A pharmacophore model for Neurokinin 2 antagonists based on compounds from several diverse structural classes

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Introduction

Most NK antagonists contain at least two aromatic ring systems connected by a linker holding a hydrogen bond acceptor. We define this part as the head fragment of the NK compounds (Figure 1). The major difference between NK1 and NK2 antagonists is that only the head fragment is required to obtain high NK1 (nM range) affinity whereas NK2 antagonists also require the presence of a tail fragment.

For the NK1 receptor there is general consensus about a pharmacophore model comprised of two aromatic ring systems in a stacked arrangement[1] (Figure 2). We present an NK2 pharmacophore model also containing two aromatic ring systems but in a different arrangement.

The NK2 antagonists used to derive and evaluate the pharmacophore model are shown in Figure 3. The set was chosen on the basis of high NK2 receptor affinity and structural diversity. Both selective NK2 and dual NK1/NK2 antagonists are included in the set.

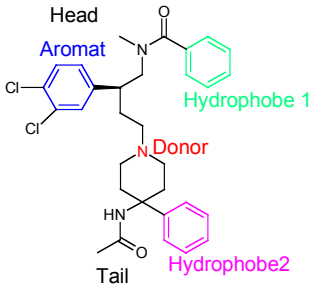

Figure 1: Definition of fragments and pharmacophoric elements in compound **2.**

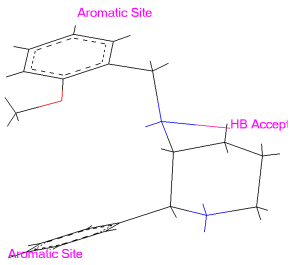

Figure 2: The generally accepted NK1 pharmacophore model. CP99994 is used as template.

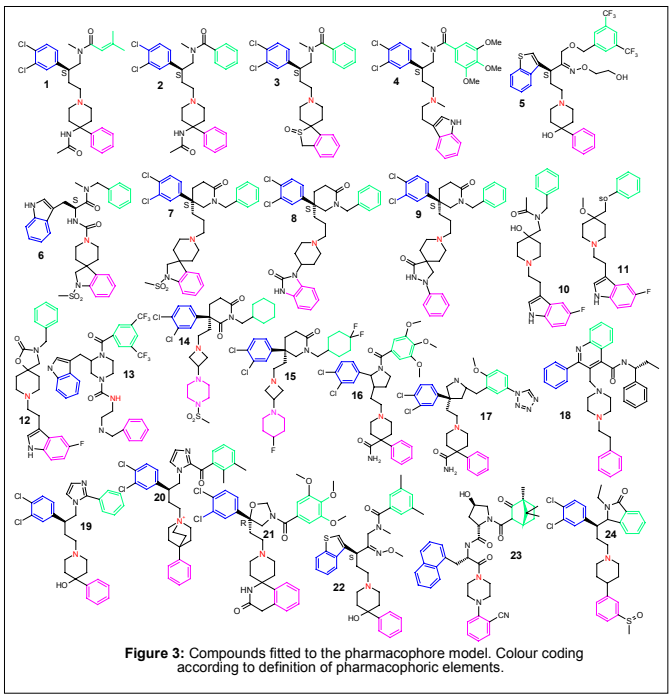

Figure 3: Compounds fitted to the pharmacophore model. Colour coding according to definition of pharmacophoric elements.

Computational Methods

1 Monte Carlo Search. The molecules were build using MacroModel 7.0. [REF] Basic amines were protonated as they would be at physiological pH. The starting geometries were minimised by use of truncated Newton conjugate gradient (TNCG) algorithm. The conformational space was search using the Monte Carlo search. The search was continued until the lowest energy conformations were found several times. The energy minimisations were carried out with TNCG and the MMFF94s [Halgren, 1999 #214][Halgren, 1999 #215] forcefield with the Generalised Born/Solvent Accessible surface (GB/SA) continuum solvation model as build into MacroModel. Default parameters were used.

2 Calculation of the conformational energy penalty. The conformational energy penalty for the putative bioactive conformation of each ligand was calculated by subtracting the internal (steric) energy of the preferred conformation in aqueous solution (i.e. the energy of the global minimum in solution excluding the hydration energy) from the calculated energy of the putative bioactive conformation.[Boström, 1998 #52] Since the conformational ensemble was represented by only the global minimum, entropy effects have not been taken into account.

