MODELLING DRUG-RECEPTOR INTERACTIONS IN AN AVERAGE BINDING SITE FOR NK2

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Abstract: A tentative procedure applied to the search for a new antagonist of the neurokinin A (NKA) is presented. In parallel a tentative 3-D model of the NK2 receptor was created, using bacteriorhodopsin (BRD) as a template. The residue substitutions were performed in BRD to obtain the sequence for NK2R_H and the seven α-helical segments were optimized forcing the α-helical backbone to match the corresponding aligned parts of BRD, while the arrangements of the side chains were model built based on available site-directed mutagenesis studies. Constrained MM and molecular dynamics simulations were carried out such a way to permit formation of H-bonds between low energy conformers of the known drugs and aminoacid residues in the receptor site. The Connolly surface for each ligand allowed to determine an "average" binding site in which all the low energy conformers, of known and prospective drugs, were docked and classified according to a statistical index. The whole procedure was repeated exploiting the lately published structure of an actual G protein coupled receptor as a better template, thus producing a cavity in the binding site to dock directly the drugs. Corollary validations of the force fields used are also mentioned. In addition intra- and intermolecular interactions suitable to produce more active drugs were evaluated.

Keywords: receptor modelling, docking, non covalent interactions, molecular mechanics, molecular dynamics, substituent effects

1. Introduction

One of the major challenges for a theoretical chemist in the design of a new drug is not represented by the wealth of factors to be taken into account, such as its stability in the physiological environment, its toxicity, its solubility, its adsorption, its fate, etc., but by the comprehension, or at least by the hypothesis to be put forward about its mode of binding at the molecular level. An effective drug obtained via *rational drug design* is a measure of how much and how well we have learned about drug-receptor interactions. When dealing with the known active sites the problem can be solved, i.e. a drug can be found which is top scoring from the point of view of both topology and potential energy. Unfortunately, the structure of most receptors is completely unknown and one has to resort to "receptor mapping", a technique attempting to evaluate the structure of a receptor binding site by regarding it as complementary to drugs which fit the receptor [1]. Such drugs

usually present the so called pharmacophore, i.e. a typical pattern (for instance in the molecular electrostatic potential, MEP [2]), or arrangement of atoms necessary for a specific interaction with that receptor. The identification of the pharmacophore however is not easy at all, because the active conformation of most drugs is hard to determine due to their conformational flexibility. This in turn prevents the possibility of receptor mapping. In these cases the only alternative is to build the receptor, even though this system is much more complex than the drug itself. Moreover, the procedure usually produces a huge number of possible structures among which it is difficult to discriminate without additional information from other sources.

The study of the drug-receptor interactions can therefore give a hint about the mode of binding of the drug and thus suggest what kind of improvements could be made in its structure or chemical composition. A validation of the methods to be used is however often necessary.

2. Methodology

The procedure employed in the search for a new antagonist of the neurokinin A (NKA) consisted in the analysis of the MEP of several stable conformers of three known drugs in order to locate the pharmacophore, since no clear-cut structural or chemical analogies existed among them. In parallel a tentative 3-D model of the NK2 receptor was created, using bacteriorhodopsin (BRD), actually 1brd [3], as a template, because at that moment a more adequate structure was not available for a "similar" seven transmembrane (TM) helix domain. The residue substitutions were performed in BRD in order to obtain the sequence for NK2R_H, according to a sequence alignment taken from the literature [4]. The seven α-helical segments, somewhat longer than in BRD, as derived from the hydropathicity profile, were optimised with molecular mechanics (MM) forcing the α-helical backbone to match the corresponding aligned parts of BRD. A few different arrangements of the side chains, suggested by available site-directed mutagenesis studies [5], were built and energy refined. Constrained MM and molecular dynamics simulations were carried out the seven transmembrane domains for forcing a low energy conformer of the known drugs to H-bond to residues in the receptor site which was found to be important to their binding from the aforementioned studies. Both the receptor site and the drugs were allowed to relax, because in our opinion not only the drugs can undergo conformational changes at approaching the receptor binding site. The Connolly surface [6] generated for each cavity around the ligands allowed to determine an "average" binding site in which all the low energy conformers of known and prospective drugs have been docked, using the DOCK code [7a], which assigns to each structure either a force field [7b-c] or a complementarity score. The drugs were then classified according to a tentative statistical index. The recent availability of a theoretical structure of rhodopsin [8] prompted us to repeat the procedure exploiting an actual G protein coupled receptor as a better template. In addition to the difference in helix packing, particularly those of TM3 and TM4, GPCRs show a highly conserved pattern of prolines in the trans membrane

domains, which on the contrary is absent in BRD. Thus, the rhodopsin based model presents a few kinks in the helices and a cavity in the binding site with a histidine and a tyrosine side chain in its walls. This will allow to dock the drugs directly into the binding site with no need to create an artificial cavity.

