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Generation of a new class of hNK_2 receptor ligands using the 'fragment approach'

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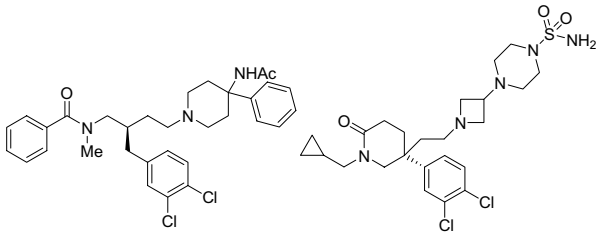
Abstract—The so called 'fragment approach' was applied in the search for new leads as selective hNK_2 antagonists. A first round of structural space exploration through the use of bond rigidity as scaffold to support the fragments, afforded **27a** as 200 nM hNK_2 ligand. Further refinement gave **MEN 14933** as a 16 nM hNK_2 ligand, selective versus hNK_1, of a novel class. Conformational analysis was used to study results and plan future work.

The human NK_2 (hNK_2) receptor has been validated as a suitable target for the development of novel drugs to be used for the treatment of a number of diseases in the respiratory, gastrointestinal and genitourinary tract.[1] Even though a number of hNK_2 antagonists have entered clinical trials (Saredutant, UK-224671, Nepadutant),[2] none has yet reached the market.



As a part of a project aimed at identifying new leads as selective hNK_2 antagonists, we set up a three component mini-library applying the 'fragment approach'.[3] We aimed to: (a) limit the molecular complexity of our fragments in order to increase the probability of a match with the receptor;[4] (b) identify a relatively rigid fragment to use as molecular anchor;[5] (c) maintain the lead-likeness of our compounds.[6]

Our initial work focused on the positioning of two aromatic rings in a relatively rigid structural motif to generate the anchoring moiety and then to add to this binary system a flexible 'drug-like' solubilizing moiety (Fig. 1).

Aromatic functional groups were chosen for their versatile binding potential, being able to participate in lipophilic, face-to-face, edge-to-face, π-cation and hydrogen bond interactions.[7]

Two kind of bonds, amide and urea, have been used to join the three fragments. The relative rigidity and defined geometry of these bonds made it possible to

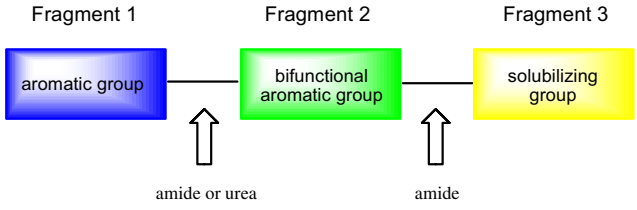


Figure 1. Working plane in the search of hNK_2 antagonists novel topology.

Keywords: NK_2; Fragment approach.
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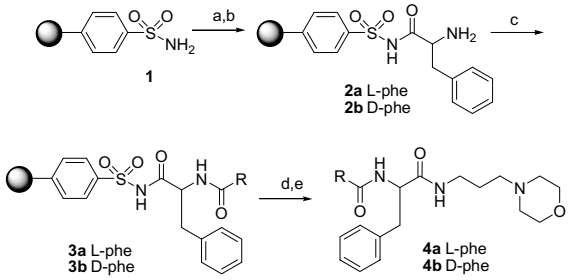


Scheme 1. Reagents and conditions: (a) Boc–PheOH (5 equiv), PyBop (5 equiv), DIPEA (5 equiv), 1 M soln in DMF; (b) TFA/DCM 9:1; (c) RCO_2H (5 equiv), DIPC (5 equiv), HOBt (5 equiv), DMAP cat., DMF/DCM 5:1; (d) 1 M $TMSCHN_2$, THF/hexane (1:1); (e) aminopropylmorpholine (1.1 equiv), then isocyanate resin, DCM.

direct the fragments into different regions of space. The choice of building blocks was mainly dictated by their shape, together with commercial availability and/or synthetic accessibility; a certain amount of synthetic flexibility must be available for improving the binding and selectivity of the ligands and to optimize the PK parameters.

In the work presented here we concentrated on fragment 1 and its attachment to fragment 2. Phenylalanine, in both configurations, was our bifunctional aromatic moiety and to begin with 3-aminomethyl morpholine was chosen as the solubilizing group.

