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Cinnamic acids and mono-substituted benzoic acids as useful capping groups for the preparation of hNK_2 receptor antagonists

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ABSTRACT

NK_2 antagonists have been reported to be potentially useful for the treatment of a number of chronic diseases, such as asthma, irritable bowel syndrome, cystitis, and depression. Starting from an in-house prepared library of capped dipeptides, we have identified a series of molecules with subnanomolar binding affinity for the hNK_2 receptor. These molecules are composed by three well-defined regions: a planar aromatic acyl system as N-terminal capping group, a rigid and quite lipophilic core, and a flexible and relatively hydrophilic C-terminal capping group. Here we report how we were able to manipulate the N-terminal capping group to obtain significant in vivo activity after iv and id administration.

Substance P, Neurokinin A, and Neurokinin B are neuropeptides belonging to the tachykinins family. They 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] The human NK_2 receptor (hNK_2) has been identified and validated as suitable target for development of novel drugs to be used in a number of 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]

As part of a project aimed at the identification of a series of small, orally available hNK_2 receptor antagonists, starting from one of our capped dipeptide libraries, we were able to identify a number of molecules with subnanomolar binding affinity for the hNK_2 receptor.[4]

All the structures can be seen as composed by three portions, each of them possessing appropriate structural features and physical requirements. The acyl system representing the capping group at the N-terminus (portion A) must be planar and lipophilic, whereas the rigid core containing the α,α-cyclopentane glycine fragment (portion B) must crucially possess strong rigidity and hydrophobic character. Finally, the hydrophilic moiety at the C-terminal part (portion C) appears to tolerate the presence of polar groups and can be quite flexible.

The first basic structures were further elaborated to produce compounds showing both low nanomolar potency on an in vitro functional test and significant antagonist activity on our animal model after iv administration at a dose of 3 μmol/kg.[5] The compound MEN 15702 reported in Figure 1 is a representative example. These compounds had generally sub-optimal aqueous solubility and a low permeability when tested in our Caco-2 cell system.

In the current contribution we report a series of modifications to portion A aimed at decreasing the size and slightly improving the polarity of the capping group.

Two kinds of capping groups were considered. Phenyls mono-substituted in meta and para position were firstly prepared (Fig. 2). Ortho substitution was avoided to maintain the planarity and

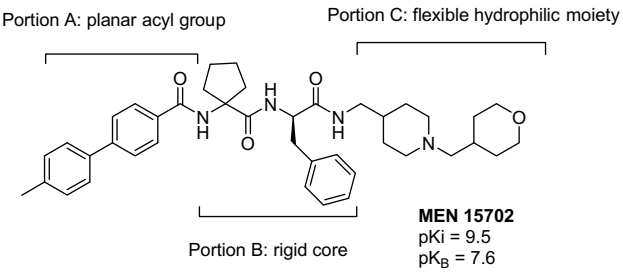

Figure 1. Representative compound derived from a library of capped dipeptides after a first round of optimization[5] and general three portions structure of this class of hNK_2 antagonists.

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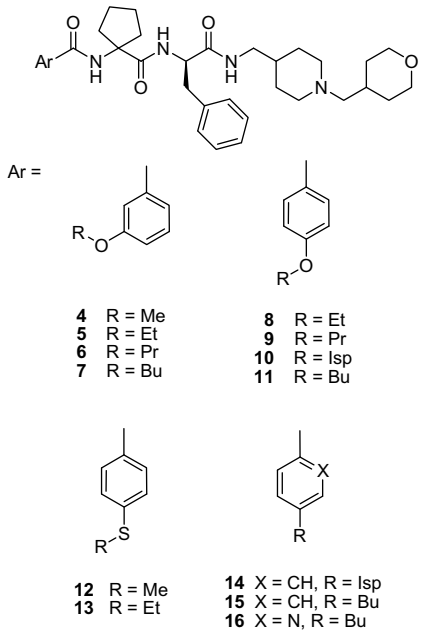


Figure 2. Para- and meta-substituted benzoic acids as capping group.

in its place an heterocyclic nitrogen was introduced in a single example (**16**).

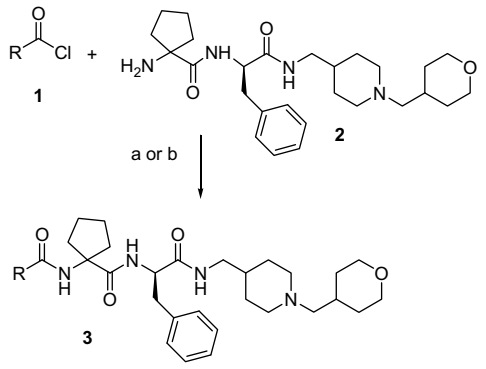
A second group of compounds was obtained capping the cyclopentane nitrogen with variously substituted cinnamic acids. We knew from previous experience that unsubstituted phenyls and cinnamic acids were generally much less potent than substituted ones.

The final products of general formula **3** were obtained through base catalyzed reaction of the preformed acyl chloride with amine **2** or by coupling of amine **2** with the opportune acid RCO_2H in standard conditions (Scheme 1).