In order to assess the reliability of the classical results the stability of a constrained peptide based antagonist of NKA was studied by several theoretical methods [9]. The preferential interactions between two specific aromatic groups (indole and 5-methyl-(δ)imidazole, the former belonging to the drug and the latter to the receptor site) were also considered in detail with high level *ab initio* calculations, including correlation and counterpoise corrections, in order to shed some light on these feeble but very important interactions [10]. After validating an MM force field to this end, the possibility of stacked and T-shaped adducts was extensively taken into account [11], while the effects on the adduct stability of a different proton position (ε) and protonation state (cationic form) in imidazole were also compared [12]. The effect of small substituents in 5 or 6 position on the properties of indole was also examined in order to evaluate which of them was best suited to produce an active drug [13].

3. Overview of the Specific Receptor Problem

Neurokinin (tachykinin)-2 receptor (NK2) is a member of the G-protein coupled receptors (GPCR) family. This class of receptors includes integral membrane proteins that transduce extra cellular optical and chemical signals to the intracellular side by coupling to specific G-proteins. This kind of receptors are predicted to have seven transmembrane regions, and are believed to interchange between a number of different conformations that can selectively bind agonist or antagonist ligands and activate G-proteins with important functional consequences [14, 15].

GPCRs exploit diverse strategies for ligand recognition, using either the transmembrane domain, the extra cellular surface, or even the N-terminal segment. The molecular details of GPCR proteins and of signal transduction processes remain largely unknown because of the difficulties involved in obtaining and purifying sufficiently large amounts of protein to produce crystals for high resolution x-ray, or electron diffraction measurements. Novel biochemical and biophysical methods are needed not only to overcome the obstacle to explore the three-dimensional (3D) structure of these receptors [16, 17], but also for investigating the dynamics of their interaction with ligands. The seven-helical structure of the transmembrane domain has recently been demonstrated by electron cryomicroscopy studies of bovine, frog and squid rhodopsin with a low resolution of 6-9 Å [18-24], that is however insufficient to obtain atomic level structure.

Experimental data and theoretical studies based on the multiple sequence alignment analysis of hydropathy and aminoacid conservation have provided more useful structural information, and have led to the construction of different approximate molecular models of GPCRs. Some of these models have been built from the structure of the non-homologous [25] seven-α-bundle membrane protein

bacteriorhodopsin [3], which differs from rhodopsin in the tilts and positions of some helices [21, 23], whereas others have used the low resolution rhodopsin electron cryomicroscopy maps, and a few experimentally derived constraints, to pack together the seven helices with arbitrarily chosen sidechain conformers [26-28].

An average 3D model of the TM seven-α-bundle of rhodopsin has recently been calculated [8], using an iterative distance geometry refinement with an evolving system of hydrogen bonds, formed by intramembrane polar side chains in various proteins of the same family and collectively applied as a set of distance constraints. This model, which is in agreement with a large body of published experimental data, deposited in the Protein Data Bank (1bok, 1boj), is available for further verification and can be used as a template to calculate seven-α-bundles of various GPCRs. Each GPCR must create a binding pocket complementary to its natural and artificial ligands and be consistent with experimental data. The earliest opsin GPCR models were built from non homologous bacteriorhodopsin structure, because these structures seemed to be evolutionarily related as reflected by a similar architecture based on seven TM α-helices surrounding a retinal chromophore attached through a Schiff base to a Lys residue on TMH7. However, the signal transduction mechanism of bacteriorhodopsin is different from the rhodopsin one, and even if evolutionary events are hypothesized [29, 30] the pattern of polarity-conserved positions through their sequences is clearly divergent [31].

Since helix-helix interactions are significantly driven by complementarity of their polar-apolar surfaces, the TMH packing arrangement is likely to be significantly different between bacteriorhodopsin and the GPCRs. The average rhodopsin model and the published refined bacteriorhodopsin structure (2brd) have been compared; they differ in the position of helices IV and V and in the tilts of helices II and III, but the spatial positions of the binding sites of these two receptors are almost identical, i.e. they have lost the similarity of their amino acid sequences, but still maintain the original 3D fold. Choosing bacteriorhodopsin as a direct template for GPCR modeling, as we did in our first NK2 model, is likely to be inappropriate [32]. A meaningful alignment of the GPCR sequences with bacteriorhodopsin is hindered by the lack of significant sequence homology among them. Many different alignments can be considered and GPCR models derived from these alignments would position residues critical for ligand binding quite differently. The superposition of a GPCR-TMH backbone onto the bacteriorhodopsin backbone is even more ambiguous because of the kinks introduced by Pro residues which position is not conserved. The kink renders the two helical portions discontinuous both in the directions of their helical axis and in the orientations of their "faces".

