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α,α-Cyclic aminoacids as useful scaffolds for the preparation of hNK_2 receptor antagonists

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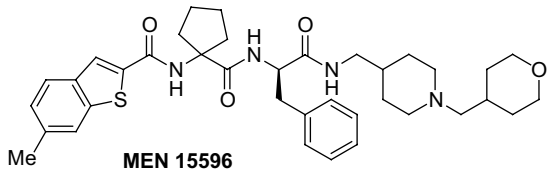
Abstract—MEN 15596 is a small molecule, potent and selective antagonist of NK_2 receptor, possessing high affinity and potency at the guinea-pig and human receptors whose pharmacological characterization has been recently published. Here we report how the corresponding class of compounds was derived from a tri-peptide library and the first optimization round to improve both in vitro activity and physicochemical properties.

The neuropeptides belonging to the tachykinins family (substance P, Neurokinin A and Neurokinin B) are widely distributed in the mammalian central and peripheral nervous systems and produce a wide range of biological effects through the stimulation of the three receptor subtypes NK_1, NK_2 and NK_3.[1] Neurokinin A, which displays the highest affinity for NK_2 receptor, has links to chronic diseases in the gastrointestinal, respiratory and genitourinary tracts.[2] The development of antagonists of the NK_2 receptor may provide opportunities for the therapy of diseases like asthma, inflammatory bowel disorders, rheumatoid arthritis, pain and psychiatric disorders.[3]



MEN 15596 is a small molecule, potent and selective antagonist at the NK_2 receptor, identified in our laboratories, which possess high affinity and potency for guinea-pig and human receptors, long duration of action in vivo experiments and good oral bioavailability.[4] Here we describe how the class of compounds from which MEN 15596 originated was selected and submitted to a first round of optimization for the in vitro potency.

As part of a project aimed at the identification of a series of small, orally available NK_2 receptor antagonists, we tested on the hNK_2 receptor one of our in-house peptide libraries of general structure shown in Figure 1.

The L- and D-forms of all the natural aminoacids, excepts cysteine, were inserted in the positions represented by α-aminoacid 1 and 2.

The hits identified during this first screening (Table 1) underlined a clear affinity pattern: phenylalanine was the best aminoacid 1, in both L and D configurations, while leucine was best suited for position 2, but in this case D-isomer was largely the preferred one. Derivative **1** was selected as starting point. For practical reasons we proceeded in our studies with L-Phe, being the affinity difference between the two enantiomers not so large, and decided to retest both the enantiomers once the overall molecule characteristics were improved.

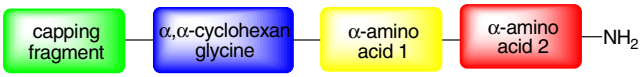


Figure 1. Schematic structure of the peptide library.

Keywords: NK_2 receptor; α,α-Aminoacids; NK_2 antagonists.
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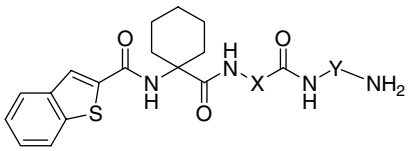
Table 1. Hits emerged from screening against hNK_2 receptor of an in-house produced tri-peptide library



Compound[5]	X	Y	pK_i[6]
1	L-Phe	D-leu	7.15
2	L-Phe	L-Leu	5.0
3	D-Phe	L-Leu	6.5
4	D-Phe	D-leu	7.3

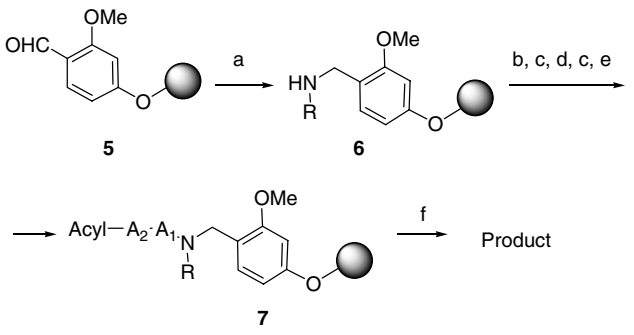
The compounds reported in this paper were prepared by solid phase synthesis, according to the sequences shown in Schemes 1 and 2.

Reductive amination of FMPB AM linker[7] (**5**) with excess of an opportune primary amine and $NaBH(OAc)_3$ converted quantitatively the formyl group into the corresponding secondary amine **6**, which was then coupled with the two subsequent Fmoc protected aminoacids A^1 and A^2, and finally capped with a carboxylic acid according to standard peptide synthesis methodology. Cleavage from the resin was obtained through treatment with a 25% solution of TFA in DCM.

