Molecular Design Using the Minireceptor Concept

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Explicit molecular binding pockets were constructed and optimized around sets of superimposed ligands using the minireceptor concept. The resulting binding sites incorporate the properties of the different ligands and were shown to be suitable for the design of molecules presenting novel interaction patterns. Two applications of minireceptor construction and/or optimization, followed by molecular design are described. In the pursuit of new ligands mimicking the action of paclitaxel, a minireceptor was constructed using the primary amino acid sequence of the target protein as a guide. The active site extracted from a homology-based model of the serotonin 5-HT_{1A} receptor was optimized around a set of three ligands using the same approach.

INTRODUCTION

In structure-based molecular design, an analysis of specific interactions between candidate ligands and their intended target is used to optimize the affinity of existing leads or to identify entirely new leads.[1] Information concerning the three-dimensional (3D) structure of the target is crucial and may be obtained through direct experimental measurements (e.g., X-ray crystallography or NMR spectroscopy)[1,2] or alternatively homology modeling using the primary amino acid sequence of the target protein together with an experimentally determined 3D structure of a close analogue. The latter approach is widely used in the area of G-protein-coupled receptors (GPCRs) employing bacteriorhodopsin as the template structure.[3−5]

In cases where no structural information on the target is available, indirect approaches have been applied in which a model of the receptor structure is inferred or mapped based on what binds to it. A well-known example of such an indirect method is the *Active Analogue Approach* (AAA):[6] a systematic way to derive pharmacophoric patterns while taking into account the conformational flexibility of the ligands. Orientation and superposition of all active ligands in their proposed receptor-bound conformation allows the determination of the *receptor-excluded volume*: the volume available to ligands within the active site. The difference between the volume of inactive ligands in the conformation consistent with the pharmacophoric pattern and the receptor-excluded volume (from all active ligands) defines the *receptor-essential volume*, volume not available to ligands.

In an attempt to translate the information from indirect models to a target structure, the minireceptor approach was developed.[7−9] This approach allows one to build a hypothetical binding pocket around any molecular ensemble of interest, such as a set of ligands superimposed in their pharmacophoric conformation. The resulting binding pockets can subsequently be used for *de novo* design of new compounds[10,11] and for searching 3D databases for structures complementary to this site[12−14] in much the same way as models of target structures derived from the methods mentioned above. In addition to the construction of completely novel binding pockets, the minireceptor approach can also be used to optimize binding pockets derived from other (computational or experimental) methods for subsequent molecular design.

MINIRECEPTOR CONSTRUCTION

A minireceptor constructed around a set of superimposed ligands need not resemble its natural counterpart but should accommodate a series of ligands in a similar binding sense. A thorough conformational analysis and an objective pharmacophore search are therefore of the utmost importance to ensure a high quality starting point and to verify whether that particular set of ligands has a common binding mode.[15]

The construction of binding pockets in the minireceptor modeling program Yak[16] relies heavily on the directionality of ligand receptor interactions which is implemented by (1) the generation of vectors associated with directional interactions (hydrogen bonds, hydrophobic interactions) around each ligand, (2) the analysis of clustering of those vectors for all superimposed ligands, and (3) the use of the Yeti force field[17] which includes directional terms for hydrogen bonds, salt linkages, and metal−ligand interactions. Points in space where many vectors cluster are considered to be relevant for receptor binding. A residue can be placed at the endpoint of a vector to provide a specific interaction, and the position of that residue with respect to all ligands is optimized using the Yeti force field. Also vectors that represent auxiliary binding sites (not necessarily present in all ligands) can be used to place appropriate residues. The choice of suitable residues to incorporate in the minireceptor model may be guided by experimental data. For example, site directed

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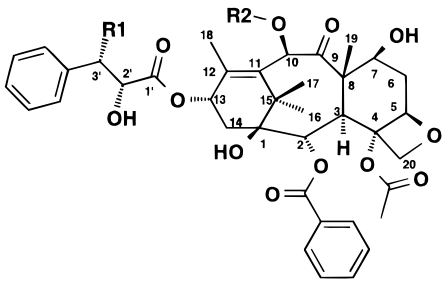


	R1	R2
1, paclitaxel	NHCOPh	COMe
2, docetaxel	NHCOO-*t*-Bu	H

Figure 1. Structures of paclitaxel and docetaxel.

mutagenesis experiments may identify specific amino acid residues which markedly influence affinity and therefore are likely to line the binding site. In the absence of such data, the choice can be guided by the electrostatic and molecular lipophilicity potentials estimated by Yak and a knowledge-base of frequently observed ligand−receptor residue pairs.

