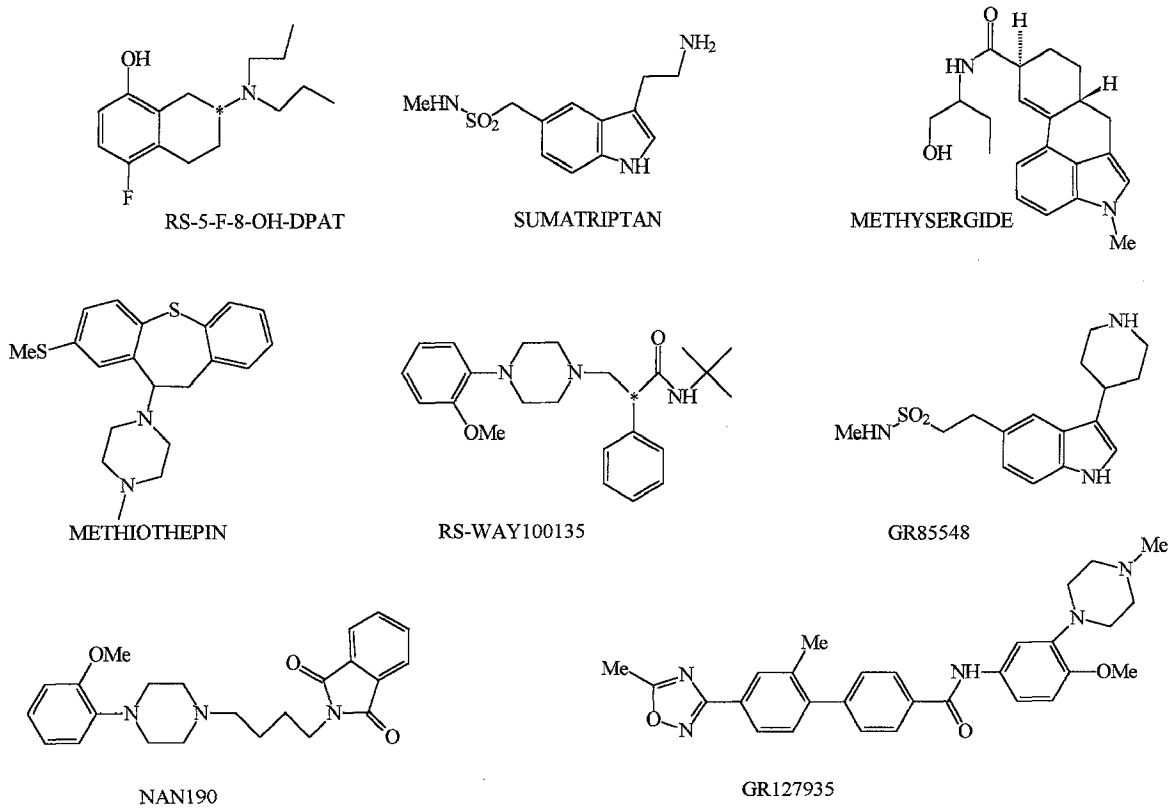


Fig. 6. Structures of the selected 5-$HT_{1A/D}$ agonists and antagonists used in this study.