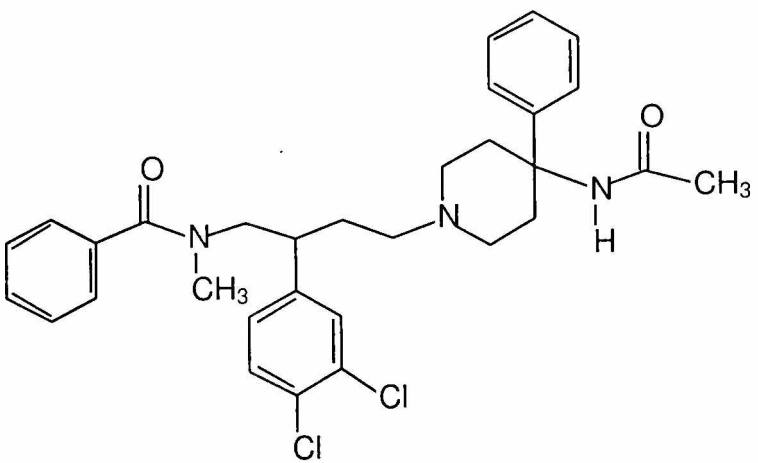
Whether kinked TMHs are superimposed onto straight TMHs or *vice versa*, the superimposition could be done for either helical portion or for a combination of both. The required consistency between the predicted (GPCR) and the observed (rhodopsin) degree of lipid exposure, for each TMH, provides additionally another criterion for testing the suitability of bacteriorhodopsin as a template for GPCR [33].

The predicted degree of surface exposure, for each TMH in GPCR, is not consistent with the observed degree of lipid exposure for each TMH in bacteriorhodopsin.

Although much progress has been made in building molecular models of GPCRs, understanding the molecular mechanism of receptor action remains unclear. Two important elements in such a mechanism are: the understanding of the specificity of ligand recognition by the receptor, and the activation of the receptor that results from the formation of a ligand-receptor complex. The importance of the first element is in the discrimination of specific ligands for the particular receptor from all other molecules. The significance of the second element can be illustrated by the consequence of the interaction with an agonist, which leads to receptor activation as opposed to the interaction with an antagonist which prevents the action of agonists. Thus the identification of the residues in the receptor, which are responsible for such remarkable selectivity, is essential for formulating a detailed molecular mechanism of receptor action. However, it is difficult to identify the binding pocket in the receptor with all the residues involved in ligand binding mainly because both the ligand and receptor are flexible molecules with many possible accessible states, and there is no clear way of deciding which are relevant to binding.

Amino acid sites are proposed to lie in direct contact with receptor ligands, based on mutagenesis experiments can be useful to predict TMH-TMH interface. However, the credibility of those predictions is rather weak, because they are not supported by evidence of direct contact.

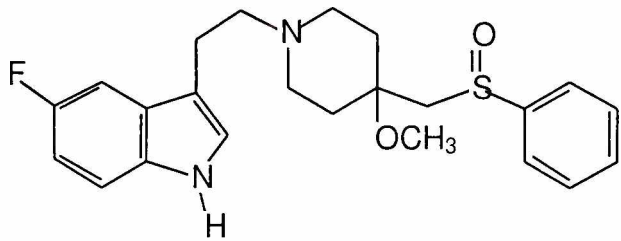
For comprehensive reviews of the whole problem related to tachykinin receptors and their antagonists we refer the reader to Refs. 34 and refs. quoted therein.



Scheme 1

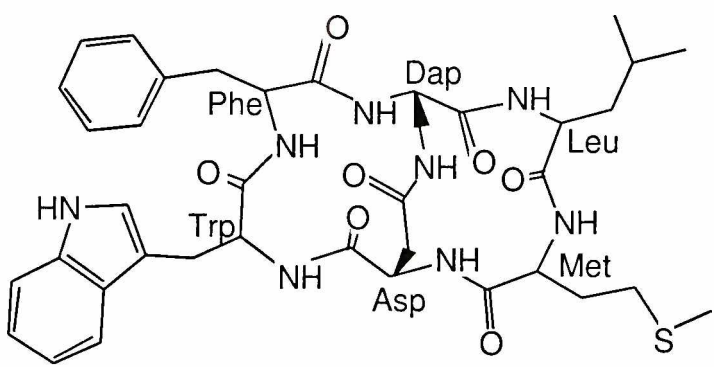
4. Search for the Pharmacophore

The three known antagonists of neurokinin A (NKA), namely SR48968 [35-37], i.e. (S)-N-methyl-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl] benzamide, shown in Scheme 1, GR159897 [38], i.e. (R)-1-[2-(5-fluoro-1H-indol-3-yl)ethyl]-4-metoxy-4-[(phenyl sulfinyl) methyl] piperidine, shown in Scheme 2, two potent and selective non-peptide tachykinin NK2 receptor antagonists, and finally MEN10627 [39], i.e. cyclo[-Asp^1-Trp^2-Phe^3-Dap^4-Leu^5-Met^6-]cyclo [1β-4β], shown in Scheme 3, a peptide-based antagonist, were considered.