All the compounds were tested for binding affinity on the hNK_2 receptor,[6] and, if possible, P_{app} on Caco-2 cells[7] was evaluated in view of a future oral delivery.

A clear trend toward increase in binding affinity with the increase of the alkyl chain length was observed for the *m*-alkoxy-substituted benzamides **4–7**. Almost a log unit improvement was gained going from the methyl to the butyl ether, very likely due to an increase of hydrophobic interactions.

A reverse, but less pronounced trend, was observed for the *p*-alkoxy-substituted benzamides **8–11**. In this case we suppose that



Scheme 1. Reagents: (a) oxalyl chloride, then amine **2** and DIPEA; (b) DCC, HOBt and DIPEA.

Table 1
Binding affinity for the hNK_2 receptor and Caco-2 permeability of variously substituted arylamides

Compound	pK_i[6]	P_{app}[7]	Compound	pK_i[6]	P_{app}[7]
4	7.6	nt	**11**	7.9	nt
5	7.7	<1	**12**	8.5	0.9
6	8.3	nt	**13**	9.1	0.6
7	8.4	4.4	**14**	8.0	nt
8	8.4	1.2	**15**	8.3	9.3
9	8.0	nt	**16**	7.6	17.6
10	8.1	0.8			

fine steric and hydrophobic interactions work in opposite directions (Table 1).

The best compound of the series (**13**), showing a subnanomolar affinity for the hNK_2 receptor, was obtained replacing the oxygen with the more polarizable and hydrophobic sulfur.

Disappointingly, the values of Caco-2 permeability were still not very high. An interesting effect on permeability was observed with the *p*-butyl benzamide (**15**) and its analogue 2-aza (**16**). In fact with the introduction of the aza group the Caco-2 permeability almost doubled, in spite of a net increase in PSA (96 vs 107 Å)[8] and a drop in calculated $\log D_{7.4}$ (4.17 vs 3.09).[9] The active transport accountability for this result was excluded by a countercheck with PAMPA permeability evaluation,[10] where **15** showed a Pe of 1.6×10^{-6} cm/s and **16** showed a Pe of 7.23×10^{-6} cm/s.

The high permeability of **16** is probably due to an intramolecular hydrogen bond that facilitates membrane permeation by lowering the desolvation energy of the amide while, at the same time, the polar groups facilitate the diffusion across the aqueous boundary layers that cover the membrane (Fig. 3).[11,12]

Unfortunately **16** also experienced a large drop in binding affinity.

A second group of compounds, the cinnamoyl derivatives **17–31** (Fig. 4), appeared to constitute a more interesting series showing higher pK_i values compared to the first series (Table 2). The pattern of ring substitution was systematically studied with *o*-, *m*-, and *p*-derivatives for F, Cl, MeO, and Me. No notable differences were observed in the binding affinity, with all the pK_i values between 8.4 and 9.5.

The compounds having a $pK_i \geqslant 9$ were tested for functional activity in guinea pig isolated proximal colon (pK_B)[13] and for in vivo activity after iv and id administration in our animal model. The potency in inhibiting colonic contractions induced by the selective tachykinin NK_2 receptor agonist [βAla^8]NKA(4–10) (3 nmol/kg iv) in guinea pig was evaluated after iv administration at a dose of 3 μmol/kg and id at a dose of 10 μmol/kg. The results, obtained after iv or id administration of the antagonist, are expressed both as maximal inhibitory effect reached ($\%i_{max}$) and as $\sum i\%$max (Table 3).[14]

The biological activities of the compounds after iv administration are in the case of **18**, **21**, and **28** equivalent to that of the reference compound MEN 15702,[5] while after id administration equivalence or a slight improvement has been obtained with **13**, **21**, **27**, and **28**.

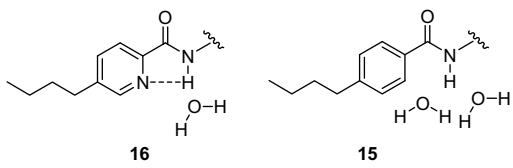


Figure 3. Intramolecular hydrogen bond in **16** facilitates membrane permeation.

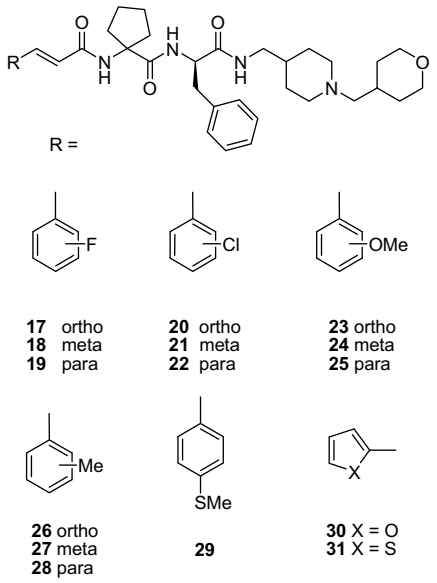


Figure 4. Substituted cinnamoyl acid as capping groups.

