6 Optimization of the pharmacophore model for 5-HT\textsubscript{7} receptor agonism and CoMFA-based modeling of the agonist binding site

6.1 Introduction

The development of rationally designed new compounds for targets lacking 3-dimensional (3D) structural information benefits by well-defined pharmacophore models. Therefore, it is essential to have a comprehensive series of ligands at one’s disposal with similar structural and pharmacological characteristics. In Chapter 2, we have developed a preliminary pharmacophore model for 5-HT\textsubscript{7} receptor agonism. This model was primarily built on the basis of the chemical structures of tryptamine-based ligands. Although it was a good starting point for the design of novel 5-HT\textsubscript{7} receptor agonists, the model proved to be inaccurate due to the lack of diversity among the set of ligands used for development of the model. Therefore, we now present an optimized model, on the basis of a set of diverse 5-HT\textsubscript{7} receptor agonists selected from the ligands developed for the present research project and from literature. This optimized model is the first pharmacophore model for 5-HT\textsubscript{7} receptor agonism reported in literature\textsuperscript{25}. Additionally, a 3D CoMFA model was developed, of which the characteristics can be correlated with the dimensions of a constructed model of the 7 transmembrane domains (TM) of the 5-HT\textsubscript{7} receptor. The combination of these molecular modeling techniques offers valuable information about the characteristics of the agonist binding site and the molecular interactions between ligands and the putative amino acid residues responsible for ligand binding.

6.2 Ligand selection

A set of 24 agonists of diverse nature was selected from previous experiments described in this thesis and from literature (Figure 6.1)\textsuperscript{6,16,17,19-21}. The ligands were selected on the basis of their diversity of core structure, hydrogen bond (H-bond) accepting and donating capabilities, and substitution pattern. Thus, the set comprehends flexible tryptamine-, 2-aminotetralin-, naphthylpiperazine-, and dihydroimidazolyl-biphenylamine-based ligands as well as the structurally restrained ergoline 20, (\textit{R})-(\textit{S})-LSD. The latter ligand served as a rigid template to identify the active conformation out of the set of calculated low energy conformations of the other ligands. Although 20 lacks a H-bond accepting group at the 6-membered ring of the ergoline nucleus, molecular modeling studies indicated that the oxygen atom of the carboxamide moiety is capable of compensating for this alleged deficiency.
Figure 6.1: Selected ligands 1-24 for development of optimized pharmacophore model and 3D-QSAR computations. All ligands are depicted in their protonated form.
It should be noted that ligands 21-24 lack a substituent at the 6-membered aromatic ring capable of accepting a hydrogen bond from a binding site residue as well. Nevertheless, the deficiency of such H-bond accepting groups in 21-24, in relation to their 5-HT$_7$ receptor binding affinities, suggests this lack can be compensated for by the presence of a lipophilic substituent instead (HYD2). This hypothesis is reflected in the dualistic character of the optimized pharmacophore model for 5-HT$_7$ receptor agonism.

6.3 3D QSAR of 5-HT$_7$ receptor agonists

6.3.1 Conformational analysis of 5-HT$_7$ receptor agonists and identification of pharmacophore

Full conformational analysis of the set of 24 5-HT$_7$ receptor agonists in their protonated form was performed in MacroModel$^1$, and followed by a pharmacophore identifying procedure through ligand overlap using APOLLO (see also Chapter 2)$^{22}$. Thus, the positively charged nitrogen of the ligands was defined as hydrogen bond donating group, and the oxygen atom of the substituent at the six-membered aromatic ring (if present) as H-bond accepting group. In (R)-(+-)LSD (20), the carbonyl oxygen of the ethyl-amide group was defined as hydrogen bond accepting group. Additionally, a centroid was defined perpendicular to the plane of the six-membered aromatic ring of the core structure. The centroid of 17 and 18 was defined for the six-membered ring not substituted with the piperazine moiety, and in 22$^{23}$ the aromatic ring of the 2-aminotetralin skeleton was used for this purpose. In case of 23$^{16}$, the protonated and positively charged amino group of the 4,5-dihydroimidazole ring served as hydrogen bond donating group, and the centroid of the methyl-bearing phenyl ring was used for overlap with aromatic rings of the other ligands. Finally, a centroid was defined for the pyridine ring of the very recently developed agonist 24$^{24}$.

