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Molecular field analysis of clozapine analogs in the development of a pharmacophore model of antipsychotic drug action

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In an attempt to elucidate some aspects of clozapine's favorable receptor binding profile, we modeled a series of 30 clozapine analogs using a pharmacophore based on the ligands octoclothepin and tefludazine. Molecular field analysis using CoMFA combined with HINT® was carried out on published D_2 receptor binding affinities. Several alternative alignments of the analogs gave r^2 values in the range of 0.8–0.95. The final model had good predictive abilities with $q^2 > 0.6$ and $r^2 > 0.9$. This provides an excellent framework to aid in the design of novel antipsychotics with diminished propensity to produce clinically limiting side effects. © 2001 by Elsevier Science Inc.

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INTRODUCTION

Schizophrenia is a debilitating disease that affects approximately 1% of the population, with the onset of the disease occurring usually in the mid-20s and persisting in many cases for the lifetime of the patient.[1] Antipsychotic drugs alleviate schizophrenia by antagonizing dopaminergic mesolimbic pathways in the brain, which are thought to be involved in the positive symptoms of schizophrenia. A significant drawback of many antipsychotic drugs is that they also antagonize dopaminergic striatal pathways resulting in extrapyramidal side-effects (EPS). Selective blockade of mesolimbic dopaminergic receptors is but one of several hypotheses thought to explain the action of antipsychotic drugs. Other hypotheses that have been made as adjuncts to the dopamine hypothesis include: a requirement for anticholinergic (M_1) activity,[2] selectivity for serotonin ($5HT_{2A}$) over dopamine (D_2) receptors,[3] a combination of $5HT_{2A}$ and α_1-adrenergic antagonist activity,[2] or a relatively higher affinity for D_4 over D_2.[4]

Many structural classes of antipsychotics have been developed; prominent among these classes is the dibenzepine type, in which the central seven-membered ring is substituted with oxygen, nitrogen, sulphur, or carbon.[5] A significant number of these dibenzepines also contain a piperidine or piperazine ring attached to the seven-membered ring. One recently reintroduced antipsychotic drug of the dibenzepine type, clozapine (Figure 1), has shown the most promise, particularly in treatment-resistant schizophrenia, due to its low incidence of EPS.[6] The exact basis for the activity of clozapine is unknown, but the fact that it acts at a range of receptors and their subtypes (H, $5HT_{2A}$, $5HT_{1C}$, α_1, M_1, D_2, D_4,)[7] suggests that a complex blend of interactions is required for its antipsychotic drug efficacy. A consequence of clozapine's affinity to such a large variety of receptors is the production of several clinically limiting side effects such as sedation, hypersalivation, and tachycardia.[8]

In this article Comparative Molecular Field Analysis (CoMFA) is used to develop a pseudoreceptor model for D_2 antagonist activity using published data from a series of clozapine analogs,[9,10] in an attempt to elucidate some aspects of clozapine's favorable receptor binding profile. The pharmacophore model was based on (R)- and (S)-octoclothepin[11] as representative molecules of the dibenzepine type, and tefludazine (Figure 1).[11] These molecules have high potency at

Color Plates for this article are on page 468.

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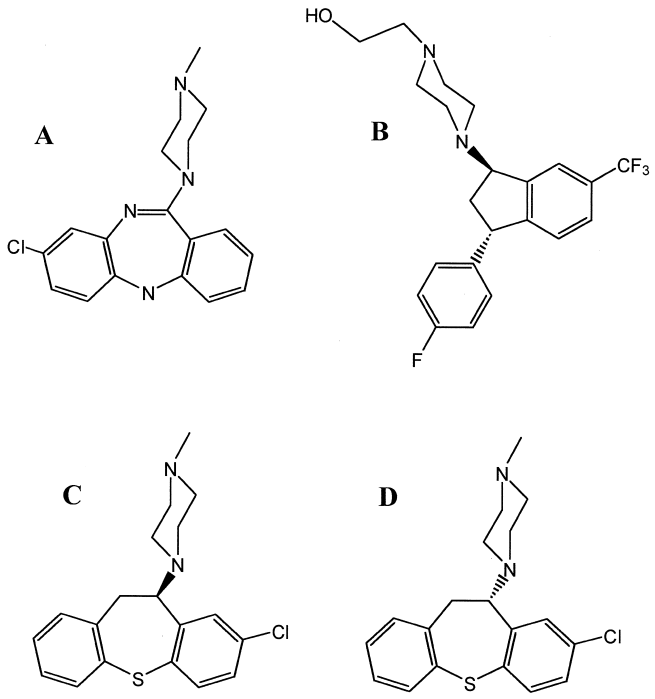


Figure 1. A clozapine, B (1R,3S)-tefludazine, C (R)-octoclothepin, D (S)-octoclothepin.

dopamine D_2 receptors and their differing stereoselective potencies have potential in establishing a binding pattern.[12] The octoclothepin isomers also provide a similar overall framework to clozapine, to which clozapine should presumably conform closely in its binding at the D_2 receptor. The structurally dissimilar tefludazine, whose potency resides in just one diastereomer (1R,3S), provides a flexible template for reducing the wide range of pharmacophore models produced by the octoclothepin conformers.