Table 2
Binding affinity for the hNK_2 receptor and Caco-2 permeability of variously substituted cinnamoyl amides

Compound	pK_i[6]	P_{app}[7]	Compound	pK_i[6]	P_{app}[7]
17	8.4	nt	**25**	8.7	nt
18	9.0	2.1	**26**	8.8	0.8
19	8.7	nt	**27**	9.0	2.1
20	9.0	1.0	**28**	9.3	<1
21	9.5	<1	**29**	9.0	4.7
22	8.9	2.8	**30**	<7	nt
23	8.9	nt	**31**	7.8	nt
24	9.1	1.4			

Table 3
Functional activity (pK_B), Caco-2 permeability (P_{app}), and in vivo activity after iv (3 μmol/kg) and id (10 μmol/kg) administration

Compound	pK_B[13]	P_{app}[7]	iv $\sum i$%max[14] (% i_{max} ± SEM)	id $\sum i$%max[14] (% i_{max} ± SEM)
MEN 15702	7.6	12	69 (79 ± 8)	42 (52 ± 4)
13	8.2	0.6	41 (77 ± 8)	44 (58 ± 6)
18	8.0	2.1	62 (83 ± 7)	15 (31 ± 21)
20	8.1	1.0	43 (73 ± 14)	0 (17 ± 9)
21	8.4	<1	61 (88 ± 3)	45 (54 ± 14)
24	7.3	1.4	49 (96 ± 3)	32 (50 ± 10)
27	7.7	2.1	35 (71 ± 15)	43 (54 ± 4)
28	7.9	<1	63 (99 ± 1)	52 (61 ± 4)
29	7.3	4.7	33 (85 ± 6)	20 (35 ± 15)

From data reported in Table 3 it is clear that permeability is not the main determinant for in vivo activity after id administration. Very likely the observed pharmacological activity is the result of a subtle balance of metabolic stability, protein binding, and tissue distribution.

In summary, through elaboration of the portion A of our series of molecules, after a first selection on the basis of binding affinity for the hNK_2 receptor and then for functional activity on guinea pig colon, we found a panel of molecules showing a consistent antagonist activity after id administration to our animal model. A further evolution through modification of portions B and C will be reported in due time.

Acknowledgments

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Supplementary data

Experimental methods and compounds analytical characterization are available at doi:10.1016/j.bmcl.2008.06.102. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.102.

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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) after 30 min incubation at room temperature. 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. In vitro intestinal absorption of synthesized compounds was evaluated using the Caco-2 cell line. Cells from passage 40–60 were seeded on Transwell inserts, allowed to differentiate and used 21–23 days after seeding. Permeability was evaluated measuring the passage of the compound from the apical toward the basolateral site as a function of time. The amount of compound transported was quantified by HPLC–MS. The apparent permeability coefficient, P_{app} value, is expressed in 10^{-6} cm/s and was obtained by the formula $P_{app} = (Q/\Delta t/(AC_0)$ where $\Delta Q/\Delta t$ is expressed in mmol/s, A is the surface area of the cell monolayers (in cm^2), and C_0 is the initial concentration of the compound in the donor side (in mmol/cm^3). All the experiments were carried out at pH 7.4, 100 μM concentration of the tested compound, and in the presence of markers of the cell monolayer integrity and of the transport systems. More details may be found in [Larger et al. *Anal. Chem.* **2002**, *74*, 5273–5281]. The mass balance of compounds, calculated as the ratio of total detected quantities of analytes at the end of the transport experiment to that originally introduced in the transwell, was around 1, indicating good recovery.
8. PSA was calculated with ACDLabs 8.0.
9. Log $D_{7.4}$ was calculated with ACDLabs 8.0.
10. Pe was evaluated according to Millipore Application Note (AN1725EN00) MultiScreen™ Permeability Plates, using carbamazepine, propanolol and warfarin as standards.
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13. $pK_B = -\log K_B$. Antagonist potency values for guinea pig NK_2 receptor estimated toward [βAla^8]NKA(4–10) induced contractions of the guinea pig isolated colon (GPC) in the presence of the NK_1 receptor selective antagonist SR140333.
14. The $\sum i$%max value is expressed as the sum of the % inhibition in comparison to the sum of the control (basal) colon contractions induced by the selective agonist at the nine times of observation (5, 30, 60, 90, 120, 150, 180, 210, and 240 min) after iv (or id) administration of the antagonist ($\sum i$), and further calculated as the sum of the theoretical maximal % inhibition ($\sum i$max-th)), which is a constant and equal to 900. This parameter gives a measure of the activity during the entire experimental period therefore allowing the evaluation of both the intensity and the duration of the antagonist effect. The maximal inhibition corresponds to $\sum i$%max = 100, while the absence of effect results in $\sum i$%max = 0.

$$\sum i\%\text{max} = \frac{\sum(\%i)}{\sum(\%i\text{max-th})} \times 100 = \text{mean \% inhibition over the entire experiment.}$$