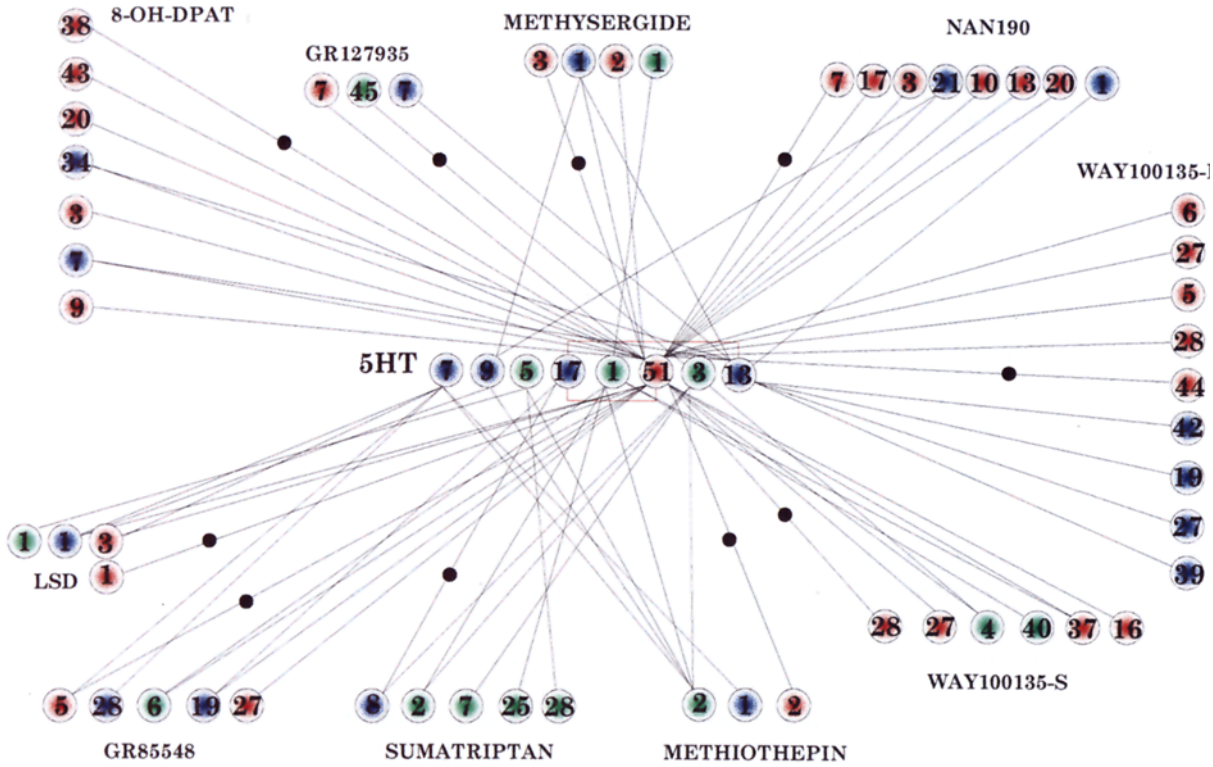


Fig. 7. Analysis of the field comparison of 5-HT conformers with a selection of 5-$HT_{1A}$ and 5-$HT_{1D}$ agonists and antagonists. Only the top selection of conformers of each drug has been included. The strongest conformer overlays of 5-HT with itself are shown in red lines (see text).

observations, nor could we discern any signs of agonist/antagonist discrimination.

The eight conformations of 5-HT which overlaid the selected drugs are reproduced in Fig. 8. The 'green' conformations, 1, 3 and 5, are related either by a $C_s$ symmetry through the indole ring or they differ only in the direction of the 5-hydroxyl proton. The 'blue' conformations are similarly related and differ from the 'green' set only in the extension of the terminal -$CH_2$-$CH_2$-$NH_2$. The single 'red' conformer is fully extended.

Although the selected drugs and the natural substrate are calculated as being neutral by virtue of the initial ion pair formation (presumably at an aspartic acid residue in the receptor), their electrical disposition in the receptor is likely to be polarised rather than symmetric. The rotation of the terminal -$NH_2$ is therefore probably relevant.

It might be hoped that, from an extended investigation of more drugs and a larger conformational set, a coherent pattern could emerge, revealing further receptor-selective features and perhaps a hint as to the reasons for agonism and antagonism.