The concept of minireceptor construction was applied in our studies aimed at the design of novel paclitaxel analogues. Anticancer activity reported for extracts of *Taxus Brevifolia* (the Pacific yew or Western yew) was attributed to the complex diterpene paclitaxel (**1**; Figure 1) in 1971.[18] This compound is considered one of the most promising leads for the treatment of solid tumors (for reviews, see refs 19−22). The cellular target of **1** is tubulin[23,24] and its mechanism of action is acceleration of polymerization of tubulin and stabilization of the resulting microtubules.[25] Photoaffinity labeling studies with analogues of **1** show a preference for incorporation of the label into the β-subunit of tubulin, although some labeling of the α-subunit seems to occur as well.[26−28] The N-terminal 31 amino acids of β-tubulin were shown to comprise the major site of photoincorporation.[27] Synthetic analogues of **1** should resolve the two main problems encountered in its therapeutic application: its limited supply from natural sources and its very poor solubility.

Methods and Results. Conformational characteristics of **1** and some analogues have been studied using a molecular mechanics force field and continuum solvation models.[29−31] A conformation corresponding to the X-ray structure of the semisynthetic paclitaxel analogue docetaxel (**2**; Figure 1) has been identified as a low energy conformation for **1** and **2**, using both a water and a chloroform solvation model.[29,30] A correspondence between the ability to assume this conformation and biological activity has been noted[29,31] which prompted us to use these conformations in a first attempt to generate a minireceptor mimicking the interaction site of paclitaxel on tubulin.

The ligands **1** and **2** were transferred to Yak version 3.5 in their putative pharmacophoric orientation. Addition of a minireceptor residue to the endpoint of a vector, originating from the superimposed ligands, was followed by global rotation: a rigid body rotation of the new residue around the new interaction in 15° increments. The position with the lowest total energy was selected. Subsequently, side-chain relaxation was performed: a systematic search in 5° increments with respect to the side-chain torsion angles of the residue (leaving the interaction with the ligands intact). The dihedral angles yielding the lowest energies were selected. A final step consisted of an energy minimization of the whole residue with respect to translational, rotational, and torsional degrees of freedom. When the residue contained multiple interaction sites, these were examined with respect to the strengths of the H-bonds or hydrophobic interactions that resulted when using these different sites. Additional interactions of the newly added residue with ligands and/or other residues were also considered when evaluating these different complexes. The vectors that were selected were chosen based on SAR considerations. The choice of residues was guided by the primary amino acid sequence of the fragment previously reported to contain the paclitaxel binding site: the N-terminal 31 residues of β-tubulin.[27]

The minireceptor resulting from this exercise consisted of 11 amino acid residues. An arginine residue interacted with the carbonyl moiety at C1′ and with the hydroxyl-O on C2′. An adjacent glutamic acid interacted with the amide NH and the hydroxyl-H on C2′. Valine and isoleucine residues formed a pocket for the C3′-phenyl moiety. A cysteine residue formed a hydrogen bond with the C4-acetyl group. An isoleucine residue interacted with the phenyl ring or the *tert*-butyl moiety of the nitrogen containing C3′ substituent. Hydrogen-bonding to the oxygen atom of the oxetane ring was achieved by adding a lysine residue. A tryptophan residue formed a H-bond to the carbonyl of the 2-benzoyl moiety (through the main-chain NH) and was engaged in aromatic interactions with this same substituent. Finally, an isoleucine residue provided hydrophobic interactions with the bridgehead methyl groups. The resulting minireceptor with **2** present in the cavity is shown in Figure 2.