METHODS

Conformational Analysis

Structures for (R)- and (S)-octoclothepin[13] enantiomers were obtained from the Cambridge Crystallographic Database.[14] Conformational analysis of the central tricyclic ring was simulated with a seven-membered ring using the subroutine *Find Transition State* in the program CAChe, version 3.1.[15] Simulated annealing, for ten cycles to 1,000K using default parameters for base temperature and cooling rate, was undertaken with Sybyl, version 6.5[16] to reveal other possible conformations of the tricyclic structure.

Using ten different local minimum arrangements we performed a systematic search using the Tripos force field[17] in Sybyl. Van der Waals radius scale factors were set at 0.65, using charges assigned by the Gasteiger and Marselli method.[18] The search was performed in 1° increments on all conformations and configurations of octoclothepin around the bond to the piperazine ring. Torsion angle versus energy plots were then produced showing the location of the local and global minima.

These local and global minima were further analyzed using MacroModel, version 5[19] and Amsol, version 6.6. [20] The MacroModel parameters used were the OPLS force field and water as solvent. The Amsol parameters used were the AM1 semiempirical method with water as solvent and heptane as solvent. This was undertaken to compare and contrast the conformational energies in various solutions.

Superimposition: Defining the Pharmacophore

To develop a pharmacophore for D_2 antagonist interaction, the different conformations and configurations generated from the previous analysis were compared by superimposition. Dummy atoms were used to represent points of likely interaction[21] at the D_2 receptor. To mimic π–π interactions dummy atoms were built 3.5 Å above and below the planes of each aromatic ring.[22] An additional dummy atom was built 2.8 Å from the distal nitrogen of the piperazine ring, along the vector of the ammonium hydrogen, for a proton donor–acceptor interaction.[22] The pK_a of the distal nitrogen was calculated to be 8.4 ± 0.7, using ACD, version 3[23] pK_a calculator, and octoclothepin isomers are therefore likely to consist of an equilibrium mixture containing approximately 90% protonated molecules at a physiological pH of 7.4. All conformations where then superimposed onto selected differing configurations using the root mean square (RMS) fit procedure within Sybyl. A weighting of 1 was assigned to the dummy atom from the distal nitrogen and weightings of 0.3 were assigned to the dummy atom pairs above and below the planes of each aromatic ring. This was done so that the tricyclic ring of the enantiomers, with four dummy superimposition points, had approximately the same priority as the dummy atom emanating from the distal nitrogen.

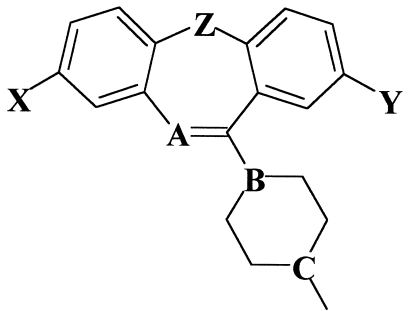
The active (1R,3S) tefludazine diastereomer[24] was built in Sybyl using standard geometries, then had dummy atoms assigned to its points of likely interaction. Superimpositioning using similar weightings of its low energy conformers upon the resulting octoclothepin pharmacophore models was undertaken. A pharmacophore model was defined from this and a search of the Cambridge Crystallographic Database for conformational information.

All different combinations and conformations of key functional groups were considered in the superimposition process with the aim of developing a consensus pharmacophore. However, it is recognized that less likely alternatives may exist for isolated compounds.

Molecular Field Analysis

CoMFA was performed on the series of 30 clozapine analogs given in Table 1 and Table 2 using their published D_2 receptor binding affinities.[9,10] The program Hydropathic INTeractions (*HINT*®), version 2.3S[25,26] was used to generate hydrophobicity fields for use in the analyses. Several alternative alignments of the analogs were developed using RMS superimposition and field fitting of the ligands. The RMS fit procedure within Sybyl was used to align the ligands to the pharmacophore developed from the conformational analysis and superimpositions described above. Two sets of superimpositions were considered, the first using five dummy atoms (Du) and the second using three dummy atoms (*Du). Additional alignments were developed using the field fit method[27] from Sybyl. Molecules were minimized with respect to the electronic and steric environment of the tightest binding ligand (compound 3B from Table 1) using *MAXIMIN2* with field fit on and the Powell minimization