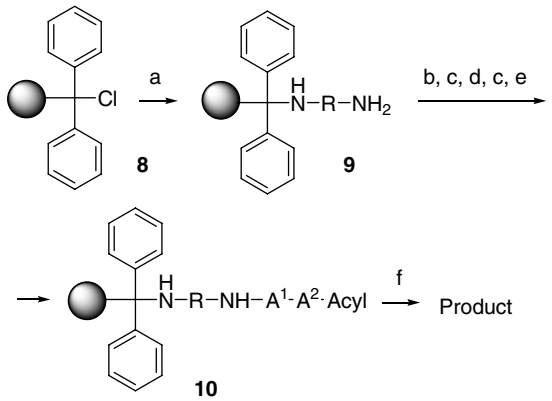
In the case of symmetrical C-terminal amines, the amine was loaded on trityl resin, and again standard peptide synthesis methodology followed by mild acidic treatment allowed the recovery of the desired product.

A general characteristic of compounds **1**–**4** was a very low aqueous solubility, very likely deriving from both stacking and hydrophobic interactions, which made them almost untractable. So we prepared a small panel of compounds where the terminal D-leucine amide was substituted with a smaller and/or flexible solubilizing group (Table 2).

The aminoethylethoxyethyl group (**15**) showed a binding affinity similar to the one of the starting hit (**1**), but was clearly much more soluble.

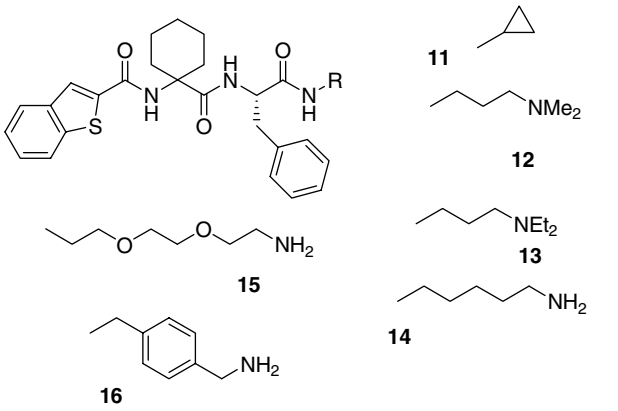


Scheme 1. Reagents: (a) RNH_2, $NaBH(OAc)_3$; (b) $FmocA_1$, DIPCD, HOBt; (c) 20% piperidine in DMF; (d) $FmocA_2$, DIPCD, HOBt; (e) carboxylic acid, DIPCD, HOBt; (f) 20% TFA in DCM, TIPS.



Scheme 2. Reagents: (a) NH_2RNH_2, DCM; (b) $FmocA^1$, HOBt, DIPC; (c) 20% piperidine in DMF; (d) $FmocA^2$, HOBt, DIPC; (e) carboxylic acid, DIPCD, HOBt; (f) 1% TFA in DMC, TIPS.

Table 2. Leucine substitution to increase solubility



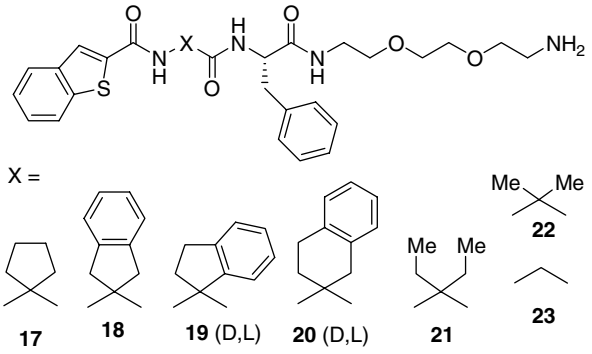
Compound[5]	pK_i[6]
11	6.5
12	6.5
13	7.4
14	6.4
15	7.9
16	6.9

Taking this C-terminal side capping group as suitable temporary solubilizing moiety, an optimization of the benzothiophene-α,α-cyclohexylglycine-phenyl alanine portion was undertaken in the form of small to medium size libraries.

A first set of compounds was prepared introducing other α,α-aminoacids, both cyclic and not, and glycine as negative control. The results are reported in Table 3.

