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Letter

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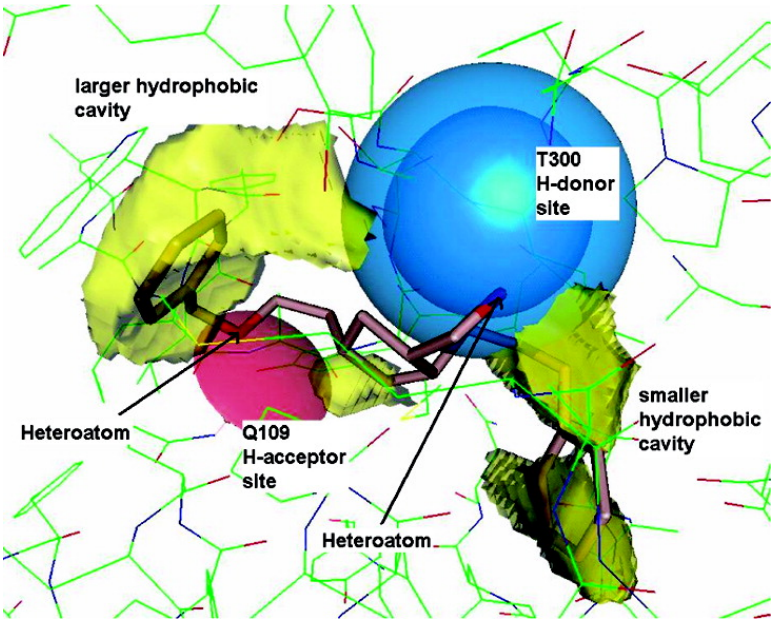

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The First de Novo-Designed Antagonists of the Human NK_2 Receptor

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Abstract: The de novo molecular design program SPROUT has been used in conjunction with a molecular model to produce a molecular template for a new class of NK_2 receptor antagonist. An efficient, stereocontrolled synthesis of a small series of molecules, designed to test the validity of this template, was developed. Competition assays using recombinant human NK_2 receptor support the structural requirements of this new designed molecular template.

The G protein-coupled receptor (GPCR) superfamily constitute perhaps the most ubiquitous signal transduction system in eukaryotic species. This family are one of the largest and most diverse groups of proteins in humans and are encoded by up to 3% of genes within the human genome. These receptors mediate vision, smell, taste, neurotransmission, hormonal and immune responses, and even viral infection.[1] It is therefore not surprising that the central role of GPCRs in cellular signaling renders them primary targets for drug therapy. Indeed, recent estimates suggest that up to 60% of modern pharmacoepia is targeted to GPCRs.[2] These integral membrane proteins all possess a heptahelical intramembranous bundle interconnected by extracellular and intracellular hydrophilic segments.[3−5]

The neurokinin (NK) receptors are an important class of GPCR that are located in the central nervous system, peripheral neurones, and smooth muscle tissue. There are at least three known NK receptors, NK_1, NK_2 and NK_3, although various subtypes and species differences have been discovered.[3] The naturally occurring neuropeptides substance P, NKA, and NKB are potent agonists for each of these receptors, respectively. The interaction of these peptides with their respective receptors is implicated in a range of biological responses including pain transmission, inflammation, and Parkinson's and Alzheimer's diseases. There is therefore tremendous potential for the development of highly specific, bioavailable small molecule antagonists.

To date, in addition to a number of peptidic antagonists,[6,7] there are currently two main classes of nonpeptidic NK_2 receptor antagonists (Figure 1); the benzamide piperidine antagonists, e.g., **1**,[8−12] and those derived from indolium piperidines, e.g., **2**[13] (Figure 1). Although much elegant work has been done to refine the structure−activity relationships within these classes, essentially all of the known antagonists have resulted from compound library screening methods. Pharmacological characterization of site-specific NK_2 receptor mutants has suggested that these two classes of ligands bind to nonidentical receptor sites which overlap partially near the extra-cellular portions of helices VI and VII.[14−15]

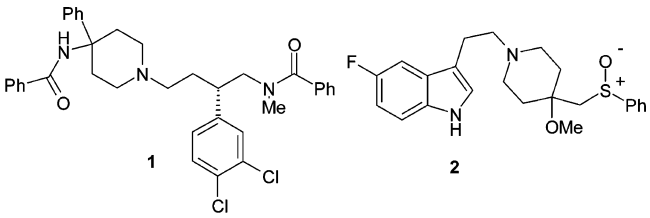

Figure 1. Examples of known NK_2 antagonists.