Scheme 2

We found however such a large number of stable conformers that it was extremely difficult to locate the pharmacophore univocally. By comparing the compounds sketched in the schemes it is in fact evident that no clear-cut structural or chemical analogies exist among them. Nonetheless, we had been able to figure out a likely mode of binding of those drugs, based on a common feature of their MEP in some conformers. To check our idea we made use of an approach completely different from that followed previously [40], where our task consisted in optimising with suitable substituents a fairly rigid lead compound, for which a patent had already been issued. Due to the different nature of these drugs, we could not use a systematic method, such as that proposed recently [41] that performs a semiautomated optimisation of a lead compound, neither the strategy put forward to design "peptoids" [42], which rely on the endogenous ligand, making a few specific steps. It is worthwhile noting that both methods have been applied as a test to design, among other ligands, the NK2 antagonists.



Scheme 3

The main assumption made in order to understand how the three NKA antagonists work is that their mode of binding is similar. But from the perusal of the structural features of the three drugs, especially for the flexibility of the two non-peptide antagonists, it was difficult to derive a clue to find another molecule with an enhanced activity, and hopefully without undesirable side effects. On the other hand, recent papers [4, 27, 43] suggested that we created a tentative 3-D model of the NK2 receptor, that should allow to evaluate the interaction of each drug conformer with the receptor site, making use of MEP in order to understand the molecular basis of ligand-receptor interactions, monitoring at the same time the effect on the MEP of the conformational changes, necessary to perform the complexation process. In this way we should hopefully either confirm or disprove our activity hypothesis. Once tentatively confirmed we could use the model of the drug-receptor interaction to design new active compounds.

5. Computational Details

Molecular mechanics (MM) energy refinements of ligands and antagonists were carried out with SYBYL [44], while those of the NK2 receptor were carried out with Discover [45], using the CVFF force field [46-47], applied also for the receptor-ligand molecular dynamics (MD) simulations. The partial charges for the electrostatic contribution in SYBYL were computed with the Gasteiger-Hückel method [48]. All the calculations have been carried out on the IRIS/4D-420-GTXB workstation at ICQEM, where also the geometry visualisations have been performed. MidasPlus [49] was used for some of the colour pictures. The Hartree-Fock *ab initio* calculations have been carried out with Gaussian94 [50] on the RS6000/590 workstation at ICQEM.

MEN10627, which was characterised in the solid state by X-ray diffraction and in acetonitrile solution by NMR spectroscopy [39], obtaining almost identical conformations in both states, was used as a test case to evaluate the response of a few methods in determining its internal energy from the equilibrium geometries of the stable conformers. Therefore, its structure was investigated using different (classical, semiempirical, *ab initio*) methods and levels [9], in order to validate also the SYBYL force field employed for this molecule.

The behaviour of CVFF was also examined with *ab initio* single point calculations at the HF/6-31G* level [51], limiting ourselves to the three different arrangements of interacting partners (retaining only the phenylmethylsulfoxide moiety and methylimidazole), and compared to the SYBYL results. A fairly good correlation between the MM and *ab initio* results was found (regression coefficients $r = 0.987$ and $r = 0.998$ for CVFF and SYBYL, respectively). The correlation between the MM results themselves is also satisfactory ($r = 0.995$).

6. Results and Discussion

Our first model of the NK2_H receptor active site was based upon techniques of homology modeling, following the low resolution 3D structure of bacteriorhodopsin, which was the only structure available at that time. From the

alignment of the sequences of ten NK2 receptors seven highly conserved segments of NK2R_HUMAN had been isolated and aligned to the bacteriorhodopsin ones [4]. Therefore, the residue substitutions were performed in BRD in order to obtain the sequence for NK2R_H. The hydropathicity profile of the NK2_H receptor (obtained using a window region of 19 and the Kyte-Doolittle [52] parameters) indicated seven trans-membrane (TM1-7) domains as in BRD, though somewhat longer; the additional residues lying outside the alignment were then included in the α-helical region. Subsequently the positions of the side chains were examined in detail. A few different arrangements of the side chains were model built, based on the available site-directed mutagenesis studies [5], chimeric approaches [53], and energy refined. The key binding residues were facing towards the inner part of the core. The overall aspect of the seven α-helix bunch is displayed in Figure 1a.