Table 1. D_2 Receptor Binding Affinities of Piperazine Analogues



Compound	X	Y	Z†	A	B	C	D_2 Binding (K_i, nM)
1A (clozapine)	Cl	H	NH	N	N	N	220
1B	H	Cl	NH	N	N	N	47
1C	H	H	NH	N	N	N	2500
1D	Cl	H	NCH_3	N	N	N	1100
1E	Cl	H	O	N	N	N	150
1F	H	Cl	O	N	N	N	21
2A	Cl	H	CH_2	CH	CH	N	560
2B	Cl	H	CH_2	CH	N	CH	1400
3A	Cl	H	CH_2	CH	N	N	520
3B	H	Cl	CH_2	CH	N	N	1
3C	H	H	$CHCH_3$	CH	N	N	94
3D	H	H	$C{=}CH_2$	CH	N	N	57
3F	Cl	H	$C{=}C(CH_3)_2$	CH	N	N	690
3G	H	H	$C{=}C(CH_3)_2$	CH	N	N	290
3H	Cl	H	O	CH	N	N	8.7
3I	H	Cl	O	CH	N	N	2.5
3J	H	H	O	CH	N	N	21
P8*	H	H	O	CH	CH	N	82

* indicates compound from Phillips *et al* 1995, numbered P1-P8 in order of new compounds presented.
† bold atom represents the atom in ring (Z).

method until they reached an RMS-displacement termination criterion of 0.001 Å. Molecules were then reminimized using *MAXIMIN2* without field fit so that the molecules were not forced into unnatural conformations. The Powell minimization method was again used for various numbers of iterations (10–1,000) and a variety of RMS-gradient termination criteria (0.01–0.5), producing a number of differing databases.

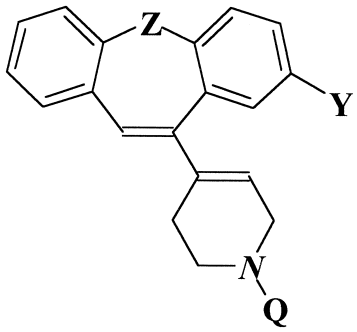
Quantitative Structure Activity Relationship (QSAR) spreadsheets were then created for each data set. The default values for CoMFA were used: a grid spacing of 2 Å, an sp^3 carbon probe with a +1 charge, box smoothing, and standard CoMFA scaling. The default values for *HINT* were used: a grid spacing of 1 Å, using all atoms, calculated partition constant, box smoothing, and a van der Waals limit of 1.0. *LogD*, the octanol–water partition coefficient at a selected pH, a measure of a compound's permeability[28] or lipophilicity was calculated using the logD calculator from ACD.[23] Partial Least Squares (PLS) analysis was performed on each database. Leave-one-out (LOO) cross-validation was used to select the number of principal components for cross-validated statistics with column filtering set at 2.0 kcal/mol. The final CoMFA model was generated using no cross-validation and the number of components suggested by the LOO validation run.[29] *HINT* fields were generated using a grid spacing of 1 Å due to the sensitivity to distance of the hydrophobic fields.[25] CoMFA electrostatic and steric fields were generated with a grid spacing of 2 Å to simplify the model, because grid spacing of 1 Å generated a multitude of smaller areas of supposable interaction.

RESULTS AND DISCUSSION

Conformational Analysis

All conformations of (R)- and (S)-octoclothepin were examined because, although often considered to be rigid, the octoclothepin structures exhibit flexibility in several regions. First, the piperazine ring is able to rotate around the connecting bond that attaches it to the central seven-membered ring of the tricyclic structure (Figure 2). Concomitantly, the tricyclic structure is also able to undergo a ring inversion,[30] resulting in A-fold and B-fold types (Figure 2) as seen from the simulated annealing process. Irurre et al.[31] experimentally determined the conformational energy inversion barrier for dibenzo[*b*,*e*]azepine derivatives to be ~12 kcal/mol and higher. This suggests

Table 2. D_2 Receptor Binding Affinities of Piperidine Analogues



Compound	Y	Z†	Q	D_2 Binding (K_i, nM)
4A	H	C=CH_2	CH_3	250
4B(r)	H	$CHCH_3$	CH_3	640
4B(s)	H	$CHCH_3$	CH_3	730
4C(pR_apS_b)	H	C═$C(CH_3)_2$	CH_3	520
4C(pS_apR_b)	H	C═$C(CH_3)_2$	CH_3	790
P1*	H	CH_2	CH_3	89
P2*	Cl	C═$C(CH_3)_2$	CH_3	440
P3*	H	CH_2	CH_2CH_3	44
P4*	H	CH_2	CH_2CH═CH_2	83
P5*	H	O	CH_3	230
P6*	H	O	CH_2CH_3	61
P7*	H	O	CH_2CH═CH_2	110

* indicates compound from Phillips *et al* 1995, numbered P1-P8 in order of new compounds presented.
† bold atom represents the atom in ring (Z).

that this ring inversion will occur under physiological conditions. Second, the piperazine ring is able to adopt either a pseudoaxial or pseudoequatorial state with respect to the tricyclic structure, shown in Figure 3. This was calculated to require an energy input of approximately 9 kcal/mole using the "find transition state" process in CAChe. This compares favorably with results obtained previously by Hendrickson,[30] who calculated that the chair/twist-chair and boat/twist-boat families in cycloheptane can be interconverted by passing over a barrier of 8.5 kcal/mol.