For both series (amides and ureas) we took advantage of solid phase synthesis. Boc–phenyl alanines, D and L, were loaded onto Kenner safety-catch linker polystyrene resin and the Boc group removed with a mixture of TFA/DCM, followed by neutralization with a solution of DIPEA in DCM.[8]

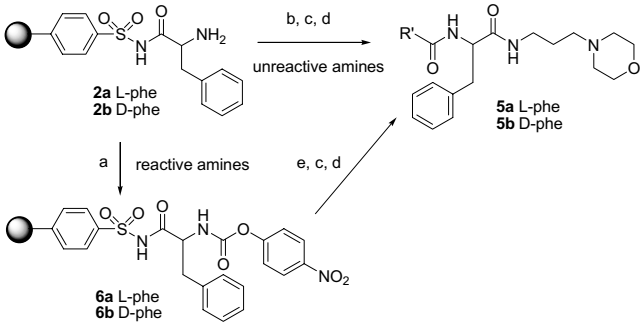
The amides were prepared on the resin by coupling with the corresponding acid with DIPC/HOBt/DMAP, followed by $TMSCHN_2$ activation and amine cleavage (Scheme 1).

For the preparation of ureas we used two different methods: with nucleophilic amines the *p*-nitrophenyl chloroformate method[9] was the preferred one, while for poorly nucleophilic amines, the corresponding carbamoyl chlorides were preformed in solution (Scheme 2).

A first set of amides was prepared with seven different building blocks (**7**–**13**). Only the couple L-phe/*p*-chlorophenyloxydimethylacetic acid (**11b**) showed a submicromolar affinity for the hNK_2 receptor (Table 1).

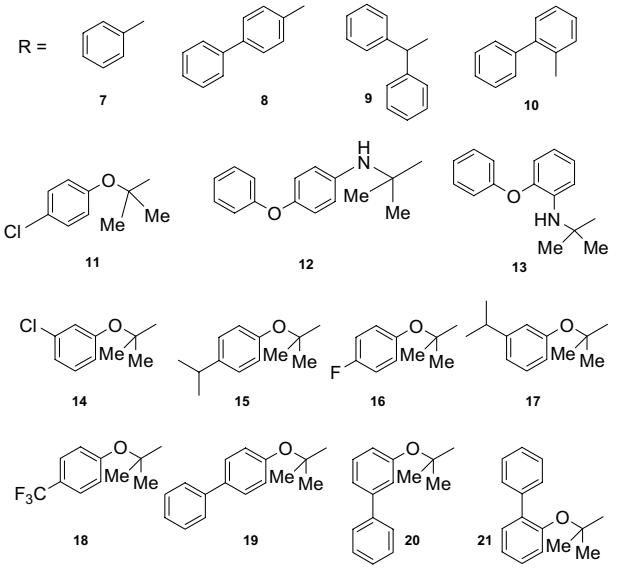
A brief SAR study of this fragment (compounds **14**–**21**) showed no significant improvement over **11b**.

For the ureas there were 10 initial fragments (**22**–**31**, Table 2). In this case four compounds (**26b**, **27a**,**b**, **31b**) showed submicromolar binding affinity. Consequently small groups of compounds containing fragments **27** and **31** variously substituted were prepared (Table 3).



Scheme 2. Reagents and conditions: (a) *p*-nitrophenylchloroformate (10 equiv) DMF; (b) carbamoyl chloride (10 equiv), DIPEA (10 equiv), DMF/DCM 1:3; (c) 1 M $TMSCHN_2$, THF/hexane 1:1; (d) aminopropylmorpholine (1.1 equiv) then isocyanate resin, DCM; (e) R′ (amine) (10 equiv), DIPEA (10 equiv), 0.5 M soln in DMF.

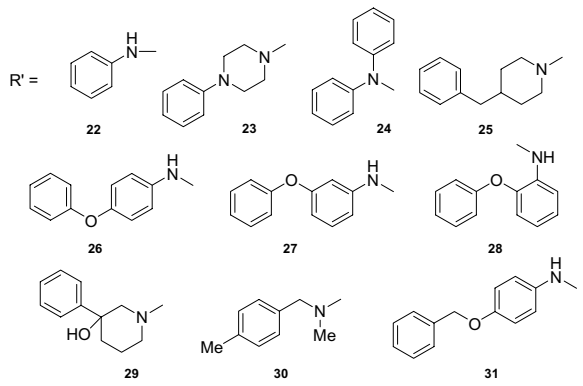
Table 1. hNK_2 binding affinities for compounds **7**–**21**



Compound[10]	K_i (μM)[11]	Compound[10]	K_i (μM)[11]
7a	>10	**14b**	0.31
7b	>10	**15a**	>1.0
8a	2.0	**15b**	>1.0
8b	10	**16a**	>1.0
9a	2.0	**16b**	>1.0
9b	>10	**17a**	>1.0
10a	>10	**17b**	>1.0
10b	3.0	**18a**	>1.0
11a	1.6	**18b**	>1.0
11b	0.6	**19a**	7.9
12a	>3.0	**19b**	7.9
12b	>3.0	**20a**	6.3
13a	>3.0	**20b**	10
13b	>3.0	**21a**	3.1
14a	>1.0	**21b**	5.0