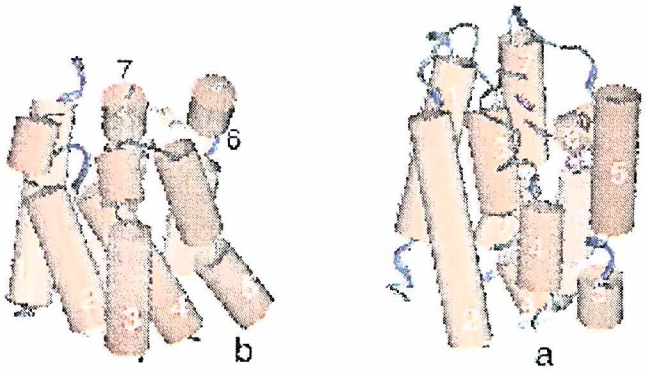


Figure 1. *View of the model for the seven-α-helices of NK2R_H (a, right hand side) derived from BRD in comparison with the model (b, left hand side) derived from the theoretical structure of rhodopsin. Few receptor site residues (His and Tyr only) are displayed in both models; notice that in the model based on rhodopsin they are buried less deeply than in the model based on BRD.*

In contrast to what expected neither through channels nor binding pockets appeared amid the seven trans-membrane domains, suggesting that one or more conformational changes should take place as the ligand approaches the binding site. The solvent exposed surface, generated using the Connolly algorithm, was colour coded according to the value of the MEP in order to suggest the best ligand orientations, or conformations for recognition with respect to the binding region. In fact we expected to find a pattern roughly complementary to that observed in some of the low energy conformers of the known drugs.

As can be seen from the MEP, displayed in Figure 2, on the three very different molecular structures (Figure 3), there are three negative lobes located in analogous mutual positions, thus suggesting a possible matching with three positive regions in the receptor site. However, as far as this interaction pattern is concerned, the colour coded receptor surface did not produce a helpful insight in this case, neither confirming or disproving our activity hypothesis. Therefore, we decided to dock the known drugs into the binding site, starting from different H-bond hypotheses. The constrained MD simulations were performed using the Verlet leapfrog algorithm

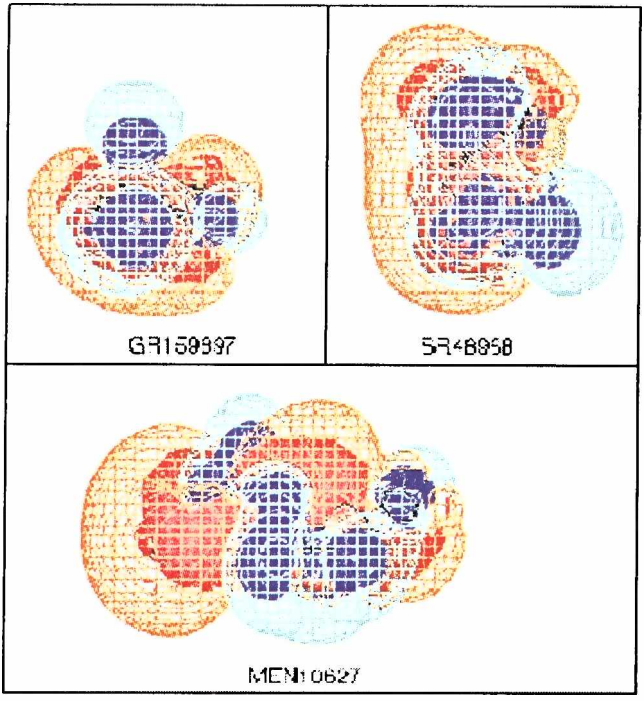


Figure 2. *Molecular electrostatic potential (red: V = +5 kcal/mol; orange: V = +1 kcal/mol; blue: V = -5 kcal/mol; light blue: V = -1 kcal/mol) of low energy conformers of GR159897, SR48968 and MEN10627.*

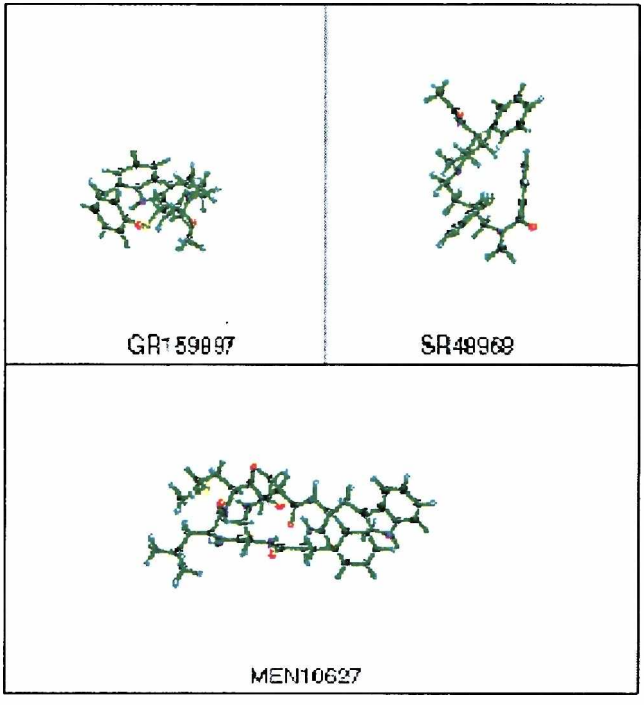


Figure 3. *Structures of the low energy conformers of GR159897, SR48968 and MEN10627, whose potential is displayed in Figure 2.*