The conformational analysis gave ten low-energy conformations for the enantiomers, shown in Figure 4. The relative energies of the differing conformations using different methods of calculation and different solvents are shown in Table 3.

The first column of Table 3 shows the heat of formation *in vacuo* calculated using the Tripos force field with Gasteiger

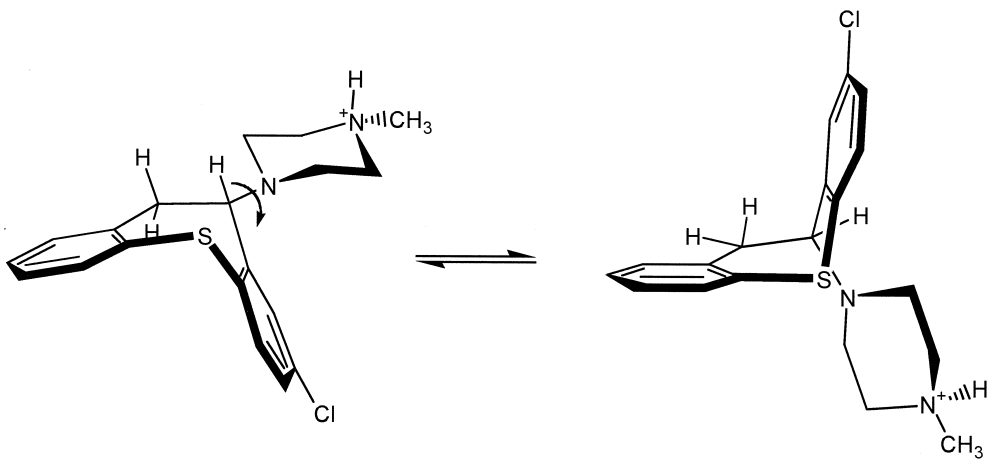


Figure 2. Piperazine ring rotation and tricyclic ring system inversion.

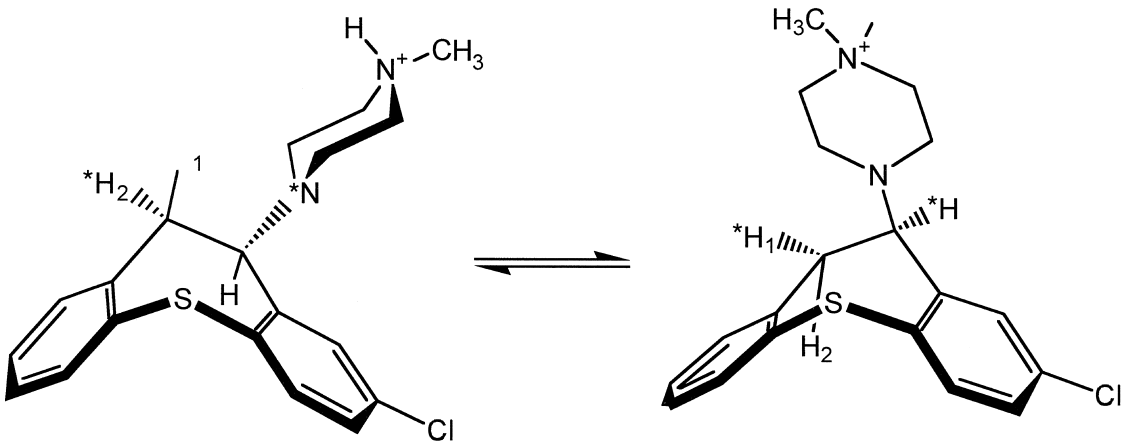


Figure 3. A-fold conformer of (R)-octoclothepin showing alternative pseudoequatorial and pseudoaxial orientations of the piperazine ring. H or N* denotes equatorial position.*