*Histamine*

The structures used are shown in Fig. 9. The biology is set out in Table 3. For the purposes of this 'methods' report, a restricted tautomer choice was used in which the 1N-(π)-tautomer of histamine was compared with the 'lowest energy' drug tautomers. Except for burimamide and cimetidine, all other tautomeric drugs were constructed as 1N-tautomers. The closeness of the two cimetidine

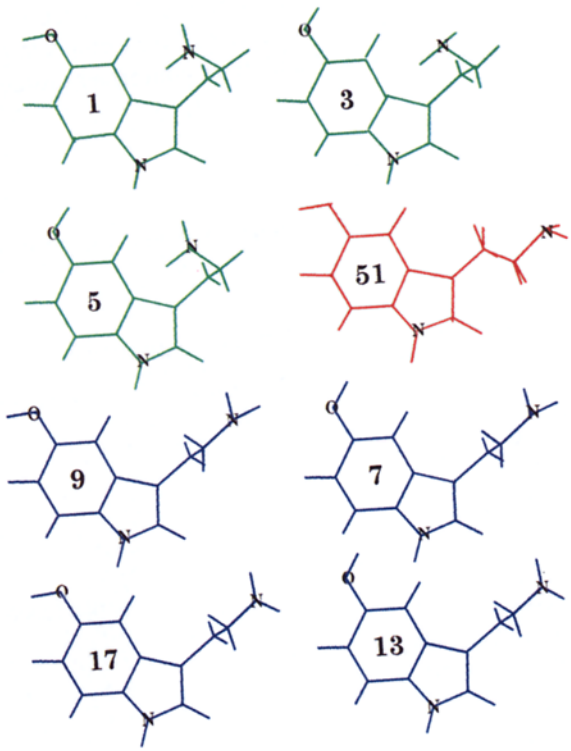


Fig. 8. Structures of the conformations of 5-HT picked out by the selected 5-$HT_{1A/D}$ agonists and antagonists used in this study.

TABLE 2
SELECTED 5-$HT_{1A/1D}$ ACTIVE COMPOUNDS

| Compound | 5-$HT_{1A}$ antagonist $pK_b$ | 5-$HT_{1A}$ agonist $pED_{50}$ | 5-$HT_{1D}$ antagonist $pK_b$ | 5-$HT_{1D}$ agonist $pED_{50}$ | No. of conformers used |
|---|---|---|---|---|---|
| 8-OH-DPAT[a] | | 8.2[b] | | 5.8[b] | 61 |
| (*S*)-5-F-8-OH-DPAT | 5.2[c] | partial agonist | | | 46 |
| (*R*)-5-F-8-OH-DPAT | | 6.1[c] | | | 61 |
| Methysergide | | 6.4[b] | | 7.0[b] | 11 |
| Sumatriptan | | 5.6[b] | | 7.0[b] | 28 |
| GR85548 | | ~7[c] | | ~7[c] | 30 |
| NAN190 | 8.9[b] | partial agonist | | | 21 |
| Methiothepin | 7.7[b] | | 7.7[b] | | 4 |
| (*R*)-WAY100135 | 6.6[d] | | | | 46 |
| (*S*)-WAY100135 | 7.6[d] | | | | 44 |
| GR127935 | 6.9[e] | | 8.6[e] | | 60 |

[a] *R*-enantiomer. [b] Ref. 15. [c] Ref. 16. [d] Ref. 17. [e] Ref. 18.

tautomer energies led us to use a pseudo-structure, protonated on both imidazole nitrogens but kept neutral.

Histamine pair comparisons across the first few best-fitting drug conformers are shown in Fig. 10 and colour coded according to common links. At this stage, the analysis had no reference to pharmacology, structure overlay or structural similarity. As with the 5-HT example, preference was given to the stronger link if two links crossed boundaries (for example, conformer 11 of famotidine has a primary link to histamine conformer 24 and was allocated 'green'). In this way, a tentative picture was built up suggesting that 'red' and 'blue' conformers dominated $H_2$ activity, whilst 'cyan' and 'green' conformers were associated with the $H_3$ receptor. It is even possible to speculate that the 'cyan' and 'red' conformers might be agonistic and the 'green' and 'blue' conformers antagonistic [25].