with a time step of 1.0 fs and an initial forcing constant of 50 kcal/(mol·Å^2), with initial velocities obtained from the Maxwellian distribution. The system was then slowly warmed to 310 K, and a constant temperature was maintained during the simulation by a weak coupling to a thermal bath. After a 10 ps equilibration, MD was continued for an additional 100 ps, while sampling of the data was peformed every 10 ps. The forcing term was gradually reduced during the simulation. All sampled conformations were minimised using constrained MM minimisations and, subsequently, allowing both the receptor site and the drug to relax.

Among the several low energy conformations of the complexes NK2R_H-SR48968, NK2R-GR159897, NK2R-MEN10627, obtained using the approach described previously, we chose for each couple the minimum energy conformation with maximum intermolecular interaction energy and H-bonding, which were used to construct a model of the active site. To verify and test our choice the Connolly surface was generated for each cavity formed around the ligands (Figure 4), in order to highlight also the geometric features of those cavities. Their shape however was remarkably different; we thus tried to find an "average" cavity (also displayed in Figure 4) able to accept whichever of them was going to enter. Details of these models are available from the authors upon request.

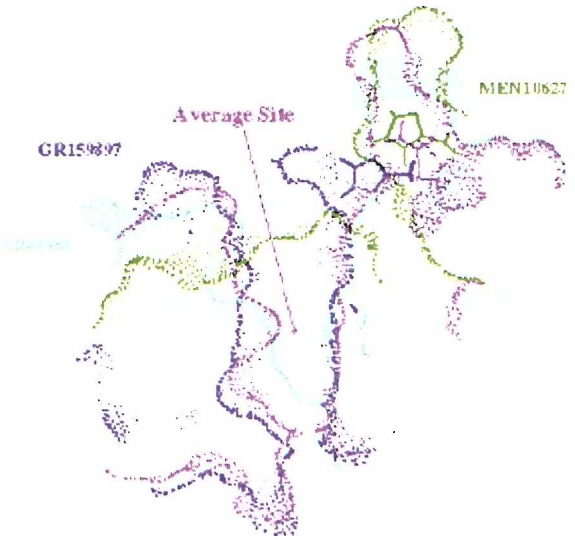


Figure 4. *Side view of the receptor pockets built around GR159897 (blue), SR48968 (cyan), and MEN10627 (green) in the model based on BRD. The "average" binding pocket is displayed in magenta.*

In order to analyse all the possible arrangements inside the average cavity of the several low energy conformers, (with ΔE 8 kcal/mol from the most stable one) obtained for the three known drugs, we used the DOCK code. The structure of the MEN10627 peptide backbone is rather rigid, whereas the side chains of its residues

are mobile with different conformations available. Thus, this bulky molecule cannot completely enter the receptor site: it remains in part on the surface, most likely interacting with residues located in the loops of the extracellular region. The non-peptide antagonists are much more flexible and less bulky than MEN10627; therefore, as shown also by site-directed mutagenesis experiments, they penetrate in depth into the trans-membrane region. The set of GR159897 conformers consists of both extended and folded structures in which the indole and phenyl rings face each other in an almost chelated arrangement. Since the residues, important for its binding from site-specific mutagenesis data, include Tyr-289, Tyr-266 and His-198, which are located on the 7, 6 and 5 helices, respectively, it is rather likely that the active conformation of this molecule is extended in order to allow its interaction with the aforementioned groups. The SR48968 enters the receptor site as well, putting its piperidine ring in the same region as that of GR159897, while part of the compound shows a strong interaction with the tyrosines located at the bottom of the pocket, it binds slightly weaker to Gln-166 and His-198. Other residues (Phe-270 and Tyr-169) might be involved in the binding of the various antagonists, but their role is still under debate [5].

Because of the large number of conformers considered for each compound, it was rather impossible to discriminate somehow among the three drugs according to their known activities. Therefore, the number of conformers displaying a favourable interaction (from the contact or force field point of view) with the receptor (f_c) out of the total number (t_c), considered for each compound, was taken as an index of binding:

$$i\% = \frac{f_c}{t_c} \times 100$$

where i can be either the contact score (*CNT*) or the force field score (*FF*) or both (*CNT&FF*), assuming that several populated active conformations should be more effective than just one, even though this only interaction might be decidedly more favourable than all the others.

