Journal of Medicinal Chemistry

Volume 43, Number 22 November 2, 2000

Communications to the Editor

Discovery of Potent Cyclic Pseudopeptide Human Tachykinin NK-2 Receptor Antagonists

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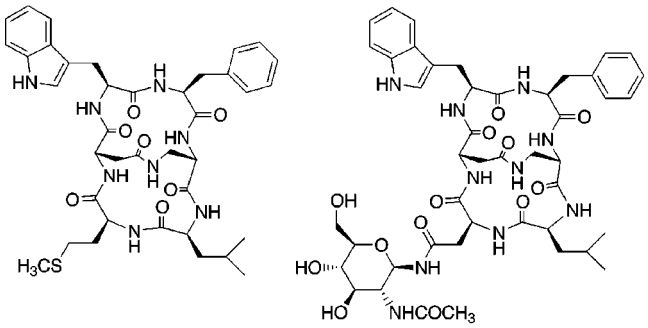
Received July 3, 2000

Introduction. The tachykinins substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) are a family of neuropeptides that are widely distributed in the mammalian peripheral and central nervous systems and produce their biological actions by activating three distinct receptor types, termed NK-1, NK-2, and NK-3. NKA exerts its biological effects mainly by activation of the tachykinin NK-2 receptor. The human NK-2 receptor has been identified and validated as a suitable target for development of novel drugs to be used for treatment of a number of diseases in the respiratory, gastrointestinal, and genitourinary tracts.[1]

Since the rational design of the bicyclic hexapeptide MEN 10627 or cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo-(2β−5β)[2,3] (Chart 1), a potent and selective tachykinin NK-2 receptor antagonist,[4,5] the glycosylated analogue MEN 11420 (Nepadutant) or cyclo{[Asn(β-D-GlcNAc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β−5β)}[6] (Chart 1) was selected for further development and is currently in phase II clinical trials. Structural studies on MEN 10627 have shown the presence of a type I and type II β-turn, with Trp-Phe and Leu-Met as corner residues, respectively.[2,7,8] In addition site-directed mutagenesis studies on labeled MEN 11420 suggested a primary role of the Trp-Phe moiety in the binding interactions with the tachykinin NK-2 receptor.[9,10] On this background a chemical program was undertaken to search for lower molecular weight compounds which maintained the same potency and selectivity of our reference compounds.

Chart 1



Initially we prepared the monocyclic compound **5a** (Scheme 1) that possesses only micromolar binding affinity; however, we considered this value sufficient to use **5a** as a lead compound for a new family of NK-2 antagonists. In fact Figure 1 shows the comparison of the type I β-turns for compounds **5a** and MEN 10627.[11] The smaller monocyclic compound maintains the structure of the larger bicyclic peptide, with exact overlapping of the Trp-Phe motif. This comparison suggests that the monocyclic compound **5a** should be the small-molecule model of the Trp-Phe β-turn for which we were looking.

It has been observed for other pseudopeptide NK-2 antagonists that an increase in lipophilicity was related to the increment of NK-2 receptor antagonist activity.[12] For this reason we introduced a benzyl group at positions 8, 9, 12, and 13 (Table 1), as a probe in order to improve the binding affinity of our lead compound **5a**. We now report on the discovery of this novel class of potent monocyclic pseudopeptides as human NK-2 receptor antagonists and part of our work aimed at optimizing the binding affinity.

Chemistry. The chemical synthesis of **5a** and of compounds containing the benzyl group at positions 9,