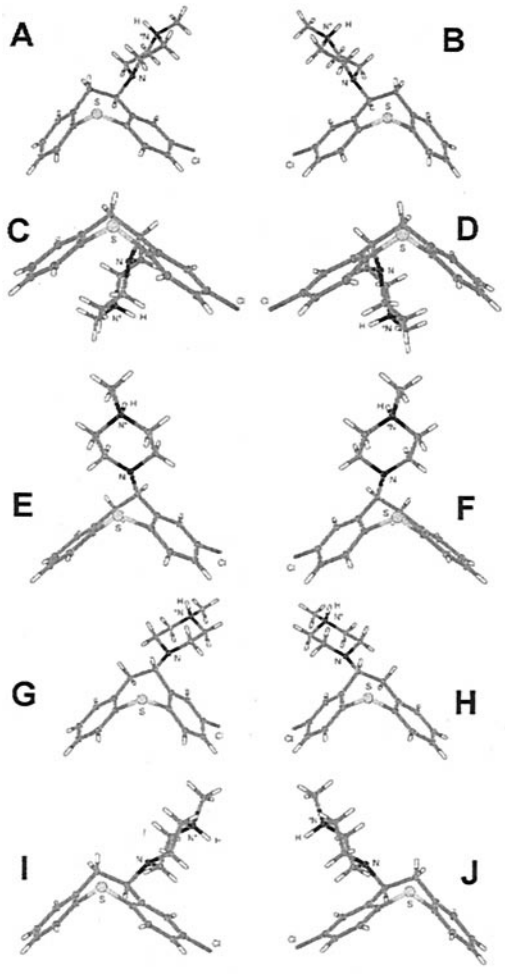


Figure 4. Ten main local and global minimum energy conformers of (R)- and (S)-octoclothepin. A, (R)- A-fold equatorial; B, (S)- B-fold equatorial; C, (S)- A-fold axial; D, (R)- B-fold axial; E, (R)- A-fold axial; F, (S)- B-fold axial; G, (S)- A-fold equatorial; H, (R)- B-fold equatorial; I, (R)- A-fold equatorial; J, (S)- B-fold equatorial.

and Marsili charges, indicating that the global minimum of (R)- and (S)-octoclothepin are the A-fold pseudoequatorial conformer (A) and the B-fold pseudoequatorial conformer (B), respectively.

The results for the final heat of formation in vacuo from Amsol were in a different order of increasing energy compared with those from the Tripos force field, although there was only a 3.9 kcal/mol difference between the lowest and highest energy conformations. The results for the conformational energies in solution (water) from Amsol gave a similar order as MacroModel (water), with a 3.7-kcal./mol range between the lowest and highest energy conformations compared with a 4.2-kcal./mol range from MacroModel. The results for conformational energies in solution (heptane) from Amsol also showed a range of less than 5 kcal./mol between lowest and highest energy conformations and gave the same global minimum as most other methods.

The results obtained from MacroModel for the conformational energies in vacuo were different from those obtained with the Tripos force field with respect to the order of increasing energy. However, the global minima were the same for both (R)- and (S)-octoclothepin.

These semi-empirical and molecular mechanics calculations are all in good agreement with one another, and all have a range of less than 5 kcal./mol between all conformations; five out of six methods give the same global minimum.

The global minimum of (S)-octoclothepin, conformer (B), was consistent with the crystal structure obtained from the Cambridge Crystallographic Database.

Superimpositions

As (R)- and (S)-octoclothepin are both active at the D_2 receptor, it is assumed here that they are acting in a conformationally similar manner. We therefore performed RMS superimpositions of the R conformers onto the S conformers to find pairs of conformers that are capable of interacting with common sites in the receptor.

The best RMS fit, with a value of 0.45 Å, resulted from the superimposition of all five dummy atoms of the A-fold (S)-octoclothepin pseudoequatorial, conformer G, on the global

Table 3. Relative Energies in kcal./mol using Differing Methods and Solvents

Ligands	Tripos (SS) Vacuum	Amsol Vacuum	Amsol Water	Amsol Heptane	MacroModel Vacuum	MacroModel Water
A & B	0	3.0	0	0	0	0
C & D	0.3	0.4	3.3	1.7	5.4	4.2
E & F	0.8	0	2.7	1.8	2.7	3.1
G & H	2.1	3.4	0.6	0.2	3.4	3.0
I & J	5.0	3.9	0.8	0.4	2.4	3.0

(SS) denotes energies from systematic searches.

minimum A-fold (R)-octoclothepin pseudoequatorial, conformer A (Figure 5), and the mirror-image B-fold (R)-octoclothepin pseudoequatorial, conformer H, on the global minima B-fold (S)-octoclothepin, conformer B, (not shown). This indicates that these are strong candidates for the relevant conformation mediating antipsychotic activity.

Defining the Pharmacophore

To differentiate between the mirror-image pharmacophore models generated above, we superimposed low-energy conformations of the D_2 active compound (1R,3S)-tefludazine onto the pharmacophores. This gave a better fit with the A-fold conformation, suggesting that this conformation is more likely to represent the biologically active one. Figure 6 shows the resulting superimposition of the R and S enantiomers of octoclothepin and (1R,3S)-tefludazine.