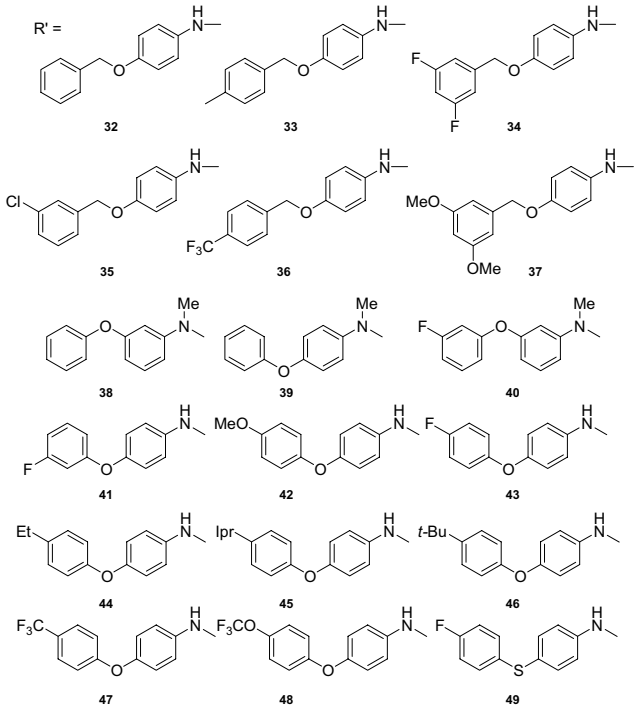
The breakthrough came with the introduction of substituents on the terminal phenyl ring of fragments **26** and **27**. The introduction of lipophilic substituents in the *para* position of the terminal phenoxy group gave an

Table 2. hNK_2 binding affinities for compounds **22–31**



Compound[10]	K_i (μM)[11]	Compound[10]	K_i (μM)[11]
22a	>10	**27a**	0.2
22b	>10	**27b**	0.3
23a	>3.0	**28a**	5.0
23b	>3.0	**28b**	>10
24a	>3.0	**29a**	6.3
24b	>3.0	**29b**	>10
25a	1.6	**30a**	2.0
25b	2.0	**30b**	3.0
26a	1.0	**31a**	>1.0
26b	0.5	**31b**	0.1

Table 3. hNK_2 binding affinities for compounds **32–49**



Compound[10]	K_i (μM)[11]	Compound[10]	K_i (μM)[11]
32a	1.2	**41a**	0.2
32b	0.63	**41b**	>1.0
33a	0.79	**42a**	0.31
33b	>1.0	**42b**	>1.0
34a	0.5	**43a (MEN 14933)**	0.016
34b	>1.0	**43b**	>0.31
35a	0.32	**44a**	0.20
35b	0.63	**44b**	0.63
36a	0.79	**45a**	0.063
36b	5.0	**45b**	0.2
37a	0.2	**46a**	0.05
37b	>1.0	**46b**	0.12
38a	>3.0	**47a**	0.25
38b	>3.0	**47b**	>1.0
39a	>3.0	**48a**	0.1
39b	>3.0	**48b**	>1.0
40a	>1.0	**49a**	0.13
40b	>1.0	**49b**	>0.2

order of magnitude improvement in K_i. The best binding was shown by **43a** (**MEN 14933**) the *p*-fluoro derivative containing the L enantiomer of phenylalanine.

A functional test on the human urinary bladder[12] showed that **MEN 14933** was an antagonist, with a pK_B 6.9.

Binding affinity for the hNK_1 receptor was evaluated against SP[11] and resulted in an IC_{50} of 621 nM, revealing a selectivity for the hNK_2 receptor over the hNK_1.

Moreover, **MEN 14933** was profiled for physicochemical parameters and showed a log $D_{7.4}$ of 4.4 and a solubility of 0.3 μmol/mL. Calculated properties[13] resulted in cpK_a of 7.5 and PSA of 91.9 A^2.

Two computational studies were then undertaken to analyze results and plan future work. In the first, the space accessible to the aromatic group (fragment **1** in Fig. 1) of each molecule was determined through a conformational analysis[14] of model compounds $RCONHCH_3$ and $R'CONHCH_3$ for the amides and urea series, respectively.

To compare the spatial disposition of the aromatic rings the conformers obtained for each model compound were aligned, superimposing the four common heavy atoms CONC. Aromatic groups of the aligned model compounds are shown in Figure 2. Although the aromatic rings of the active and inactive compounds share a region of structural space, an area exists, which is inaccessible to those with poor binding affinity, whereas all those with high affinity place one or more or the aromatic groups in this region.