HOMOLOGY MODEL OPTIMIZATION USING THE MINIRECEPTOR CONCEPT

A large number of neurotransmitters and hormones interact with membrane-bound receptors that are coupled to guanine-nucleotide-binding regulatory proteins (G-proteins). This class of G-protein-coupled receptors (GPCRs) has been extensively studied, and molecular cloning and mutagenesis studies have provided information with respect to the primary amino acid sequence and residues involved in ligand-binding and/or receptor-activation.[32,33] Members of this superfamily of receptors share a considerable sequence homology and hydropathy analysis has revealed that they all contain seven hydrophobic regions, probably corresponding to seven membrane-spanning helices. A similar pattern has been observed in bacteriorhodopsin, one of the few membrane-bound receptors of which a detailed experimental structure is available.[34] The bacteriorhodopsin structure has been used as a template for homology modeling of GPCRs, associating the transmembrane regions in such a way that the hydrophobic areas face the cell membrane and the hydrophilic parts form a pore, in which the ligand binding site is located.[3−5] An interesting combination of the AAA and homology-modeling has been reported.[35,36] The binding site of the homology-based model could be defined by probing it with the receptor-excluded volume and the orientation of pharmacophoric points obtained by applying the AAA. This combination is especially interesting because it includes more experimental data than a purely homology-based model. It

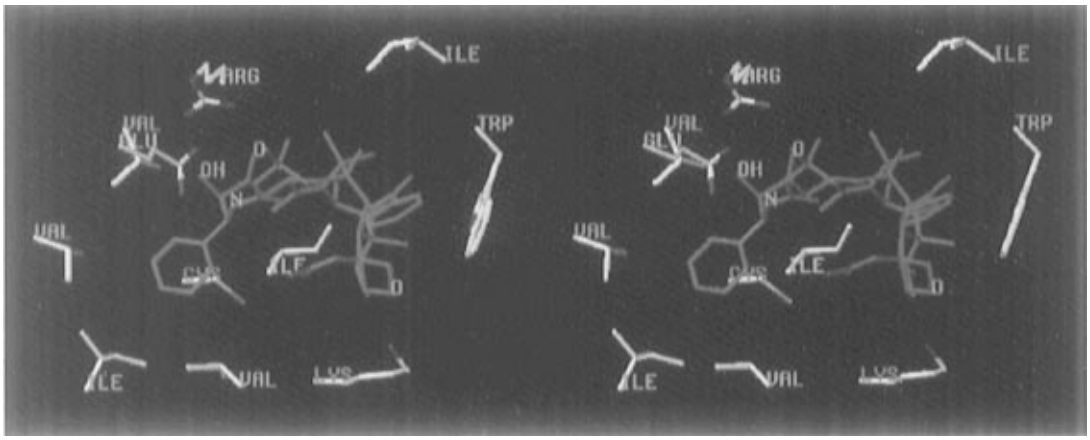


Figure 2. Stereorepresentation of the paclitaxel minireceptor model containing ligand **2**. The hydrogens and main chain N and CO are omitted.

ensures that the resulting binding site can accommodate a large number of (structurally diverse) ligands and can rationalize their affinity and activity.

In order to make the derived models more suitable for molecular design we decided to optimize the binding site extracted from the homology-based model and to treat this truncated site as a minireceptor. This approach allows one to optimize the amino acid side chains in the presence of several superimposed ligands while at the same time optimizing the positions of the ligands. The added constraint imposed on the protein side chains by considering several ligands simultaneously increases the ability of the resultant minireceptor model to generalize to other ligands not included in the training set. This makes the minireceptor models not only suitable for molecular design but also for the docking of individual ligands and the examination of alternative binding modes. An application to the optimization of the binding site for the serotonin 5-HT_{1A} receptor is described below. Agents acting at 5-HT_{1A} receptors are thought to have clinical potential in the treatment of anxiety and depression.[37,38] A series of aporphine-based ligands has recently been shown to exhibit high affinity to these receptors and a remarkable selectivity *vs* dopamine D2 receptors (Table 1).[39−41]