Initially, fitting of all ligands possessing H-bond accepting substituents at the 6-membered aromatic rings of the core structures were aligned with 20 using this feature. However, when the number of ligands lacking this feature appeared to be growing during the process of research, an alternative alignment rule was applied, resulting in an improved correlation of experimentally derived and predicted pK$_i$ values. This alternative alignment rule encompassed only the positively charged nitrogen atom and the centroid of the 6-membered aromatic ring of the core structures. Notable differences in alignment were expected for tryptamines only, since the 2-aminotetralins were already superimposed very accurately onto the structure of 20, due to more similarities between the rigid bicyclic moieties with respect to the ergoline structure. Since this latter alignment resulted in a CoMFA model with the highest correlation coefficient between experimentally derived and predicted pK$_i$ values, the results of all molecular computations presented in this chapter are based on this rule.
6.3.2 CoMFA model of 5-HT\textsubscript{7} receptor agonists

The set of superimposed ligands was subsequently used for computation of the 3D CoMFA model in Sybyl\textsuperscript{2}. From the spatial orientation of the alignment, the distance between the two H-bond donating and accepting receptor dummies (i.e. the mutual interaction points) was measured and was found to be 8.0 Å. Other geometric parameters, describing the minimum pharmacophoric parameters for 5-HT\textsubscript{7} receptor agonist binding of the set of 20 ligands, are listed in Table 6.1. The mutual interaction points of all ligands that were calculated by APOLLO are mimicked by means of water molecules. The relative orientation of these interaction points (i.e. putative receptor binding site dummies) was subsequently used in our receptor model to locate and orientate the side chains of the amino acid residues that presumably play an important role in ligand binding.

6.4 Receptor modeling

6.4.1 Model of the 7 transmembrane domains of the 5-HT\textsubscript{7} receptor

The sequence of 449 amino acids of the human 5-HT\textsubscript{7A} receptor was taken from the SwissProt database (entry P34969; http://us.expasy.org/sprot/). Based on the identification of evolutionary highly conserved amino acids within the rhodopsin-based family of G-protein coupled receptors, the helical parts of the receptor were manually aligned with a template of alpha-carbon atoms of the transmembrane domains\textsuperscript{4}. Initial minimization of the helices separately, and subsequent minimization of the ensemble of the 7 transmembrane helices resulted in our model with a total energetic value lower than the sum of energies of the individually minimized helices\textsuperscript{25}.

6.4.2 CoMFA mapping onto the 5-HT\textsubscript{7} receptor binding site

The 3D CoMFA model was projected onto the binding site of the 5-HT\textsubscript{7} receptor to identify conceivable amino acid residues that could account for the contour maps as computed by the comparative molecular field analysis. For this purpose, the 3D orientation of mutual interaction points, as calculated from the preceding alignment procedure by APOLLO, was used to locate the ligand binding amino acid residues at the receptors binding site. Careful examination of this projection onto the inner sphere of the binding site basically indicated the most distinguishing amino acid residues. The combination of these molecular modeling techniques –one ligand-based and one structure-based approach– offers the opportunity to correlate results obtained by either one of these techniques and help to characterize the agonist binding site and the molecular interactions between ligands and the putative amino acid residues responsible for ligand binding.
6.4.3 Docking of 5-HT\textsubscript{7} receptor agonists at binding site

Manual docking of the agonist into the binding site was guided by the 3D orientation of the mutual interaction points surrounding the set of ligands from the APOLLO fitting procedure. The mutual interaction point of the H-bond accepting groups of the agonists was oriented at the position of threonine 244 of TM5 (Thr\textsuperscript{5.43}; numbering according to highly conserved amino acid residues throughout family of GPCRs\textsuperscript{5}), while the mutual interaction point of the positively charged nitrogen atom was super positioned at aspartate 162 in TM3 (Asp\textsuperscript{3.32}). Subsequent minimization of the receptor-ligand complex resulted in properly docked ligands, and revealed several interesting ligand binding interactions additional to the supposed interactions with Asp\textsuperscript{3.32} and Thr\textsuperscript{5.43}.

6.5 Results and discussion

The geometries of the conformations of the ligands selected by the pharmacophore identifying program APOLLO are listed in Table 6.1. The results of the fitting procedure are graphically depicted in Figure 6.2 as well. APOLLO was able to identify good fits of all ligands well within the range of 50 kJ/mol, relative to the global minimum conformation of every single ligand.