In the absence of X-ray crystal structural data for these receptors, a 3D molecular model has been developed[16] which utilizes the projection structure of the light-activated GPCR rhodopsin as a template.[17]

The resultant model accommodates data derived from sequence analysis algorithms for the putative transmembrane segments and indicates the relative orientation of amino acid residues within each α helix. We have previously applied a computational automated docking procedure to this model in order to identify the nonpeptidic antagonist binding region.[18] This was predicted to be a broadly cylindrical region spanning the space enclosed by helices III, VI, and VII and bounded by residues N86 (helix II), Q109 and A116 (helix III), H198 (helix V), Y266 (helix VI), and W294 (helix VII) (Figure 2). Evidence supporting the validity of this proposed binding region was obtained from a study of the binding affinities of a series of ligands based on **2** which were designed to test the predicted mode of binding of **2** to the receptor.

The structure-based design of small-molecule enzyme inhibitors is a powerful tool in drug discovery, particularly when there is an X-ray crystal structure of the target enzyme available.

Recent reports involving the application of de novo design indicate that this approach is a powerful alternative to these classical drug discovery approaches.[19−23]

Here, by using the structural features present within the enzyme only, new inhibitor designs are built-up sequentially according to the requirements of the targeted binding site. Therefore, in principle, de novo design should be a far more useful technique than virtual high-throughput screening, because a good de novo design program will examine structure space larger by many orders of magnitude than that of most virtual libraries currently used for this purpose.

To design a completely new class of NK_2 antagonist, we decided to apply a de novo design approach to this putative antagonist binding cavity. We were aware that such an objective, based solely upon a molecular model of a biological target, as opposed to an X-ray or NMR-derived structure, was ambitious. Nevertheless, such a structure-based approach offers tremendous potential in terms of the ability to tailor the design to increase antagonist potency and selectivity for a particular receptor.

Here we report the application of the de novo molecular design program SPROUT[24] to the design of novel

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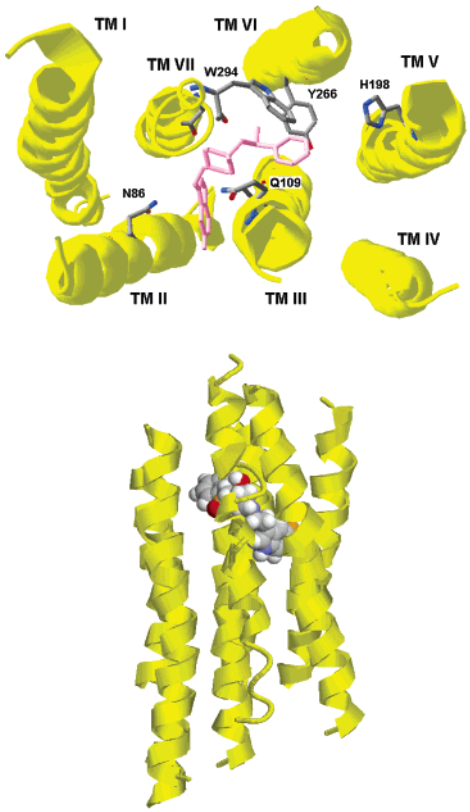


Figure 2. Model of transmembrane region of the NK_2 receptor showing putative antagonist binding region containing **2**. (Top) View looking down into receptor from extracellular side (antagonist shaded pink). (Bottom) Side-view with ligand represented in space-fill and extracellular section of receptor facing uppermost.

antagonists of the human tachykinin NK_2 receptor. We also describe the development of efficient and stereoselective syntheses of these antagonists as well as their biological evaluation.

SPROUT[24] is a powerful suite of software modules for de novo structure-based molecular design. SPROUT has modules for (i) characterizing regions within a protein, (ii) detecting 'hotspots' where ligand atoms would form strong interactions with the protein, (iii) docking molecular fragments to these sites, (iv) joining these fragments with a molecular backbone to give a complete ligand, and (v) ranking the set of predicted ligands by criteria including predicted binding energy, structural complexity, and synthetic accessibility.

To utilize a de novo approach for the design of new NK_2 antagonists, we applied SPROUT to the putative antagonist binding region within our model[25] of the NK_2 receptor.

To design structurally simple antagonists which were predicted to show reasonably good affinity for the enzyme, we wished to utilize only a small number of antagonist−receptor contacts in our SPROUT design strategy. Our earlier work had revealed the presence of two regions of relatively high hydrophobicity that we wished to utilize in antagonist design. Additionally, SPROUT analysis revealed the presence of a number of potential H-bonding sites within the putative binding cavity. From these, a side-chain NH from Q109 helix III (termed a 'donor' site) and a backbone carbonyl from T300 helix VII (termed an 'acceptor' site) were chosen due to their close proximity to the hydrophobic regions. The resulting designed antagonist template consisted of a five-membered cyclic core possessing two chains, 1,3-disposed, and terminating in hydrophobic groups designed to interact with the hydrophobic regions within the cavity. Additionally, the design called for the presence of two specifically located heteroatoms. One of these forms part of one of the hydrophobic chains and accepts an H-bond from Q109 helix III. The other is located as part of a small chain on the central framework. Both of the hydrophobic chains and this smaller chain are required to have an all-cis relationship (Figure 3).