3 Pharmacophore Definition. Three hydrophobes and a hydrogen bond (HB) donor were chosen as pharmacophore elements. The hydrophobes was aromatic rings or cyclohexane. For each of the rings centroids were constructed. The HB donor was a protonated basic nitrogen or a amide. A vector was constructed from the N-atom to a dummy atom 2.8Å from the N-atom in the direction of the nitrogen-proton bond. The centroids and the nitrogen of the hydrogen bond donor were used for superimpositioning of the ligands.

4 Flo96 flexible superimpositioning search: Structures were build and imported from MacroModel into the automatic fitting program Flo96. [REF] Only two structures were fitted at a time. Either one structure was used as a template or both structures were kept flexible. The output from Flo98 was exported back into MacroModel where each structure was relaxed using flat bottom constrain and the energy was calculated using the MMFF94s forcefield.

Results and Discussion

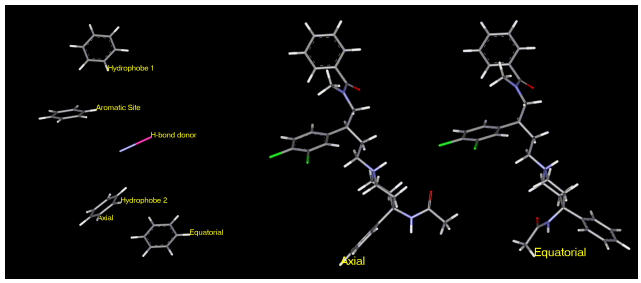

Figure 4: Left: Arrangement of pharmacophore elements. Right: The putative bioactive conformation of **2**.

The pharmacophore model is outlined in Figure 4. The antagonists bind in an extended conformation where "hydrophobe 1" and the aromatic site form an L shape. "Hydrophobe" 2 can be in an equatorial or an axial conformation. The two conformations have similar energy penalties. The hydrogen bond donor is represented as a vector. Figure 5 is a superimposition of 9 ligands, showing that the vector is pointing in the same direction. These results agree with a previously published NK1 and NK2 receptor study[2].

It was possible to fit all compounds onto the pharmacophore model in a low energy conformation (<12kJ/mol) with low RMS values (<0.8Å).

The less active (R)-enantiomer of compound **2** was fitted to the putative bioactive conformation of **2** using Flo96. A conformation that superimposed very well with the template was found (Figure 6). This conformation was not a local minimum and had a conformational energy of 20kJ/mol above the bioactive conformation of the (S)-enantiomer.

The change in free energy, ΔG, can be described by Equation 1, where K_i is the binding constant.

$$\Delta G = RT\ln K_i \qquad \textbf{(Equation 1)}$$

For each energy penalty of 5.8kJ/mol, K_i will increase with a factor of 10. The energy difference between the S- and R-enantiomers corresponds to a drop in affinity of a factor of $2.5*10^3$. This agrees well with the experimental value of $2.0*10^3$ [3].

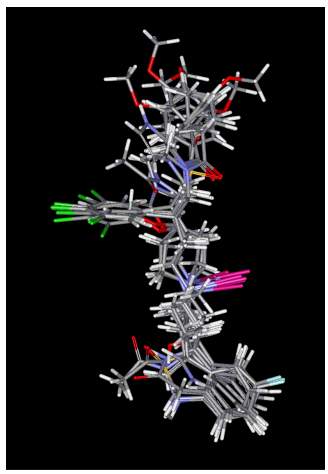

Figure 5: A superimposition of **1**, **2**, **3**, **4**, **10**, **11**, **12**, **19** and **21**. Notice how well the dummy atoms superimpose.

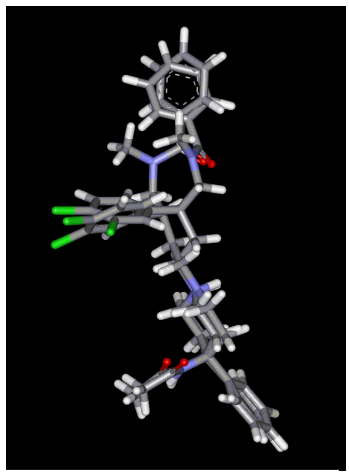

Figure 6: A superimposition of (R)- and (S)-**2**.

Conclusions

- A pharmacophore model for NK2 antagonists has been derived. The model consists of a hydrogen bond donor represented as a vector and of three hydrophobic groups of which only two are necessary for high affinity.
- Two of the hydrophobes are in an L-configuration, and the third hydrophobe can be either equatorial or axial.
- The model has been evaluated against 24 structurally diverse high affinity NK2 and dual NK1 and NK2 antagonists. For all compounds, a low energy conformation was found that fitted into the model with low RMS values.
- The model was successfully able to explain the stereoselectivity of compound **2**.

References

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