![Figure 6.2: 3-Dimensional orientation of superimposed ligands with mutual interaction points. Ligand 20 with tryptamines (left), 20 with 2-aminotetralins (middle), 20 with remainder ligands (right). Hydrogen bonds between mutual interaction points and ligands depicted by dotted lines. Non-essential hydrogen atoms are not depicted.](image)

Obviously, all the indole nuclei and ethylamine side chains of the tryptamine-based ligands are orientated similarly, and match well with the rigid structure of 20. In case of the 2-aminotetralins, the core structure of the 8-substituted derivatives is almost perfectly superimposed onto the structure of 20, whereas in case of the 5- and 6- substituted derivatives, the rings are oriented equiplanar with the 8-substituted aminotetralins, but are rotated around the centroid of the aromatic ring. This deviation is most obvious from the orientation of 9, which is not able to form a H-bond with its methoxy group and the mutual interaction point, and which could be a good reason
for its relatively low 5-HT₇ receptor affinity. The superimposed ligands 17-19 and 23-24 are oriented predominantly equiplanar with their aromatic rings and with respect to the 6-membered aromatic ring of 20, while the hydrogen bond donating nitrogen atoms are oriented in the direct vicinity of the positively charged nitrogen atom of 20. The orientation of the hydrogen bond donating NH group of the 4,5-dihydro-imidazole group of 23 is well aligned with the structure of 20, while the unsubstituted aromatic ring is oriented similar to the 5-substituents of 21, 22, and 24.

On the basis of the series of tryptamines and 2-aminotetralins, it can be concluded that the substituents at the central 6-membered aromatic ring do not function as H-bond donor. As mentioned before in Chapter 2, the high binding affinity of 4 can be explained by the ability of the carboxamide group to form a double hydrogen bond with the putative binding site residue Thr⁵.⁴³. Notably, there appears to be no additional effect of the H-bond accepting substituent of 17 as compared to 18. This observation is not in line with the lower binding affinity of 1 (pKᵢ = 6.8⁶) compared to 2 and 3, but could be explained as a result of unfavorable steric interaction with the boundaries of the binding site due to the larger geometry of these ligands. On the other hand, the absence of a H-bond accepting group in case of 18, and the recently presented⁶,¹⁶,²⁰,²⁴ ligands 21-24 appears not to be detrimental for 5-HT₇ receptor affinity. Apparently, the deficiency of a H-bond accepting (HBA) moiety at the central aromatic ring system can be compensated for by the presence of a flat aromatic ring system (HYD2) at a distance of 4.04 - 4.28 Å from, and almost perpendicular to the central aromatic ring system (HYD1), that can occupy a hypothesized lipophilic pocket hosted by lipophilic residues of TM5 and TM6. This observation is reflected in the dualistic character of the optimized pharmacophore model for 5-HT₇ receptor agonism (Figure 6.3). In both cases, the region hosting the positively charged nitrogen atom (PI) is located at a distance of between 5.3 - 6.1 Å from HYD1.

![Figure 6.3: Optimized pharmacophore model for 5-HT₇ receptor agonism (left panel). Examples of 2 (middle) and 21 (right) fitting the pharmacophore model. Non-essential hydrogen atoms are not depicted.](image)

Although the specific role of Thr⁵.⁴³ with respect to the conformational change of the 5-HT₇ receptor upon activation remains unclear, it is suggested that these different types of ligands might cause the same conformational change at the binding site. Ligands having a H-bond accepting
group would cause the residue to turn counterclockwise by attractive forces forming a hydrogen bond with the hydroxyl group of this residue, while ligands having a hydrophobic substituent (HYD2) can initiate the same rotation by causing repulsive steric interactions with (the methyl group of) Thr$^{5.43}$ as illustrated in Figure 6.4. This hypothesis is confirmed by docking experiments indicating that the hydrophobic substituents (HYD2) of 21-24 are orientated in the vicinity of the H-bond donating amino acid residue Thr$^{5.43}$ as well, and the conformation of the binding site residue is almost identical in both cases.