A search of the Cambridge Crystallographic Database revealed one conjugated diene of the type in the Phillips data set,[9] with an A-fold conformation that supported the model used in this article. All other structures of conjugated dienes[32–36] similar to the type in Table 2 that were neither sterically hindered nor co-crystallized with counter ions, crystallized in a pseudo-trans arrangement about their single bond, adding more weight to the proposed conformations of these ligands.

Comparison with Other D_2 Antagonist Pharmacophores

Figure 7 shows the distances between the likely points of interaction in the derived D_2 pharmacophore. This model is an extension of the model developed by Bøgesø et al.,[11] with the exception that we use the global minimum energy conformation of (R)-octoclothepin and dummy atoms representing likely points of interaction for superimpositions. Differing weightings were also used in the RMS alignment of the ligands. Although the resulting models look similar, the position of the distal nitrogen in the model generated here covers a circular area with a diameter of approximately 2.0 Å, resulting in distances from the center of the aromatic ring to the nitrogen of between 5.4 and 6.7 Å. These distances are in agreement with some of the

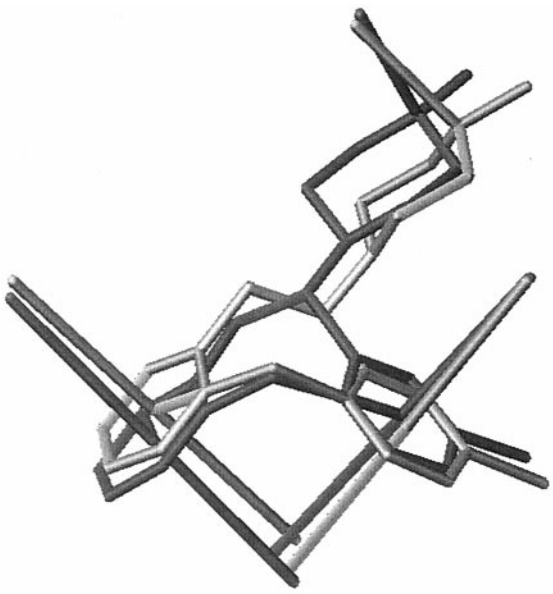


Figure 5. Superimposition of A-fold (R)-, light shading, and (S)-, dark shading, octoclothepin showing dummy atoms.

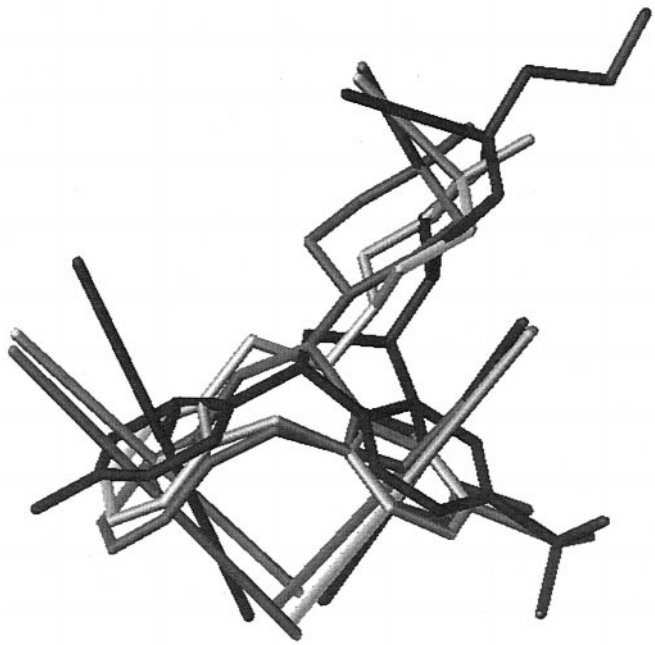


Figure 6. Tefludazine, black, superimposed on A-fold (R)-octoclothepin, light shading, and (S)-octoclothepin, dark shading.

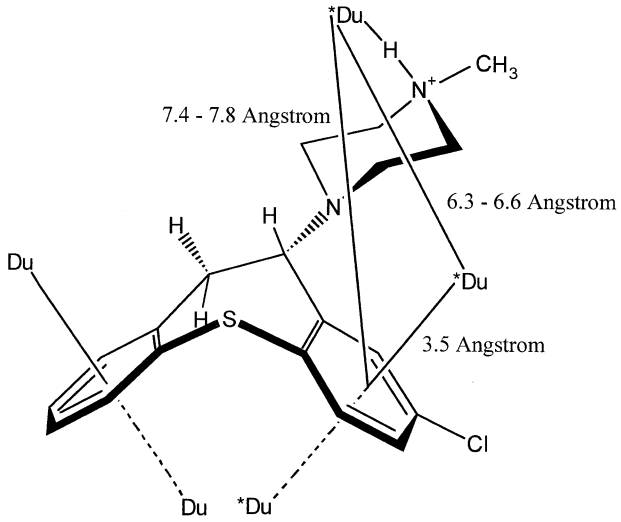


Figure 7. Proposed D_2 antagonist pharmacophore.

older models for antipsychotic activity that have been generated, such as the Lloyd /Andrews model,[37] which has a distance of 5.2 Å from nitrogen to aromatic centroid.