As expected glycine (**23**) was inactive, losing all the entropic gain introduced by the constraint and, very likely, also beneficial hydrophobic interactions. All the other scaffolds revealed to lead to the same range of activity of **15**, with two interesting exceptions. The first one is **20**, which has a drop in K_i of over one order of magnitude versus **15**, very likely due to steric factors. The second is the difference in affinity of **21** (α,α-diethyl) versus **22** (α,α-dimethyl). In this case we could ascribe

Table 3. Rigid scaffold scanning



Compound[5]	pK_i[6]
17	7.3
18	7.7
19	7.9
20	6.4
21	<6
22	7
23	<5

the effect to the overall quite rigid molecular conformation.

Substitution of phenylalanine with a large panel of non-natural aminoacids gave no improvement, as did a scanning of the *N*-capping acyl group (data not reported).

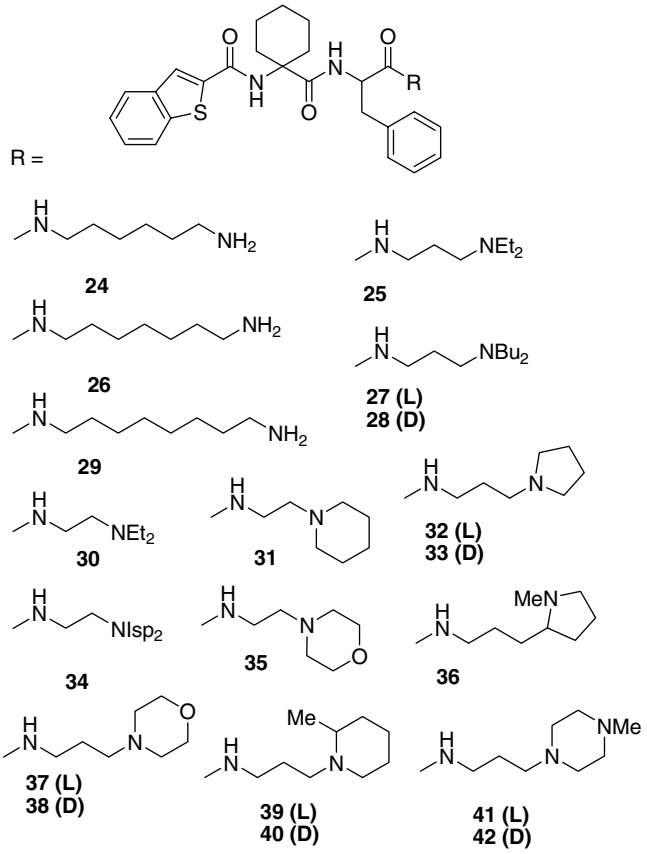
All these results in terms of binding affinity confirmed our suspicions that the anchoring part of the starting hit was the quite rigid benzothiophene–cyclohexane–phenylalanine motif. Very likely improved potency could be obtained by searching for additional interactions with the receptor of substituents on the carboxy-terminal section of the molecule. Pursuing this objective, compounds were first prepared with L-Phe, and for the better ones the D-Phe isomer was also tested (Table 4).

Indeed, with the scanning of C-terminal substituents we found a general net increase of binding affinity. Moreover, differently from what happened with the couple of analogues **1/4**, moving from L-Phe to D-Phe, caused an increase in the pK_i values, in some cases of more than one log unit (**39** vs **40**; **37** vs **38**). A final panel of compounds was prepared inserting the rigid scaffolds found to be equivalent to cyclohexane in the previous scanning (Table 5).

A number of compounds displayed nanomolar and even subnanomolar (**43**) affinity values. The best ones, in view of evaluation in our in vivo animal model,[8] were tested for functional antagonist potency in guinea pig isolated proximal colon (GPC-pK_B, Table 6).[9]

Compound **43** was evaluated for solubility (0.1 mg/mL at pH 7.4) and for in vitro PK parameters (Papp 9.0×10^{-6} cm/s in Caco-2 cells; 4% left after 30 min incubation with GP liver S9 fraction). Its antagonist potency measured in the human urinary bladder smooth muscle contractily assay resulted in a pK_B of 8.3.[10]