![Docked ligands 2 (upper left) and 22 (upper right) at 5-HT$_7$ receptor binding site. Schematically depicted hypothesized molecular interactions between Thr$^{5.43}$ and HBA-containing ligands (lower left) and HYD2-containing ligands (lower right). Non-essential hydrogen atoms are not depicted.](image)

Initially, it seemed puzzling that within the series of tryptamines, larger substituents attached to the positively charged nitrogen atom reduce the affinity for the 5-HT$_7$ receptor, while the opposite appears to be true for the series of 8-substituted 2-aminotetralins. However, this might be explained in terms of steric hindrance and lipophilicity. From the geometries listed in Table 6.1 it can be observed that the average distance between the positively charged nitrogen atom (PI) and the central aromatic ring (HYD1) within the series of tryptamines and 8-substituted 2-aminotetralins equals 6.02 and 5.27 Å, respectively.
### Table 6.1: Ligand geometries, experimental pharmacological data, and predicted pKᵢ values from CoMFA computations.

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It is hypothesized, that within the series of 2-aminotetralins, larger substituents can occupy a lipophilic pocket located near Asp³ thirty-two with increasing affinity for the receptor. Within the series of tryptamines, these larger substituents might contrarily experience increased steric interactions with the boundaries of this lipophilic pocket as a result of the more distant orientation of the nitrogen atom substituents with respect to the central aromatic ring system. Furthermore, as a result of the inverted chirality of the 2-aminotetralins 14-16 in relation to ligands 10-13, the alignment of these latter ligands with template 20 is less optimal, resulting in lower binding affinities for the 5-HT₇ receptor.

The results of the 3D-QSAR computations are graphically depicted in Figure 6.5, illustrating the binding affinities of 4, 7, 20, and 22 in relation to the orientation of the CoMFA fields. Both 4 and 20 share the orientation of the carbonyl group near the area where negative charge is favored (NCF). In case of 7, the distance between the methoxy group and this area is larger, while the 2-methyl substituent is pointing into the direction of areas where bulk is disfavored (BD). The absence of a H-bond accepting group in 22 is compensated for by the occupation of a large area that represents the favored presence of bulk (BF) by the naphthyl substituent.
The areas pointing out of, and in the direct vicinity of the protonated nitrogen atoms also indicate the favored presence of bulky substituents (BF), while the numerous smaller areas surrounding it indicate the negative effect of bulk with respect to the binding affinity (BD). The area indicating the harmful effect of negative charge close to the 6-membered aromatic ring of the core structures of the ligands can be ascribed to the orientation of the methoxy group of 2-aminotetralin 9, a ligand with low affinity for the 5-HT\textsubscript{7} receptor (see also Figure 6.2, middle).

By mapping the areas of the 3D CoMFA model onto the model of the seven TM domains of the 5-HT\textsubscript{7} receptor, the nature of these areas could be correlated with the presence and nature of the amino acid residues forming the binding site. As explained in Section 6.4.2, this process was guided by the 3D orientation of mutual interaction points, as calculated from the preceding
alignment procedure by APOLLO. The mutual interaction point of the H-bond accepting groups of the agonists was oriented at the position of Thr$^{5.43}$, while the mutual interaction point of the positively charged nitrogen atom ($\text{PI}$) was super positioned at Asp$^{3.32}$. This way, the area close to the H-bond accepting moieties ($\text{NCF}$) fits well with the H-bond donating hydroxyl group of Thr$^{5.43}$. The areas pointing out of, and in the direct vicinity of the protonated nitrogen atoms of the ligands represent a hypothesized lipophilic pocket which boundaries are formed by residues of TM3 (Val$^{3.33}$ and Ile$^{3.29}$) and TM7 (Phe$^{7.48}$). The areas that cause steric repulsion of the 2-methyl substituent of 7, and analogously probably also with the differently orientated ethylamine side chain of 1,2,3,4-tetrahydroisoquinoline-based ligands (see Chapter 3), can be ascribed to Leu$^{7.41}$. Furthermore, the aromatic substituents of ligands 21-24 show stabilizing $\pi-\pi$ stacking interactions with Phe$^{5.47}$. Finally Phe$^{6.44}$, Leu$^{6.49}$, Ala$^{5.44}$ and Ala$^{5.46}$ enclose the cavity these aromatic substituents occupy.

The numerical outcome of the CoMFA computations, based on the set of superimposed ligands, is listed in Table 6.2. The high value of the correlation coefficient of the non-cross-validated run ($R^2 = 0.99$), and the small standard error (s.e. = 0.16) of the predicted $pK_i$ values with respect to the experimentally derived values are indicative of a model with excellent correlation between experimentally derived and calculated $pK_i$ values. The correlation between experimentally derived $pK_i$ values and predicted $pK_i$ values is listed in Table 6.1.