Using this statistical approach we again examined the correlation of the activity (pK_i) vs i %, the percentage of favourably interacting conformers, that showed a satisfactory trend, reported in Table I, for active and inactive molecules. The inactive compounds (pK_i<6) present an activity index (*CNT&FF*%) lower than 9, in contrast with the most active ones that show an activity index larger than 17. The (R)-form of SR48968 (the mirror image of (S)-SR48968) and CAMACETIL have been used on purpose, in order to evaluate the performance of the index chosen. The overall regression coefficient is about 0.95. A second set of molecules (all containing both a fluoroindole and a piperidine ring, derived from GR159897 by substituting one or more groups), which activity was available from the literature, have been inserted in the average binding site with the DOCK program. They have been grouped according to the orientation of the indole ring inside the binding site: INT and EXT (toward either the inner or the outer part of the site, respectively). In this case the same interaction threshold as previously was used as well and the conformers below the threshold have been discarded. The results obtained are

reported in Table II, together with the reference pK_i values. The correlation with pKi is slightly better for the conformers with the indole ring oriented toward the inner part of the site than for the EXT ones (regression coefficient r = 0.966 vs r = 0.961).

Table I. *Correlation among the contact (CNT), force field (FF), contact and force field (CNT&FF) scores and the activity (pK_i) of a few drugs.*

Compound	*CNT* (%)	*FF* (%)	*CNT&FF* (%)	pK_i
(S)-SR48968	39.1	26.1	17.4	9.6[a]
(R)-SR48968	26.1	17.4	8.7	<6[a]
(R)-GR159897	60.7	39.3	25.0	10.0[b]
MEN11_	25.0	25.0	12.0	7.6[c]
CAMACETIL	19.0	19.0	4.8	<6[c]

[a] *From Ref. 36a;* [b] *From Ref. 38a;* [c] *A. Menarini Pharmaceuticals, private communication*

Table II. *Correlation between the force field (FF) scores and the activity (pK_i) for a few compounds derived from GR159897 in two different main orientations: INT and EXT (toward either the inner or the outer part of the receptor site, respectively).*

Compound	$FF\ (\%)_{INT}$	$FF\ (\%)_{EXT}$	pK_i
GR159897	80.0	83.0	10.0[a]
GR159897H	50.0	62.5	9.5[a]
GR0 (OH, S)	37.5	60.0	8.4[a]
GR1 (OH, SO_2)	16.7	28.6	7.5[a]
GR2 (OH, NH)	11.0	22.0	7.0[b]

[a] *From Ref. 38a;* [b] *From Ref. 54*

A few new compounds have already been suggested to our industrial partner (A. Menarini Pharmaceuticals) based on the rationale of activity put forward, but syntheses, biological activity tests, and patent registrations are much more time consuming than the MM computational studies, the only level affordable with such large and flexible systems. The same kind of analysis was performed on the prospective drugs, docking them inside the determined average cavity and scoring their arrangement therein. Because of the industrial interest in those molecules we cannot report their data. Their behaviour to the binding was fairly similar to that displayed by the three known drugs, thus supporting a potential good activity.

However, as put forward in the previous sections, the choice of BRD as a template could be the main source of errors in the whole process.

In order to overcome this problem, we have recently constructed a molecular model of the trans-membrane region of the NK2_H receptor using the theoretical model of rhodopsin [8] as a template. The technique used was similar to that employed previously. The multiple sequence alignment of the G-protein coupled receptors and rhodopsins has been chosen, to keep known highly conserved residues of the receptor highly within the trans-membrane helical regions. The molecular model of the trans-membrane helix bundle for the NK2_H receptor was assembled on the rhodopsin template, side chains have been adjusted to remove bad steric contacts between helices, and the structure was subjected to energy minimization using a distance-dependent dielectric constant ($\varepsilon = 4R$) throughout this simulation. The receptor model was minimized using constraints on the backbone atoms and on the side chains of conserved residues.

Comparing this final model, displayed in Figure 1b, with our old model (Figure 1a) the rms deviation of C_α atoms of about 5 Å is observed. The spatial position of the side chains of important residues involved in ligand binding, such as His 198, Tyr 266, His 267, Tyr 289 is quite different. In the model based on rhodopsin these residues are closer and define a portion of a visible pocket (reported in Figure 5) not present in the first step of the construction of the earlier NK2_H model. Also the Ramachandran's plots for the two models are somewhat different (Figure 6) in that, there are less points with positive ψ values in the rhodopsin based model than in the BRD based one.

The docking procedure of the conformers, of known and proposed drugs, into the visible pocket should be repeated with either a rigid or a flexible binding site in order to see whether the interaction is more favorable than with the former model.