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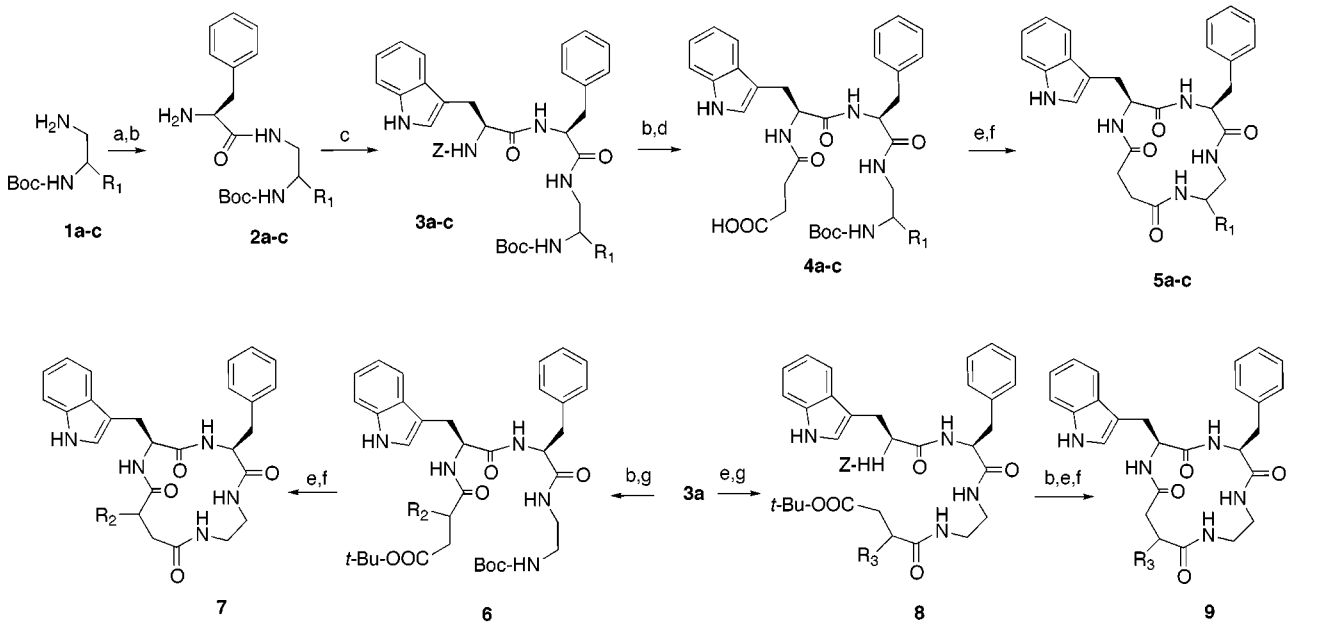
10.1021/jm0010217 CCC: $19.00
Published on Web 10/13/2000

Scheme 1. Synthesis of Compounds **5a**−**c**, **7**, and **9**[a]



[a] Reagents: (a) Z-Phe-OH, EDC, HOBT, DMF (80%); (b) H_2, Pd/C, MeOH (90−97%); (c) Z-Trp-OH, EDC, HOBT, DMF (95%); (d) succinic anhydride, DMF (95%); (e) TFA, CH_2Cl_2 (75−85%); (f) EDC, HOBT, DMF (75−85%); (g) 2-benzylbutanedioic acid 4-*tert*-butyl ester, EDC, HOBT, DMF (70−80%).

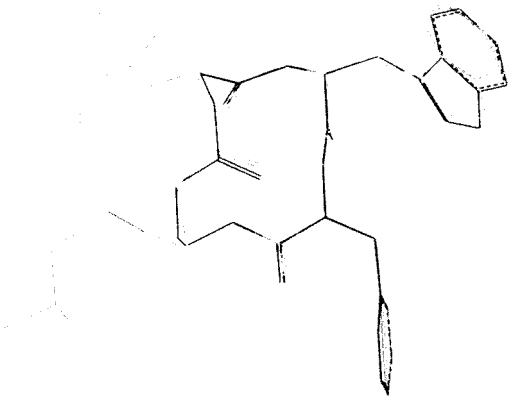


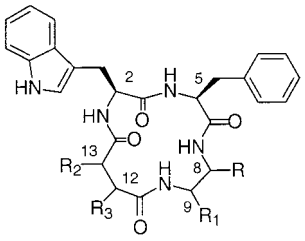
Figure 1. Superposition of MEN 10627 (light) and **5a** (dark).

12, and 13 was performed as described in Scheme 1. The synthesis of compounds containing the substituent at position 8 is described in Scheme 2.

The diamines **1** were prepared starting from the corresponding Boc-amino acids using known procedures[13−15] and were reacted with commercially available protected amino acids under standard peptide coupling conditions.