Table 4. C-terminal substituent scanning

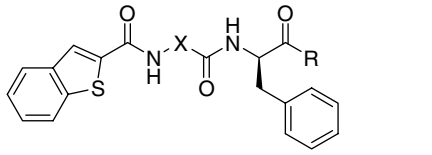


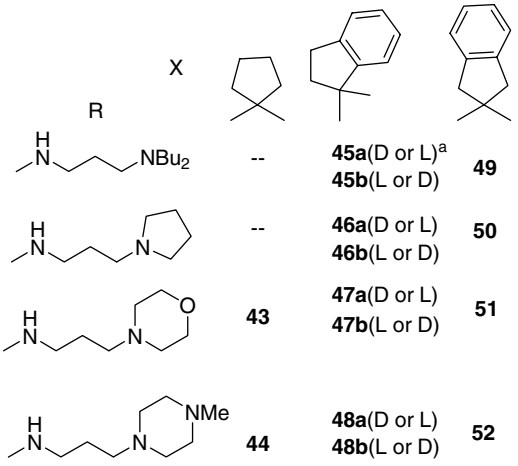
Compound[5]	pK_i[6]
24	6.6
25	7.0
26	7.0
27	8.2
28	8.9
29	6.9
30	7.0
31	7.2
32	7.7
33	8.4
34	7.5
35	7.0
36	7.3
37	7.6
38	9.1
39	8.1
40	9.4
41	7.0
42	8.4

Starting from a hit derived from constrained and capped tripeptide library, we were able, through iterated structural modifications, to obtain compound **43**, with a subnanomolar binding affinity for the hNK_2 receptor, with acceptable aqueous solubility and Caco-2 cells permeability. In vivo assessment on our model after iv administration gave no detectable activity, very likely because of its very low metabolic stability.

Nevertheless, its activity profile in vitro made **43** a good starting point for further optimization of the in vivo activity. The results of this work will be reported in due time.

Table 5. Scaffold modulation





Compound[5]	pK_i[6]
43	9.2
44	8.7
45a	7.5
45b	8.8
46a	8.2
46b	8.9
47a	7.0
47b	8.7
48a	<7
48b	9.0
49	8.8
50	8.9
51	8.7
52	8.6

[a]Diastereoisomers were separated, but not assigned.

Table 6. Functional activity of selected compounds

Compound[5]	pK_i[9]
28	6.7
33	<6
38	7.0
39	<5.5
40	6.7
24	<6.0
43	7.6
47b	7.7
48b	7.6
49	6.7
50	6.7
51	7.0
52	6.9

Acknowledgments

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5. All final compounds were characterized by MS. Purity was evaluated by analytical HPLC ($\lambda = 214$ nm) and was >95%. Compound **43** was also characterized by ^{1}H NMR.
6. Binding experiments were performed with membranes of CHO-K1 (hNK_2) cells stably transfected with the human NK_2 receptor. Compounds were tested for their ability to displace [^{125}I]NKA, 0.15 nM. The affinity of the test compounds for the tachykinin NK_2 receptors determined in these competition experiments was expressed in terms of pK_i.
7. 4-(4-formyl-3-methoxyphenoxy)butyryl AM (Novabiochem).
8. Male Dunkin–Hartley guinea-pigs (Charles River, Italy), weighing 350–400 g, were anaesthetized with urethane (1.5 g/kg, sc) and the left jugular vein cannulated for drug administration. The abdomen was opened and a latex balloon, connected to a PE90 polyethylene catheter, was inserted into the proximal colon at about 2–3 cm from the caecum, filled with 0.5 ml of saline and connected to a pressure transducer (Transpac IV, Abbott) for intraluminal pressure recording by means of a MacLab/8s data acquisition system (ADInstruments, UK). The activity of the antagonists was determined as inhibition of the response induced by intravenous administration of the selective tachykinin NK_2 receptor agonist [βAla^8]NKA(4–10) at 3 nmol/kg repeated at 5, 30 and then every 30 min until 4 h after treatments.
9. The experiments were performed on guinea-pig (Dunkin–Hartley, Charles River, Italy) isolated proximal colon circular smooth muscle preparations (GPC). The activity of test compounds at tachykinin NK_2 receptors in GPC was assessed against the selective NK_2 receptor agonist [βAla^8] NKA(4–10) in the presence of the NK_1 receptor-selective antagonist SR140333 (1 μM). Cumulative concentration–response curves to the agonists were obtained in all preparations, each concentration being added when the effect of the preceding one has reached a steady state. The antagonist affinity of all the test compounds (15 min pre-incubation period) was expressed as pK_B (negative logarithm of K_B, the antagonist dissociation constant) which was estimated as pK_B (negative logarithm of K_B, the antagonist dissociation constant) which was estimated as the mean of the individual values obtained with the equation $pK_B = \log\ [\text{dose ratio} - 1] - \log[\text{antagonist concentration}]$.
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