## Discussion

An attempt has been made to define the properties which contribute to nonbonding molecular attraction in a convenient and manageable way. Both Coulombic and surface interactions have been distilled down to four-dimensional points in space and energy. These points have been observed to be exquisitely sensitive to conformation and substitution, providing far more criteria for variability than structure alone. Structures can be regarded as just the skeleton on which these points interact with each other and their surroundings. The methodology begins to reveal why apparently similar structures can show such different pharmacological behaviour and diverse structures are apparently active at a specific site.

Because we are trained in structure perception, it is very difficult to look at a molecule and estimate the form of its interaction fields. It is therefore necessary to define the field and test each structure on this 'field template' before a judgement can be made. If the active 5-$HT_{1D}$ conformations are extracted, and overlaid on their fields, such a template can be created for the 5-$HT_{1D}$ receptor and the conformational composite of a proposed new drug can quickly be compared (the computation is greatly

TABLE 3
SELECTED HISTAMINE $H_{2/3}$ ACTIVE COMPOUNDS

| Compound | H2 antagonist $pK_i$ / $pK_b$ | H2 agonist $pA_{50}$ | H3 antagonist $pK_i$ / $pK_b$ | H3 agonist $pA_{50}$ | No. of conformers used |
|---|---|---|---|---|---|
| Cimetidine | 6.1[a] | | 4.5[b] | | 32 |
| Ranitidine | 6.8[a] | | 4.9[b] | | 28 |
| Famotidine | 7.7[a] | | | | 46 |
| Histamine | | 6.0[b] | – | 7.0[b] | 26 |
| Dimaprit | | 5.0[c] | 5.5[c] | – | 105 |
| Impromidine | 7.7[c] | partial agonist | 7.2[b] | – | 47 |
| Amthamine | | 6.0[d] | | weak[d] | 45 |
| *R*-α-methyl-histamine | | 3.0[e] | | 8.2[e] | 27 |
| *S*-α-methyl-histamine | | 3.3[e] | | 6.1[e] | 28 |
| Imetit | | 3.0[e] | | 8.8[e] | 46 |
| Thioperamide | | | 8.5 | | 21 |
| Burimamide | 5.1 | | 7.2 | | 51 |

[a] Ref. 21. [b] Ref. 22. [c] Ref. 20. [d] Ref. 24. [e] Ref. 23.