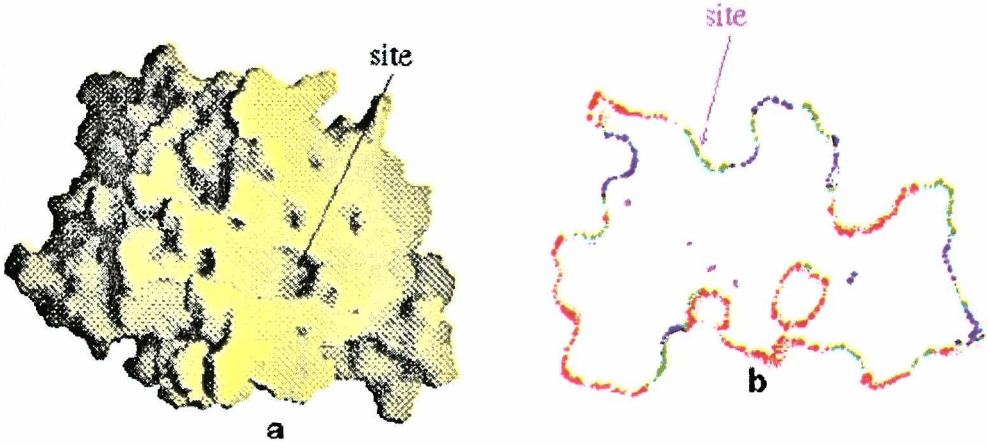


Figure 5. *Receptor binding pocket (indicated as "site") (a) front view and (b) side view, for the model based on rhodopsin.*

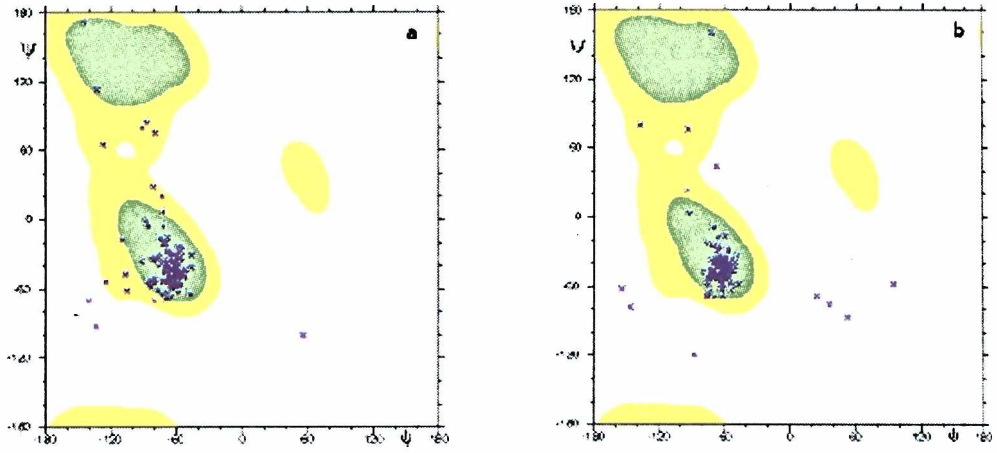


Figure 6. *Ramachandran's plot superimposed to the α-helix one (light grey and dark grey regions) of the models based (a) on BRD and (b) on rhodopsin; the crosses correspond to the NK2R_H residues.*

7. Conclusions

The possibility of building a receptor from the structure of a similar protein helps somehow to overcome the difficulties, linked to the drug flexibility that prevents the identification of the pharmacophore and consequently receptor mapping. On the contrary the drug flexibility may be exploited to dock a large number of structures inside the receptor site, thus trying to figure out which is the active conformation of a given compound. The receptor pocket shaped on the most stable interacting arrangement of the partners is still a rough approximation to the real binding site. An "average" site, shaped to host different drugs, can be a better approach in studying the receptor-ligand interactions, because it retains the main features of the small pockets, even though the binding might be reduced due to its larger dimensions. Under this respect the DOCK program can give a major contribution, checking both the various drug orientations and the corresponding values of the interaction energy, despite the fact that the receptor flexibility cannot be taken into account. A good compromise could be devised as well by considering several conformations of the receptor binding pocket. However, by using rhodopsin as a template, a binding pocket can be found in the likely proper location. The thorough analysis of the interaction energy with the latter binding site, for the various drug orientations, is still in progress, though preliminary results with rigid ligand structures are promising.

We tried to identify the pharmacophore in the three different drugs described to be active toward NK2R_HUMAN, using MEP as a rationale because their structural features are considerably different. A few other molecules, that MEP showed the typical pattern for a number of conformers, were studied and docked in the average cavity. Their activity was estimated using a simple statistical formula. An analogous procedure will be carried out for the model based on rhodopsin.

Though we tried to keep the number of arbitrary choices in our modeling to a minimum, putting the rhodopsin in place of BRD as a template, the side chain orientations, the docking of the ligands, based on mutagenesis experiments and the

set of conformations used can be questioned. Only a good activity and binding of the proposed drugs will give us a hint that this is a valuable route.

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