Methods and Results. A homology-based model of the 5-HT_{1A} receptor had been constructed previously,[39,40] according to a strategy first described for modeling the muscarinic m1 receptor.[35] Manual docking of a series of C11-hydroxylated aporphine ligands into this model led to the identification of three key-interactions:[39] (1) a reinforced electrostatic interaction between the protonated nitrogen of the ligands and Asp116 in helix 3, (2) a hydrogen-bond between the C11-hydroxyl and Ser199 (as a donor) in helix 5, and (3) an aromatic edge-to-face interaction between the aromatic A-ring of the aporphines and Phe362 in helix 6. This model was refined by minireceptor optimization (Yak version 3.9) applied to the isolated active site in a stepwise manner using high-affinity ligands **3**−**5** in order of increasing size.[40]

The receptor site was extracted from the homology-based model by deleting all residues further than 7.5 Å from any of the bound ligands. Only those receptor site residues which are within 5 Å of the ligands were optimized with Yak. The remaining residues between 5 and 7.5 Å were rigidly fixed in space thereby preserving the overall shape of the binding site. All Yak minimizations were performed on the ligands and residues simultaneously using the all-atom Yeti force field[17] while keeping the backbone atoms fixed. The latter constraint, together with the presence of an outer shell of fixed residues, ensured that optimized active sites would be compatible with the homology-based receptor model. Translational and rotational degrees of freedom were considered for the ligands, whereas only torsional degrees of freedom were considered for the amino acids.

An optimization of the active site with **4** and **5** simultaneously (using 5 and 3 Å spheres) yielded a generalized model to be used for subsequent docking of a whole series of aporphines.[40,41] This minireceptor model agreed well with the previously described homology-based model,[39] the main difference being the use of Ser168 instead of Ser199 to hydrogen bond to the C11-oxygenated ligands. Ser199 is still close enough to interact with **4** but has to be rotated in order to make room for the larger C11 substituents. It should be noted at this point that the generalized minireceptor models should provide a complimentary shape and distribution of polar functionality with respect to the whole set of ligands considered. Which of the residues provides a hydrogen bond is not crucial at that stage, and no conclusions about the importance of Ser168 *vs* Ser199 should be drawn based on this model.

It appeared that **5** had to bind in a different orientation compared to **4** in order to accommodate the C11-phenyl moiety and at the same time maintain an interaction with Asp116. The C11-phenyl ring is situated in a relatively large and hydrophobic pocket lined by residues from transmembrane helices 4, 5, and 6. Optimization of **6** in the minireceptor model indicated that this compound might assume an intermediate orientation. The gradual change in binding mode depending on the nature of the C11 substituent is shown in Figure 3, resulting from the simultaneous optimization of **3**, **5**, and **6**. In contrast to **5**, the C11-oxygenated compounds **3** and **6** interact through hydrogen bonding with Ser168 and, because of a different position of their protonated amine functionality, choose to interact with the other oxygen of Asp116.

Examination of a series of 10-substituted, 11-oxygenated compounds revealed the presence of a small hydrophobic pocket which could hold the 10-methyl substituent of **4** but no substituents that presented more bulk.[39,40] The slightly different orientation of aporphines with a C11-methoxy substituent compared to a C11-hydroxy substituent postulated above (and shown in Figure 3) implies that their respective