Molecular Field Analysis

CoMFA analyses were performed on more than 20 data sets using *HINT*, steric and electrostatic fields, and logD. Initial studies of the data sets, created from three- and five-point superimpositioning, obtained reasonable r^2 values varying between 0.65 and 0.85. However, the predictive abilities of these models (q^2) were only fair with typical values being between 0.25 and 0.4. Nevertheless, the steric and hydrophobic CoMFA fields predicted by these models were relatively consistent, with the only significant variations being in the placement of the electrostatic fields that made a lower contribution to the model. When only steric and electrostatic fields were included in the model, the contributions were approximately 60 and 40%, respectively. This indicated that more work was needed on the alignment of the molecules to improve predictive abilities.

In an attempt to improve the initial model, we investigated the field fit method. Field fitting molecules into the steric and electronic environments of the tightest binding ligand (compound 3B, Table 1) causes several problems if the molecules in question are not of comparable architecture. For example, in the case of molecules 2B and 4C(pS_apR_b), 2B lacks a protonated distal nitrogen. The program therefore tries to fit the proximal protonated nitrogen of the piperidine ring into the electronic environment of the protonated distal nitrogen of 3B, causing major structural abnormalities. 4C(pS_apR_b) also has problems as it is in the B-fold conformation and is badly deformed when fitted into the steric boundaries of the A-fold ligand 3B. These problems were overcome by using molecule 2A as a template for 2B, and by using the mirror image of molecule 4C(pR_apS_b) as the 4C(pS_apR_b) template.

As field fitting distorts the molecules into the steric and electronic environments of the tightest binding ligand, some minimization must take place after field fitting to ensure that a high-energy, unnatural conformation is not obtained. Various termination criteria and ranges were tried with the best CoMFA model being obtained after a minimization run of 100 iterations. Other minimizations of lesser numbers of iterations produced conformations that were 15 kcal/mol or more above their local minimum. As a 10 kcal/mol difference between a conformer and its local minima is considered to be a reasonable cutoff,[38] these conformers were not considered. When the molecules in the databases were minimized for more than 100 iterations, the piperazine ring often rotated around the bond linking it to the seven-membered ring so that the dummy atom from the distal nitrogen no longer fitted within the constraints of the pharmacophore.

Table 4 shows four combinations of fields generated from the field fit method after 100 iterations, used in the generation of the CoMFA model. Analyses I, II, and III all show good predictive capabilities, with $q^2 > 0.54$, indicating that the steric

Table 4. Field Fit after 100 Iterations for Different Combinations of Fields

Analysis	I	II	III	IV
Components	Steric Electrostatic HINT	Steric Electrostatic	Steric HINT	Electrostatic HINT
q^2	0.584	0.601	0.549	0.321
SEP	0.552	0.540	0.563	0.719
N	4	4	3	5
r^2	0.886	0.904	0.808	0.866
SEE	0.289	0.265	0.367	0.320
F	48.6	59.1	36.1	30.9
Contributions				
Steric	0.391	0.505	0.505	
Electrostatic	0.302	0.495		0.490
HINT	0.307		0.495	0.510

The LOO cross-validated r^2 is denoted q^2; SEP is the standard error of prediction; N is the number of components used in the PLS analysis; r^2 is the non cross validated value; SEE is the standard error of estimation; F is the F-statistic for the analysis; "Contributions" gives the relative contributions of the fields.

contribution to the model is important. Analysis IV does not have a steric contribution to its model; although the r^2 of 0.866 is good, the predictive capabilities of the model are poor.

The *HINT* hydrophobic/polar fields do not improve the model. However they do provide an insight as to other possible contributions. Models generated including logD as a lipophilicity parameter, at pH 7.4, produced logD contributions in the range of 2 to 10%. This may indicate that the lipophilicity parameter, logD, was of little importance. However, this could be because activity testing is done in vitro and not in vivo, and the drugs do not have to permeate any barriers. This does not seem to be the case when *HINT* is used to three-dimensionally model lipophilicity parameters, giving contributions of around 30%.