The aim of the second study, was to compare our molecules with a pharmacophore model (Fig. 3) for the hNK_2 receptor derived by Poulsen et al. (model 2 in Ref. 15). This model is characterized by three hydrophobic elements (red spheres), and four putative counterparts (yellow spheres) of a hydrogen bond donor and two hydrogen bond acceptors, one of which is bidentate (atoms in ball and stick).

Low energy conformers of **MEN 14933**, obtained through a conformational search,[16] were evaluated against the model. A small group of them was found to fit the model with two hydrophobic features and three of four H-bond donor acceptor systems. One of these conformers is reported in Figure 3.

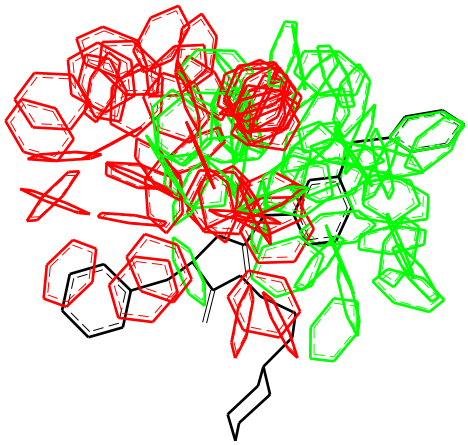


Figure 2. Spatial disposition of the aromatic groups. Red: inactive compounds with L-phe (K_i > 1 μmol); green: active compounds with L-phe (K_i < 1 μmol). To identify the position of the ring in relation to the entire molecule a conformer of **27a** is reported in black.

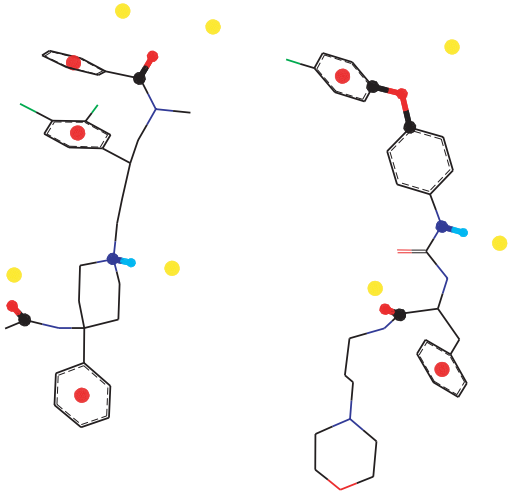


Figure 3. Left: Saredutant according to Paulsen et al.[15] fits all the elements of the pharmacophore model. Right: Conformer of **MEN 14933** that fits five of seven pharmacophoric points with an RMS value of 0.6.

Starting from the idea of maintaining two aromatic rings in a relatively rigid geometry and in the search for a new topology, we performed the synthesis of a number of compounds according to Figure 1. Our attention focused on fragment 1 and its connection with fragment 2. This lead us to identify **MEN 14933**, a 16 nM ligand for the hNK_2 receptor, which showed in vitro antagonist functional activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.11.045.

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10. All final compounds were characterized by ^1H NMR and MS purity, evaluated by analytical HPLC (214 nm), was >95%.
11. Binding experiments were performed on hNK_1 or hNK_2 receptors transfected onto membranes of U-373MG (hNK_1) or CHO-K1 (hNK_2) cells. Compounds were tested for their ability to displace the opportune radioligand from the receptor: [^{3}H][Sar^9]SP sulfone, 1.2 nM (hNK_1), [^{125}I]NKA, 0.15 nM (hNK_2). The affinity of the test compounds for the tachykinin receptors determined in these competition experiments was expressed in terms of K_i.
12. Functional experiments on human urinary bladder. Mucosa-free strips of detrusor muscle were excised from the urinary bladder dome of patients undergoing cystectomy because of carcinoma of the bladder base. The strips were placed in 5 mL organ baths filled with oxygenated Krebs–Henseleit solution at 37 °C, under a resting tension of 10 mN. Mechanical activity developed by the preparations was recorded isometrically. The test compounds were tested for their ability to block neurokinin A-induced contractions. Antagonist affinity was expressed as pK_B; pK_B = log[dose ratio − 1] − log [antagonist concentration].
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14. The conformational analyses were carried out using the grid search module of SYBYL 6.91 (Tripos Inc.). The resulting conformers were minimized in SYBYL using the MMFF94s force field with a water dielectric constant of 78.5. Structures located more than 3 kcal/mol above the minimum were discarded.
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16. The computational study of **MEN 14933** was performed using MACROMODEL 8.5 (Schröedinger, L. L. C.). The conformational space was searched using the Monte Carlo method (5555 steps). All rotable single bonds and the flexible morpholine ring were included in the search. The energy minimizations were performed using the MMFF94s force field with the GB/SA solvation model. Conformations (337) within 3 kcal/mol of the minimum, were obtained.