We have previously reported a simple and convenient method for the synthesis of 1,3-cis disubstituted tetrahydrofuranyl rings based upon the oxidative ring-opening of the adducts derived from cycloadditions of 3-oxidopyrilium betaines.[26] We reasoned that molecules possessing the structure required by the designed template could be readily accessed using this chemistry, and a short synthesis of a range of such systems **6** designed to test the validity of the antagonist template in Figure 1 is shown below (Scheme 1).

Ketone protection of bicycle **3** (obtained along with the C-3 diastereoisomeric ester as a separable 1:1 mixture; total yield 76%, from cycloaddition of 3-oxidopyrilium betaine with methyl acrylate using the literature procedure[27]) followed by ester reduction and protection gave ether **4** in good yield. Ozonolytic cleavage and reduction then gave diol **5** which was directly alkylated to yield ethers **6**. Use of the more bulky 2-(bromomethyl)naphthalene as alkylating agent resulted in predominately monoalkylation on the chain attached to C-5 of the THF ring. This selectivity of alkylation was used to prepare ethers **6** which possessed differing aromatic substituents. An analogous reaction sequence applied to the C-3 epimeric ester of compound **3** was also used to give access to the C-3 epimeric alcohol corresponding to **6**. We were surprised to find that all attempts at hydrolysis of ketals **6** to reveal the corresponding ketones were unsuccessful, mild acid hydrolysis failing completely and more vigorous acid hydrolysis or the use of Lewis acids giving complex reaction mixtures.

To ascertain the likely effect of incorporation of the ketal unit upon receptor binding, the computer model containing the designed template was modified so as to incorporate the ketal unit. Following energy minimization within the receptor model, comparison of this structure with the original template revealed that the ketal moiety is located in a space near to helix VII between M297 and S299. Although no additional bonding interactions are present, this modification has very little effect on the positioning of the original template (Figure 4), and we were satisfied that structures **6** would be a good representation of the SPROUT-designed antagonist template.

Biological evaluation[28] of the designed antagonists **6** was performed using competition assays with [^{3}H]-SR48968 **1** and recombinant human NK_2 (Table 1).

As can be seen from Table 1 entry 1, the compound corresponding to the SPROUT-designed antagonist template shows the greatest affinity for the NK_2 receptor. Changing the stereochemistry at the C-3 atom in structure **6** results in greater than a 500-fold decrease