CoMFA Field Interpretations

Analysis II in Table 4 was found to be the best model with the highest q^2, r^2 and the lowest standard error of prediction. The predicted activities of all compounds except for one are within ± 0.5 pK_i log units of their measured values. The exception is compound 3A, with a residual of -0.63. The electrostatic map (Color Plate 1) shows regions where partial negative charge enhances activity (red) in the following locations: near functional group Y, indicating the benefits of chlorine, as is the case for iso-clozapine (1B) compared with compound 1C; near position B from an unprotonated nitrogen heteroatom; and above position Z, indicating the favorable interaction due to lone pairs from contributing heteroatoms.

The electrostatic map (Color Plate 1) also shows regions where positive charge enhances activity (blue): on the carbon near position Y from an electron withdrawing chlorine substituent imparting a partial positive charge; on the carbon atom to the left of position Z from an electron withdrawing substituent imparting a partial positive charge; and a very small contribution near position X showing the possible detrimental effect of having a chlorine substituent.

The steric map (Color Plate 2) shows a yellow region indicating areas where steric hindrance occurs above position Z, indicating unfavorable interactions that are occurring with bulky alkyl groups. This can easily be seen when comparing compounds 4B(R) and 4B(S) with compound P1, where the activity drops almost tenfold with the addition of a methyl group at position Z.

The steric map (Color Plate 2) also shows green regions where extra bulk is favorable. Around position Y there is a large area indicating where a bulky substituent (chlorine) would be favorable, and to the top and right of the piperazine ring there is an area for favorable ring rotation around the connecting bond.

Although analysis I is not quite as statistically significant as analysis II, it does shed a different light on the model in showing other possible contributions. The *HINT* map (Color Plate 3) shows purple regions where hydrophobic interactions are more favorable to the binding of the ligand around position A, indicating that a nitrogen in this position does not aid binding. This is seen when comparing compounds 1E to 3H and 1F to 3I, and around position Y. However, a favorable hydrophobic interaction in this location is of some concern since no compounds with, for example, a methyl group at position Y, have been included in the analyses. A compound of this type would have to be included for a more definitive analysis. Overall, the variation of hydrophobicity at this site does appear to have some bearing on activity.

The *HINT* map (Color Plate 3) shows orange regions indicating where hydrophilic or polar interactions are favorable to binding, particularly above position Z, indicating a possible polar interaction from the lone pairs from contributing heteroatoms.

CONCLUSIONS

We developed a pharmacophore model that shows how (R)- and (S)-octoclothepin can fit together using superimpositioning of the points of interaction of the compounds. This was then used to develop a CoMFA model considering three- and five-point superimpositions and the field fit method.

An improvement in the predictive capabilities of the models going from five point to three point to the field fit method, indicates that a significantly better alignment and CoMFA model of the molecules is attained by the field fit method.

The resulting model provides an excellent framework to aid in the design of new antipsychotics and D_2 antagonist ligands. Extensions of the themes produced by the model could be undertaken to increase the binding affinity, such as adding larger, more electronegative hydrophobic substituents at position Y, ensuring that a hydrophobic group is kept at position A, keeping nitrogens in positions B and C, and maintaining a small electronegative heteroatom at position Z.

Clozapine medications usually require a high-dose regimen[1] compared with typical antipsychotic drugs. By manipulating the balance between hydrophobic and hydrophilic group requirements at different positions in accordance with this CoMFA analysis, it may be possible to develop a clozapine analog with optimal bioavailability at reduced dose. This may have the advantage of reducing side effects through decreased interactions at the receptors involved.

The results of this CoMFA study also suggest that a small electronegative hydrophilic substituent in place of the nitrogen at position Z in clozapine may produce a clozapine analog of comparable D_2-receptor binding affinity. A consequence of removing this nitrogen could be the potential to diminish clozapine's major clinical side effect, since there is evidence that formation of a nitrenium ion at this position is involved in the mechanism of agranulocytosis.[39] Replacement of the nitrogen with other moieties predicted by this model may give diminished propensity to produce agranulocytosis.

Clozapine interacts with a multitude of receptors and exhibits limbic selectivity, which could be due to its preference for D_4 receptors and their location mainly in the mesolimbic regions.[40] Further studies are being undertaken at alternative receptors (D_1, D_3, D_4 and $5HT_{2A}$, $5HT_{2C}$ and $5HT_3$) using the binding affinities from the studies of Phillips et al.,[9,10] to define the pharmacophore requirements at these receptors. In this respect, another CoMFA study that has been conducted on heterocyclic analogs of clozapine,[41] could, in combination with the current study help identify pharmacophore binding groups capable of interacting at complementary sites in proposed receptor models.[42]

Overall, this work should hopefully lead to the development of novel antipsychotics that have greater efficacy against treatment-resistant schizophrenia and are devoid of some of clozapine's clinically limiting side effects.

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