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<td>non-cross-validated run</td>
<td>0.99</td>
<td>6</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 6.2: Results of CoMFA computations.

The results of the docking experiments indicate that all ligands are capable of forming molecular interactions with residues Asp$^{3.32}$ and Thr$^{5.43}$ after minimization of the receptor-ligand complex, similar to 2 and 22 as depicted in Figure 6.4. In all cases, the total energy of the receptor-ligand complexes is lower than the sum of the energies of the free receptor and the ligand.

Remarkably, Trp$^{6.48}$ and Phe$^{6.44}$, being part of the aromatic cluster of TM6 (Phe$^{6.44}$-Trp$^{6.48}$-Phe$^{6.52}$), and therefore likely to play an important role in the activation of the receptor$^{16}$, were able to form $\pi-\pi$ stacking interactions with the aromatic cores of all ligands. The minimized receptor-ligand complexes show that the side chains of the amino acid residues of the aromatic cluster form a well-ordered stack of aromatic rings together with the central aromatic moiety present in all ligands of the set. The 1,2,3,4-tetrahydroisoquinoline-based ligands discussed in Chapter 3 and
other ligands that possess a core structure that is not completely flat and in line with the ergoline structure of 20 (like indoline 20a of Chapter 4) tend to disrupt this well-ordered stacking. This might be one of the reasons these ligands show no affinity for the receptor.

We wish to emphasize the choice for the 3D model developed by Baldwin of alpha-carbon atoms of the transmembrane domains of G-protein coupled receptors as a template for our 5-HT$_7$ receptor model, instead of the X-ray structure of bovine rhodopsin with a 2.6 Å resolution, published in 2000$^{15}$. In our view, rhodopsin, with its low degree of homology with the 5-HT$_7$ receptor (ca 24 % for the sequences of the TM domains), is not the ultimate template for homology modeling of all GPCRs. Initial study of the X-ray structure of bovine rhodopsin revealed that the distance between the hypothesized ligand binding amino acid residues Asp162 and Thr244 of TM3 and TM5, respectively, is far too big (> 16 Å) for serotonin and other agonists to be spanned. Furthermore, Lopez-Rodriguez et al.$^{14}$ very recently argued for a different orientation of (the upper part of) TM3 of the 5-HT$_{1A}$ receptor, according to molecular dynamics studies with a model based on the X-ray structure of rhodopsin, and used this methodology as well to develop their recently published model of the 5-HT$_7$ receptor$^{13}$. In fact, this different orientation resembles more the structure of the Baldwin template with respect to the relative distances of TM3, TM5, and TM6 – especially near the binding site – than it resembles the X-ray structure of rhodopsin. Since the endogenous ligands for this family of receptors range from small ions to polypeptides$^{18}$, it is very unlikely that this particular model of the 5-HT$_7$ receptor should fit the structure of an X-ray structure of a very specific type of GPCR, namely one with a covalently bound agonist (retinal), without any modifications. We acknowledge the significance of the recently elucidated X-ray structure, but refuse to accept this as the Holy Grail in homology modeling of GPCRs. The template used in our study incorporates structural information more relevant to neurotransmitter-mediated receptors, derived from the analysis of ca. 500 sequences of GPCRs. According to the procedure used to build our model, the relative distance and orientation of the hypothesized ligand binding amino acid residues mentioned above, is more likely to be able to interact with serotonin and other agonists with comparable geometry.

In our view, a receptor model for a specific receptor subtype has to be adapted to a particular conformation of the neurotransmitter involved; otherwise there is no reason for this subtype to exist. This adaptation process can involve small rotations and translations of some helices, in such a way that a perfect match is found for this particular conformation in the receptor. The specific conformation of the neurotransmitter can be deduced from the resemblance of this conformation with that of some agonists. Previously, this procedure was successfully followed in the investigation of compounds with mixed D$_2$ and 5-HT$_{1A}$ receptor affinities$^9$. 
6.6 Conclusions

On the basis of a comprehensive set of 24 5-HT$_7$ receptor agonists of diverse nature, the preliminary pharmacophore model developed in Chapter 2, has been optimized, resulting in a bivalent model that discriminates ligands, possessing either hydrogen bond accepting substituents (HBA) or lipophylic substituents (HYD2) at a distance of approximately 3.0 Å and 4.2 Å from the core structure, respectively. Both models share the properties of a hydrophobic domain (HYD1) hosting the central 6-membered aromatic ring present in all ligands of the set, and a positively ionizable nitrogen atom (PI) at a distance of between 5.3 Å and 6.1 Å.