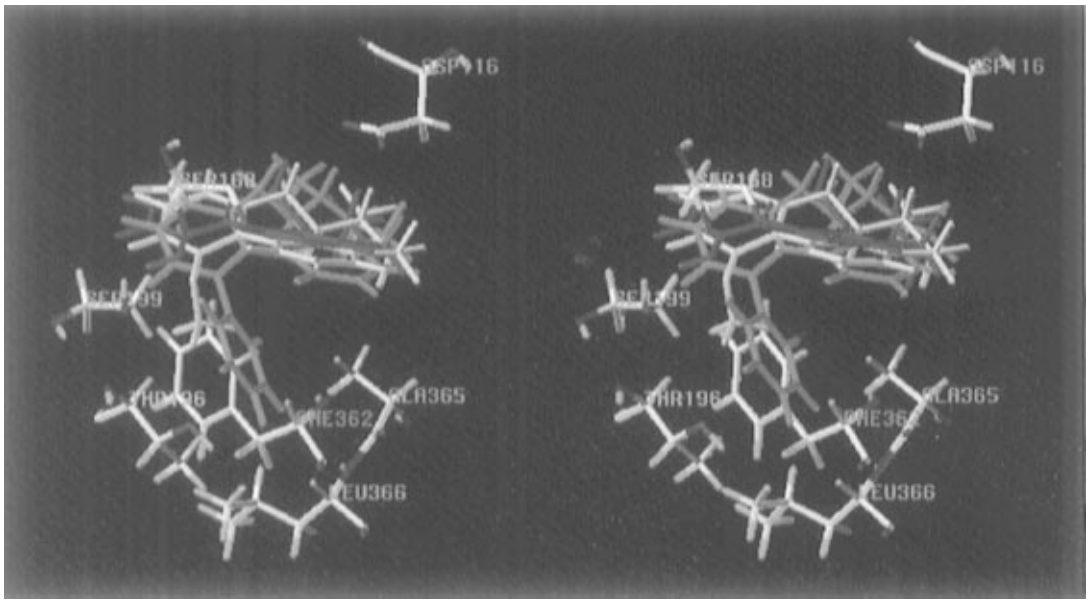
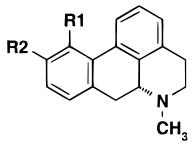


Figure 3. Stereorepresentation of part of the 5-HT_{1A} minireceptor model, obtained by simultaneous optimization of **3** (red), **5** (green), and **6** (yellow).

Table 1. Structures and Receptor Binding Data for Selected Aporphine-Based Ligands



compd	R1	R2	K_i 5-HT_{1A} (nM)[a]	K_i D_{2A} (nM)[b]
(*R*)-apomorphine	OH	OH	296	42
3	OH	H	10	59
4	OH	CH_3	0.5	1070
5	C_6H_5	H	2	233
6	OCH_3	H	5	551
7	OCH_3	CH_3	8	248

[a] Displacement of [^{3}H]8-OH-DPAT, data from refs 40 and 41. [b] Displacement of [^{3}H]Raclopride, data from refs 40 and 41.

C10 substituents have a different accessibility to this so-called "methyl pocket". The observation that the effect of C10-substitution on the 5-HT_{1A} affinity varies with the substituent on C11 (Table 1) is then in accordance with the postulated change in binding mode depending on the nature of the C11 substituent.

The development of binding site models for the dopamine D_{2A} receptor by the same procedure as the one outlined above for the 5-HT_{1A} receptor, allowed us to rationalize the selectivity data presented in Table 1.[39,41] The models showed that in contrast to the D_{2A} receptor, the 5-HT_{1A} receptor could accommodate both H-bond forming moieties and hydrophobic moieties at C11 by allowing the binding mode to change while maintaining an interaction with the aspartate residue in helix 3. Another distinct difference between the two receptor models concerns the presence of the "methyl pocket" in the 5-HT_{1A} receptor which can accommodate a C10 methyl group. Such a moiety is not tolerated by the D_{2A} receptor. The low affinity of (*R*)-apomorphine for the 5-HT_{1A} receptor has been explained by an electrostatic repulsion between the C10 OH-substituent and the carbonyl of a glycine residue in helix 5.

MOLECULAR DESIGN

Two different computational methods for finding new lead structures can be distinguished: 3D-database searching[12−14] and *de novo* design.[10,11] In 3D-database searching, databases of three-dimensional structures of existing compounds are explored, with the prospect of selecting compounds that might exhibit yet undiscovered biological activities. Use of 3D-database searching techniques does not depend on the availability of a structure of the binding site. Pharmacophore points based purely on the AAA can provide quite satisfactory search queries. However, in the presence of a target structure, one can include receptor based extension points that ensure proper directionality of the interactions. The *de novo* design strategies generally rely on a structure of the binding site, which is used to propose complementary structures that have not yet been synthesized.

The binding site cavities used for molecular design should have a generalized appearance, ideally containing features consistent with binding of several (different) high affinity ligands. Only then can the design process be expected to yield novel combinations of interaction patterns. This philosophy is exemplified in the minireceptor approach which allows the construction and/or optimization of binding sites around a set of superimposed ligands. It should be noted that the approach is limited by the assumption that all those ligands share a common pharmacophore.