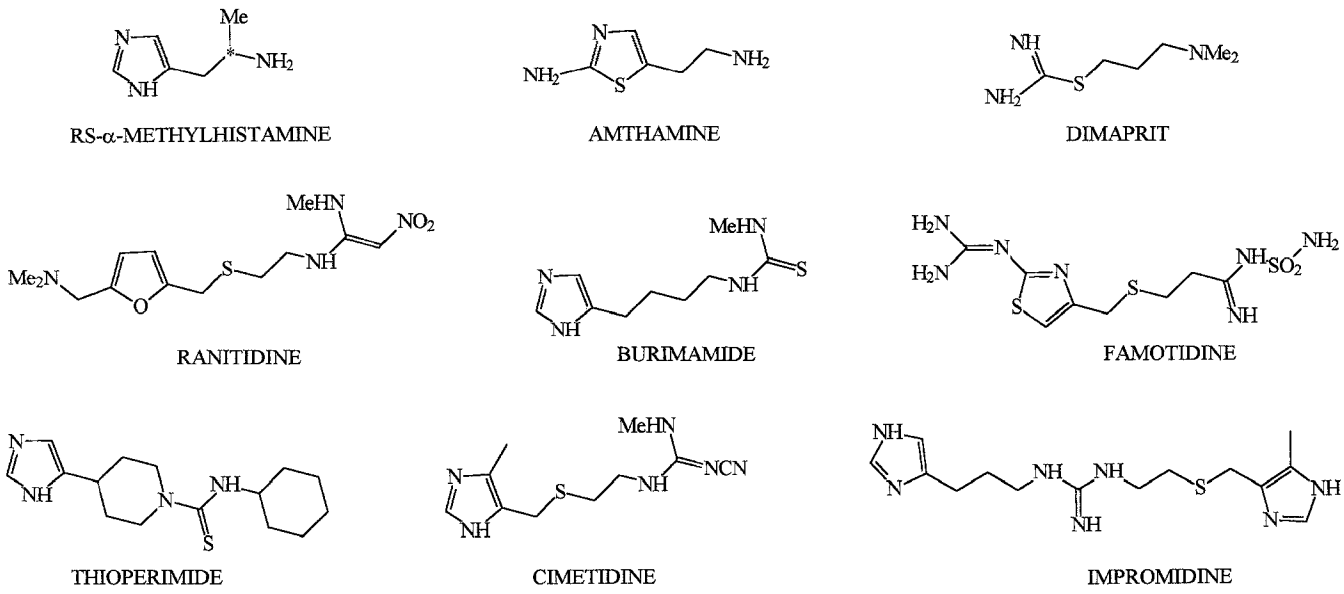


Fig. 9. Structures of the selected histamine $H_{2/3}$ agonists and antagonists used in this study.

decreased when one of the pair of comparison files contains just one 'field conformation'). This process has been carried out for the natural peptide agonist gastrin and its synthetic peptidomimetic antagonists and for the isoenzymes of phosphodiesterase. These studies will be reported in due course.

Although some agonist/antagonist discrimination can be made for the histamine receptors, it is not yet an obvious outcome of the present technique. This may be due to the possibility that histamine uses subtle conformational and tautomeric changes to bring about agonism [25], whilst 5-HT uses some non-ground-state configuration. Alternatively, the technique is not yet sophisticated enough to distinguish agonism and antagonism.

In the exercise reported here, structures that are assumed to act on the same (undefined) site have been investigated. To this end, the polarity of one of a pair is reversed and the pair is then laid one over the other. Preliminary work on docking species, similar to those already reported for XEDs [1], has been attempted where two fields have been free to interact directly (e.g., water onto acetone). The direction and mode of attack have been correctly sustained to within the van der Waals distance, but the final docked arrangement has not always been found to be as close as experiment dictates. This is not surprising. As two docking species close in on each other, the fields distort and reform to reflect the new species. Only when the fields are weak, for example during π–π stacking, do the final field point overlays match the experimental observations. Implicit in this observation is the conclusion that the quantitative prediction of binding strength and possible efficacy is unlikely to be reflected by the Coulombic overlay 'energies' used in this report, although they may prove useful qualitatively. The relationship between overlay energy and affinity is being investigated, but it is worth re-emphasising that the present study cannot be used predictively with any reliability (see below). For example, both cimetidine and ranitidine overlay the supposed $H_3$ conformers of histamine, but their very low affinity cannot be anticipated from Fig. 10.

Comparisons across systems show some interesting phenomena. For example, 5-HT overlays conformer 3 of histamine, as do several conformers of both NAN190 and sumatriptan, but the interactions are restricted to a very limited set of conformers (Fig. 11) and their calculated overlay energies are usually found to be small compared with those across the accepted targets. However, we suspect that some of these cross activities may be 'real' and may either have not been experimentally investigated or are pharmacologically weak but nevertheless present [26]. Indeed, the comparison of Coulombic overlay energies across a range of drugs does seem to have some bearing on the experimental binding values and is a current topic in our research.

We are aware that the methodology has the ability to allow smaller molecules to fit over larger ones, even though they are known not to be pharmacologically active. As already noted, the overlay energies in cases like these are comparatively small. There is probably more specific surface (SIP) control by the receptor than we have at present incorporated, or perhaps the binding strength or the minimum number of binding links has not been accounted for properly. The problem may not be resolvable without more information about the structure and behaviour of the receptor, which leaves our present methodology underdefined.