The CoMFA model based on the same set of ligands shows a good correlation between experimental and predicted pK$_i$ values ($R^2 = 0.99$, s.e. = 0.16). Subsequent mapping of the CoMFA fields onto the binding site of the model of the 7 TM domains of the 5-HT$_7$ receptor attributed important roles in ligand binding to Asp$^{3.32}$ and Thr$^{5.43}$. Amino acid residues of the aromatic cluster of TM6 are hypothesized to play an important role in ligand binding as π-π stacking moieties. Ligands possessing an aromatic substituent (HYD2) instead of a H-bond accepting group, seem to bind the receptor with high affinity as well by occupying a lipophilic pocket hosted by residues of TM5 and TM6.

6.7 Experimental part

6.7.1 Molecular computations

General remarks

For general remarks on conformational analysis, pharmacophore modeling, CoMFA computations, the reader is referred to Section 3.7.1

CoMFA computations

Selected conformations of the ligands were imported into a Sybyl$^3$ database as pdb-files, as selected by the APOLLO molecular modeling and extracted from the RMSFIT output file using the MMDFIT module. Atom types were checked and adjusted if necessary, extension vectors and centroids were deleted, and atomic charges were calculated (Gasteiger-Hückel). Based on this database, a new MSS was opened, and pK$_i$ values were entered manually. CoMFA parameters calculated with Sybyl standard parameters and with steric and dielectric cutoff values of 15 and 10 kcal/mol, respectively (distance dependant dielectric function), were inserted as separate column. Partial least squares (PLS) calculations (non-cross-validated) resulted in the described CoMFA model, which was subsequently used for mapping onto the binding site of the 5-HT$_7$ receptor to identify conceivable amino acid residues.

Homology modeling of the receptor

The sequence of 449 amino acids of the human 5-HT$_7$ receptor was taken from the SwissProt database (entry P34969; http://us.expasy.org/sprot/). Based on the identification of evolutionary highly conserved amino acids within the rhodopsin-based family of G-protein coupled receptors, the helical parts of the receptor were manually aligned with a template of alpha-carbon atoms of the transmembrane domains$^9$. Initial minimization of the helices separately (Tripos force field, Gasteiger-Hückel charges, dielectric constant 5.0, distance dependant, conjugate gradient 0.1 kcal/mol/Å),
and subsequent minimization of the ensemble of the 7 transmembrane helices resulted in the model with a total energetic value lower than the sum of energies of the individually minimized helices.

**Mapping of CoMFA model onto receptor binding site**

The CoMFA model was projected onto the binding site to identify conceivable amino acid residues that could account for the contour maps as computed during the comparative molecular field analysis. For this purpose, the 3-dimensional orientation of mutual interaction points, as calculated from the preceding alignment procedure by APOLLO, was used to locate the ligand binding amino acid residues in the receptor binding site. The mutual interaction point forming H-bonds with the HBA groups of the ligands was superimposed onto Thr244 (Thr$^{5.43}$), and the H-bond accepting interaction point forming H-bonds with the PI moieties of the ligands was superimposed onto Asp162 (Asp$^{3.32}$).

**Docking of ligands in binding site**

Ligands were manually docked into the binding site and minimized to analyze the ligand binding interactions. This docking procedure was guided by mapping the mutual interaction points as calculated from the preceding alignment procedure by APOLLO onto the previously mentioned amino acid residues. As a starting point for minimization, the conformations of the ligands used for super-positioning in APOLLO were used, and the side chains of the hypothesized amino acid residues were adjusted, if necessary, to adopt the 3 dimensional orientation of mutual interaction points in the most optimal manner. Minimization of the merged complex of ligand and receptor (Tripos force field, Gasteiger-Hückel charges, NB cutoff 8.0, dielectric constant 5.0, distance dependant, conjugate gradient 0.1 kcal/mol/Å) resulted in properly docked ligands.
List of references