The minireceptor models described above for paclitaxel and the 5-HT_{1A} receptor are used in the following to illustrate their applicability to molecular design. The model for the paclitaxel binding site consisted of 11 amino acid residues which were supplemented by so-called "Lennard−Jones particles".[42,43] These particles can be added in Yak version 3.94 around the ligand−minireceptor complex to mimic a hydrophobic environment. In this particular case they were added to close the active site, preventing the generation of ligands in areas not defined by the minireceptor. The particles included were those that had a distance between 3 and 5 Å from the highest affinity ligand **2**. The model for the 5-HT_{1A} receptor was the one optimized around **3**, **5**, and **6** simultaneously. The 19 residues considered in the design

Table 2. Characteristics of Leapfrog Runs

	no. of ligands[a]	% successful moves[b]	molecular weight[c] mean	st dev
paclitaxel				
dream mode	112	47	219	137
optimize mode	158	33	330	176
5-HT_{1A}-receptor				
dream mode	39	35	108	44
optimize mode	132	24	421	186
(2-aminopyridine as initial ligand)				
optimize mode	160	29	256	103
(fragments of docked ligands as initial ligands)				

[a] Total number of ligands resulting from three Leapfrog runs, each performing 1000 moves. [b] Percentage of successful moves (i.e., moves that result in a valid ligand), averaged over all three runs. [c] Mean and standard deviation (st dev) of the molecular weight of the ligand population after combining the results from the three separate runs.

runs were the ones that had atoms within 3 Å of any of the ligand atoms.

De Novo Design: Methods. The Leapfrog module present in the Sybyl molecular modeling software[44] is a fragment-based *de novo* design module. Application of genetic operations (like mutation and crossover) results in molecular evolution of a ligand population with gradually improving interactions with the active site. Two different approaches to ligand design were investigated, one starting with only the active site present (Dream mode) and one in which fragments of docked ligands were used as the forebears of the evolved ligand population (Optimize mode). For operations in the Dream mode, Leapfrog generates sitepoints within the binding site cavity, centered at binding energy maxima with respect to electrostatic and (optionally) hydrophobic interactions. These receptor "hot-spots" serve as the starting position for the initial fragment added to the cavity, and they are placed at the optimum position for putative ligand atoms to interact with cavity residues. Default Leapfrog settings were used throughout except for the number of steps, which was set to 1000, and the inclusion of lipophilic sitepoints. Sitepoints outside the active site cavity were manually removed from the sitepoint files. Dictionary AMBER charges (Kollmann_all_atom) were assigned to the amino acid residues. All runs were performed in triplicate.

De Novo Design: Paclitaxel. Characteristics of the Leapfrog runs performed with the paclitaxel minireceptor are included in Table 2; some of the resulting ligands are shown in Figure 4. The Dream mode runs did not yield many interesting ligands. Ligand **a** contains a carboxylic acid moiety interacting with the lysine residue. The left most cyclohexane ring of this ligand overlaps with the phenyl ring of the 2-benzoyl moiety of **2**. Ligand **b** not only exemplifies the complexity of the structures one can obtain but also indicates the potential of generating novel frameworks capable of filling an active site. Again, a carboxylic acid interacts with the lysine residue. The CF_3 and aldehyde moieties are in the vicinity of the cysteine residue.

The middle and bottom rows of Figure 4 contain structures resulting from Optimize mode runs using the following fragments of **2** as initial fragments: the oxetane ring, $C_6H_5C(O)OCH_3$ (from C2), $CH_3C(O)OCH_3$ (from C4), CH_3-$CH(OH)C(O)CH_3$ (the C1′ and C2′ functionality), and CH_3C-$(O)N(H)CH_3$ (the amide functionality on C3′). The amide