The applications of this approach can be extended; for

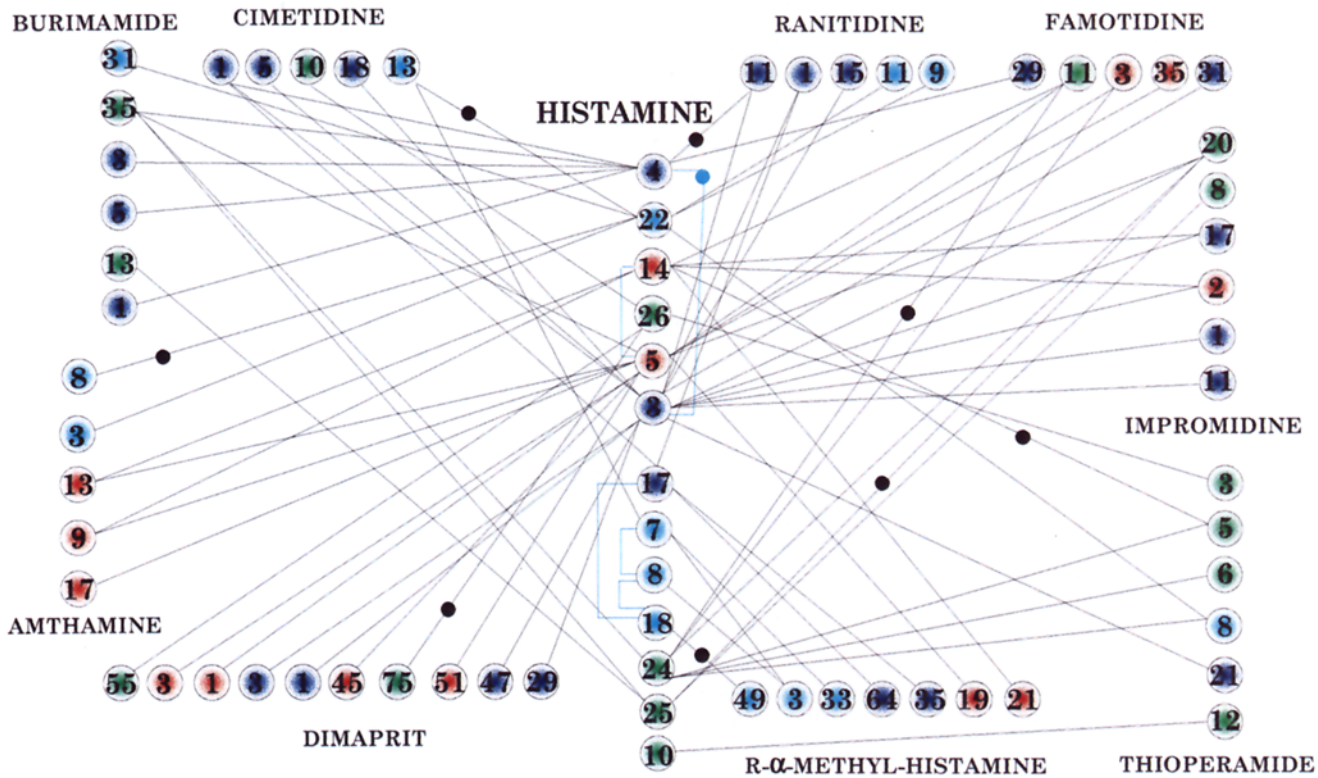


Fig. 10. Analysis of the field comparison of 1N-histamine tautomer conformers with a selection of $H_2$ and $H_3$ agonists and antagonists. Only the top selection of conformers of each drug has been included. The strongest conformer overlays of histamine with itself are shown in cyan lines (see text).