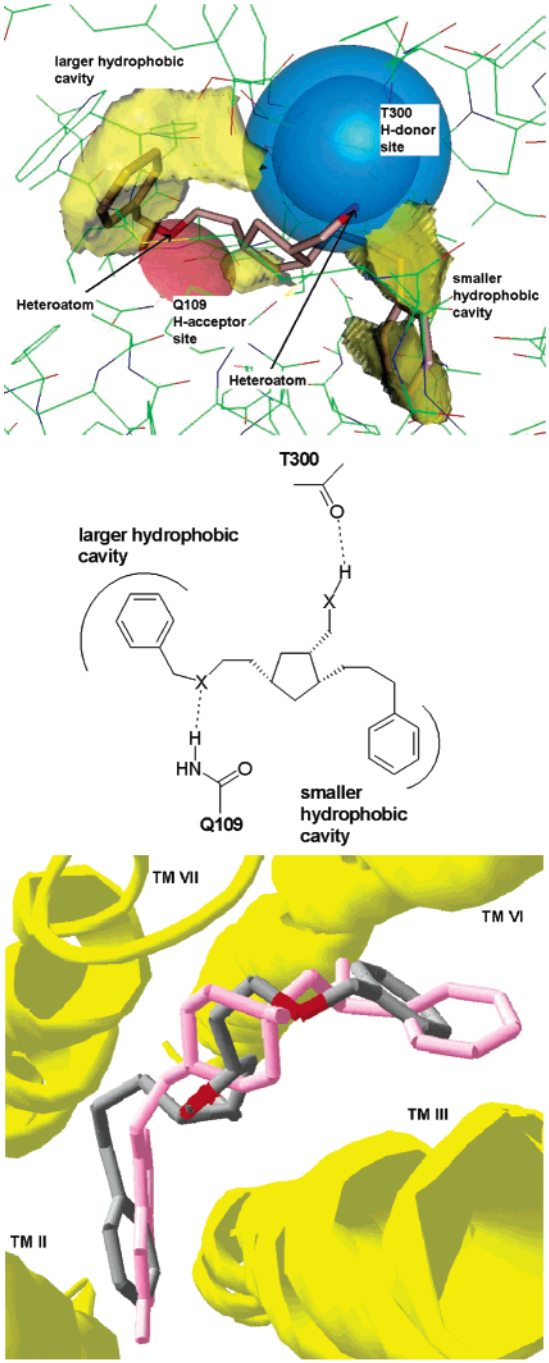


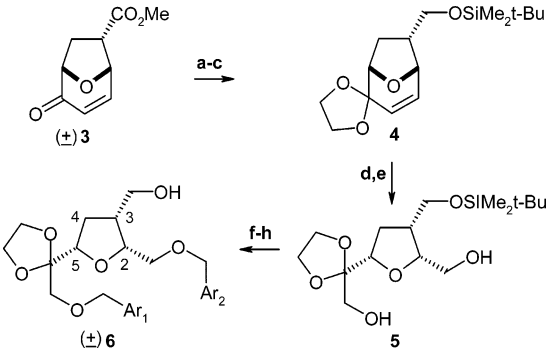
Figure 3. SPROUT-generated NK_2 antagonist template. (Top) The hydrophobic cavities (yellow) and the H-bonding sites (blue and red) utilized in the design. (Middle) Schematic showing contacting residues (X = N or O). (Bottom) Overlay of designed template and docked ligand **2** (shaded in pink).

in activity (entry 2), in keeping with the requirement for an 'all-cis' stereochemical relationship.

Interestingly, increasing the bulk of the hydrophobic substituent attached to C-5 of the ring from phenyl to naphthyl (entry 4) reduces the affinity by greater than 200-fold whereas changing both hydrophobic substituents to naphthyl leads to more than a 300-fold decrease in affinity (entry 3). These observations appear to indicate the size-constraints within the hydrophobic cavities.

Clearly, the designed template is for a single enantiomer of absolute stereochemistry shown in Figure 3. In these proof-of-principal studies, however, we have

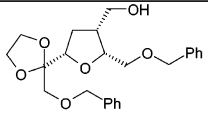
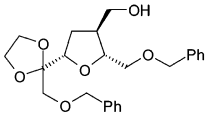
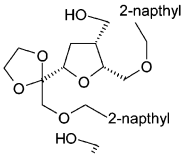
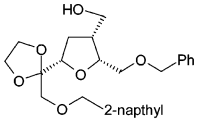
Scheme 1. Synthesis of Designed Antagonists **6**[a]



[a] Reagents and conditions: (a) ethylene glycol, TsOH, 61%; (b) $LiAlH_4$, Et_2O, 72%; (c) t-$BuMe_2SiCl$, DMF, 71%; (d) O_3, MeOH; (e) $NaBH_4$, MeOH; (f) NaH, THF; (g) $ArCH_2Br$, THF, 53−65% over steps d−g; (h) $^nBu_4NF$, THF, 87−98%.

Figure 4. Overlay of SPROUT-designed template (darker shading) with ketal-modified system (lighter shading), both located within the NK_2 receptor model (not shown).

Table 1. Structures and Activities of Designed Antagonists

Entry	Structure[b]	IC_{50}(μM)
1		2.18
2[a]		1230
3		810
4		504

[a] Obtained from the C-3 diastereoisomer of **3** using the synthetic sequence shown in Scheme 1. [b] Obtained as racemic mixtures.

chosen to perform biological evaluation using readily available enantiomeric mixtures containing this designed template along with the enantiomer. Therefore, although the measured binding affinities of these mixtures to NK_2 are very encouraging, it is likely that the enantiomerically pure versions corresponding to the designed molecular template are even more potent in the presence of NK_2.

In conclusion, the de novo design program SPROUT has been used in conjunction with a stereoselective synthetic route, to produce a simple molecular template for a new class of NK_2 antagonist. We believe that this methodology is complementary to the use of high-throughput screening and is particularly attractive where such screening methodology is not available or where access to large collections of library compounds of sufficient molecular diversity is limited. This approach clearly holds tremendous potential for the identification of useful leads for the development of new GPCR-targeted drugs.

Supporting Information Available: Experimental procedures, including analytical, spectral, and X-ray crystallographic data for the preparation of antagonists **6**, and details of the NK_2 receptor binding assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) Assays performed as described in reference 12 and in the Supporting Information.

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