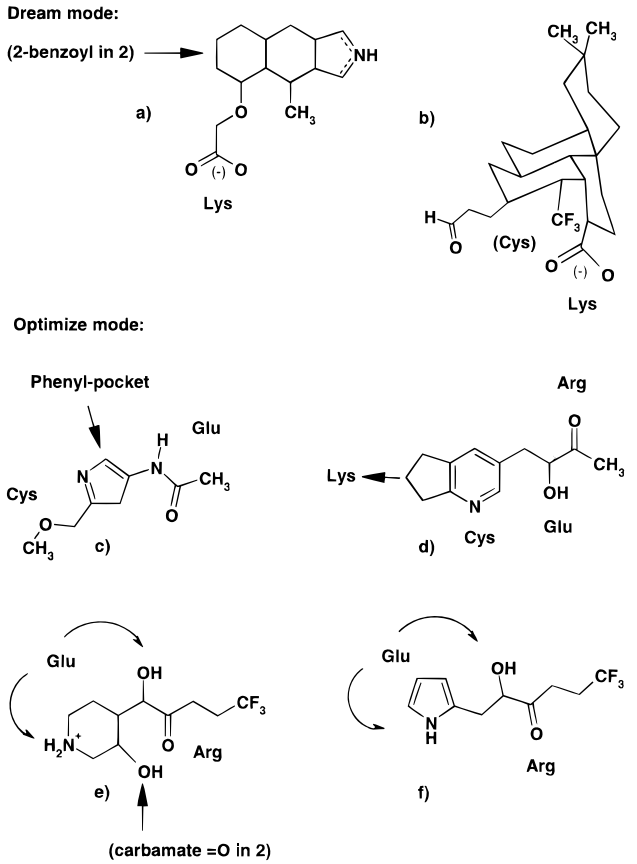


Figure 4. Leapfrog results using the paclitaxel minireceptor. The top row shows two structures obtained by running Leapfrog in the Dream mode; the next two rows show some results when using fragments of **2** as initial ligands in Optimize mode.

fragment in ligand **c** (interacting with the glutamic acid) comes from one of the starting fragments. The ether−O interacts with the cysteine residue, and, in related ligands, substituents on the position indicated by the arrow fill up the pocket occupied by the C3′-phenyl of **2**. The starting fragment for ligands **d**−**f** was $CH_3CH(OH)C(O)CH_3$, interacting with arginine and partly with glutamic acid. In ligand **d**, the aromatic N interacts with the cysteine residue, and the position indicated by the arrow is close enough to the lysine to consider appropriate substitution with a hydrogen bond acceptor. In ligand **e**, a protonated N was generated that interacted with the glutamic acid residue. The new OH moiety overlaps with the carbamate═O of **2**, and the ring structure partly fills the cavity occupied by the C3′-phenyl of **2**. Ligand **f** shows yet another way of achieving an interaction with the glutamic acid, and the aromatic ring again partly fills the cavity occupied by the C3′-phenyl of **2**. Although none of these ligands achieved interactions with all pharmacophoric moieties that were mapped onto the minireceptor, they do represent starting points for subsequent design and indicate novel interaction modes.

De Novo Design: 5-HT_{1A} Receptor. Characteristics of the Leapfrog runs performed with the 5-HT_{1A} minireceptor are present in Table 2. None of the Dream mode runs yielded a particularly interesting ligand. Since Leapfrog by default tries to match a carboxy group in the cavity with a 2-aminopyridine fragment, we used that fragment from a Dream mode run in a subsequent Optimize mode run, restricting evolution to C3 and C4 which are the positions tentatively pointing toward Ser168, Ser199, and the pocket