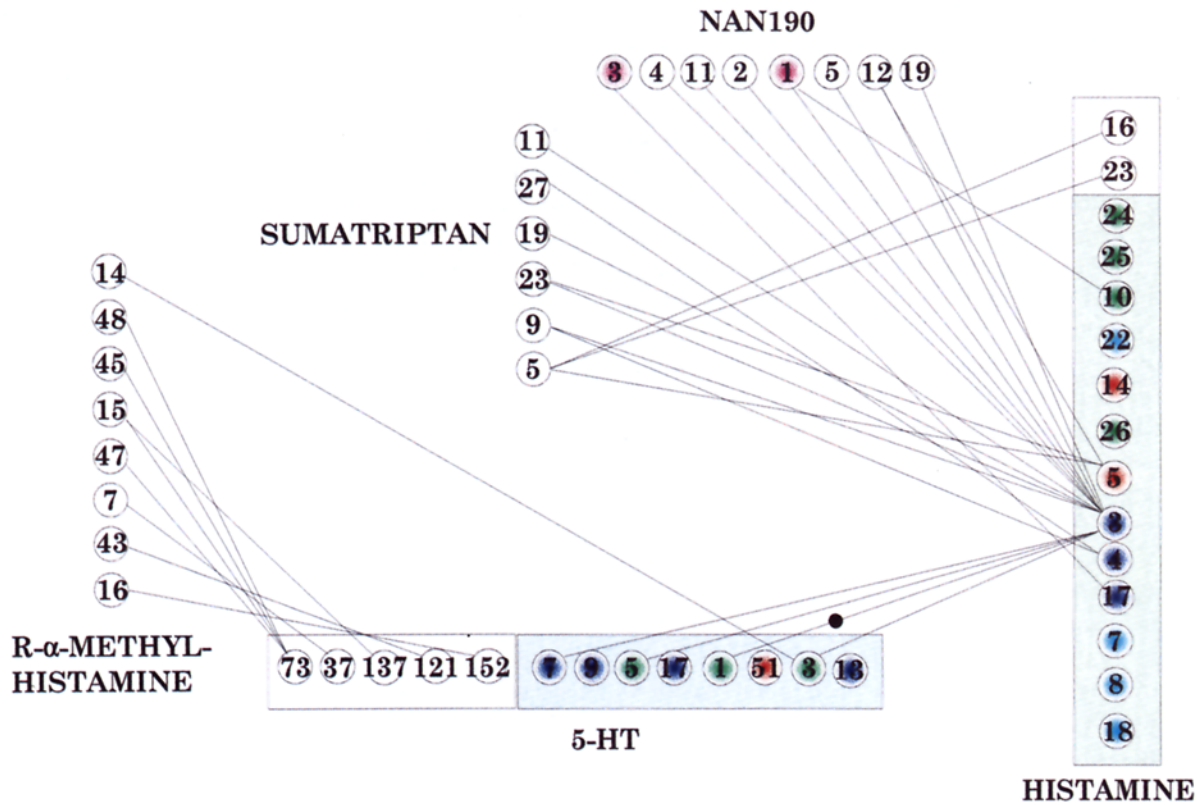


Fig. 11. Cross-correlations of the field comparison of several histamine and 5-HT drugs. The shaded areas contain conformers of histamine and 5-HT which correlate with their accepted drugs (see Figs. 5 and 8).

example, the technique is likely to be more useful than structure comparisons in hunting databases for active compounds. If the technique continues to hold, we will need to check on the importance of single structures (even inflexible molecules) and sometimes regard the interacting molecule more as a 'quantum probability distribution' of structures acting as one binding or reacting entity.

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13 In accordance with the Reaction Isotherm, less than 0.1% of the higher energy conformer will exist at body temperature when it lies 2.7 kcal/mol above the lowest energy conformer.
14 The primary sorting factor, to select the best 50 overlays, uses the Coulombic overlay energy only. Although this is regarded as an 'energy', its quantitative value has no physical significance because such interactions would not occur in nature. Fields of this kind reorganise themselves as the two species closely approach and finally dock. However, recent work (not reported here) has suggested that Coulombic overlay energies may reflect a qualitative binding strength order. Secondary factors, used to order the 50 overlays in different ways, need to (i) take account of the excess or relief of energy necessary for the receptor to incorporate a given conformation; and (ii) consider that an overlay between drug conformers and a specific common conformer may occur more than once and be included in some 'entropic' adjustment. Furthermore, Boltzmann statistics need to be applied across the comparisons. These issues are being addressed.
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