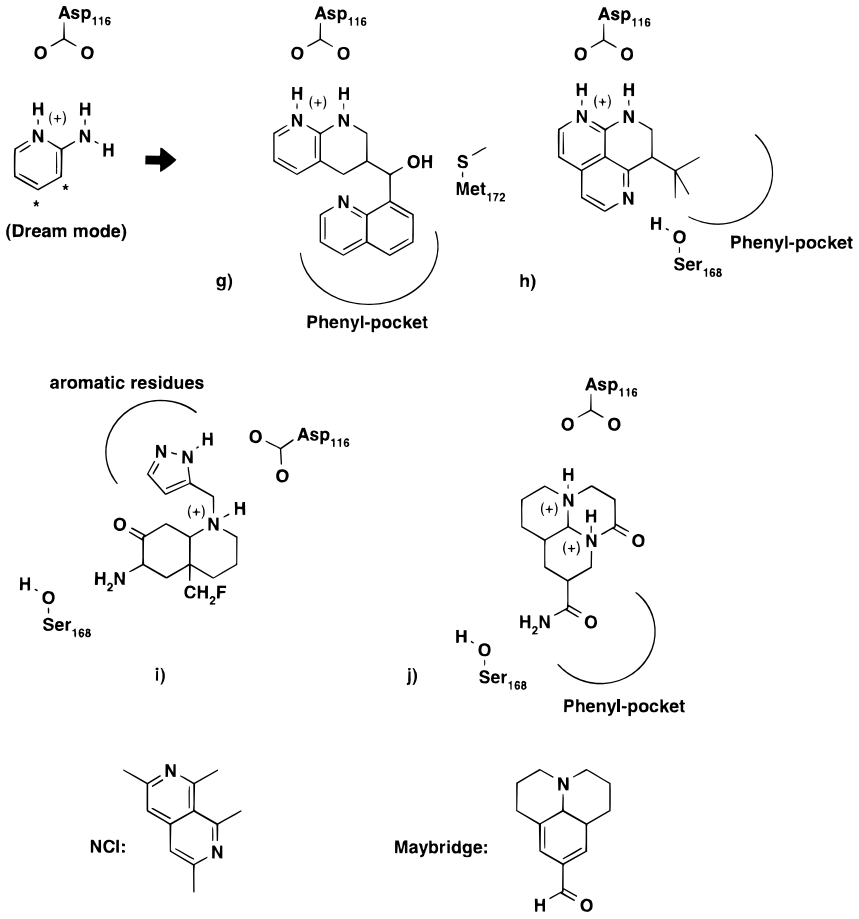


Figure 5. Leapfrog results using the 5-HT_{1A} minireceptor. The top row shows some results obtained in an Optimize mode run, using 2-aminopyridine from a Dream mode run and restricting evolution to the positions indicated with an asterisk. The middle row shows two ligands resulting from Optimize mode runs using fragments of docked ligands **3** and **5** as initial fragments. The bottom row contains structures resulting from searches of the NCI and Maybridge databases for parts of the suggested frameworks.

in which the C11-phenyl substituent of **5** is located. This Optimize run yielded some interesting new frameworks shown in Figure 5 (ligands **g** and **h**). Both these ligands interact with Asp116. Ligand **g** in addition fills the pocket also occupied by **5** and interacts with a methionine residue. Ligand **h** forms a hydrogen bond with Ser168 and also partly fills the phenyl pocket.

Optimize mode runs using as initial fragments the phenol moiety of **3**, the C11-phenyl of **5**, and $C_2H_5NH(CH_3)_2^+$ as extracted from **3** yielded several ligands, two of which are included in Figure 5. Noteworthy in ligand **i** is the substituent on the protonated nitrogen. It was shown in the previous binding site modeling of the aporphines that the space available for N-substituents is limited.[40,41] However, the aromatic substituent in **i** found a small pocket lined by several aromatic residues. Both ligands **i** and **j** interact with Asp116 and Ser168, while ligand **j** in addition partly occupies the phenyl pocket.

Although the protonation states suggested by Leapfrog are highly questionable, the proposed ligands again provide good starting points for subsequent design. In addition to indicating novel interaction patterns, the suggested ligands picked up interactions with residues that are likely to be present in the vicinity of the key residues (ligands **g** and **i**). A search in available databases for parts of the suggested frameworks (using the Tripos Unity software) yielded compounds from the NCI and Maybridge databases which are also included in Figure 5.

CONCLUSION

Application of the minireceptor approach in both the construction and optimization of active sites has been shown to yield binding pockets with good potential for subsequent design of novel ligands. The major advantage of this approach is the fact that it can generate and optimize binding pockets around a set of superimposed ligands, thereby yielding a more generalized model. In addition, the possibility to optimize ligand position and side-chain conformation simultaneously was shown to be advantageous when examining binding modes in the 5-HT_{1A} receptor project.

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