As described in Scheme 1 the diamines **1a**−**c** were condensed with Z-phenylalanine, and after removal of the Z-protecting group, the resulting intermediates **2** were condensed with Z-tryptophan to give products **3**. For the 9-substituted benzyl derivatives, compounds **3a**−**c** were converted to tetrapeptides **4a**−**c** by Z-deprotection and reaction with succinic anhydride. After Boc-deprotection of the diamine moiety of the molecule, the corresponding zwitterions were finally cyclized to give the products **5a**−**c**. For the 13-substituted benzyl derivatives, the compound **3a** was reacted after Z-deprotection with 2-benzylbutanedioic acid 4-*tert*-butyl ester[16] to give **6** where subsequent Boc-deprotection and cyclization furnished **7** as a diastereoisomeric mixture. For the 12-substituted benzyl derivatives, the intermediate resulting from Boc-deprotection of **3a** was reacted with 2-benzylbutanedioic acid 4-*tert*-butyl ester to give **8**. This was submitted to Z- and *tert*-butyl ester deprotection and cyclized to give the cyclopeptide **9**, as a diastereoisomeric mixture.

Table 1. Chemical Structure and Affinities for the Tachykinin NK-2 Receptor Evaluated in Binding Experiments (pK_i values) and Functional in Vitro Experiments (pK_B values)



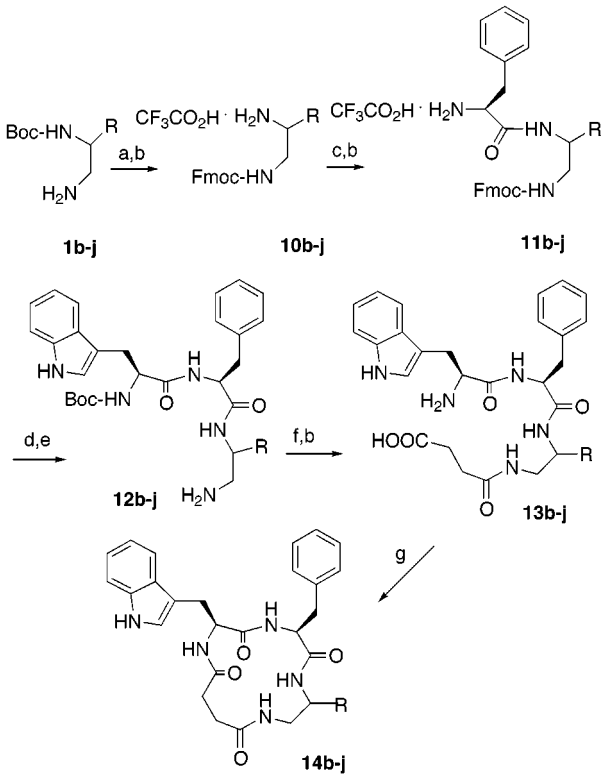
compd	R	R_1	R_2	R_3	config	pK_i[a]	pK_B[a]
5a	H	H	H	H		5.9 ± 0.1	6.3 ± 0.15
5b	H	Bzl	H	H	(*R*)	5.9 ± 0.1	5.4 ± 0.19
5c	H	Bzl	H	H	(*S*)	5.9 ± 0.1	5.5 ± 0.18
7	H	H	Bzl	H	(*R*,*S*)	6.2 ± 0.1	5.7 ± 0.2
9	H	H	H	Bzl	(*R*,*S*)	6.1 ± 0.1	6.1 ± 0.09
14b	Bzl	H	H	H	(*S*)	5.9 ± 0.1	5.8 ± 0.05
14c	Bzl	H	H	H	(*R*)	8.7 ± 0.3	7.6 ± 0.09

[a] pK_i and pK_B are −log of K_i and K_B, respectively; see refs 17 and 18.

The cyclization step on these compounds was performed in DMF at 0.01 M concentration in order to minimize intermolecular condensation reactions.

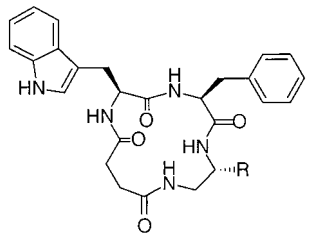
Results and Discussion. The binding[17] data and the

Scheme 2. Synthesis of Compounds **14b**−**j**[a]



[a] Reagents: (a) Fmoc-OSu, THF (70−75%); (b) TFA, CH_2Cl_2 (85−95%); (c) Boc-Phe-OH, EDC, HOBT, DIPEA, DMF (82−94%); (d) Boc-Trp-OH, EDC, HOBT, DIPEA, DMF (87−97%); (e) Et_2NH, DMF (66−75%); (f) succinic anhydride, DMF (90−95%); (g) EDC, HOBT, DMF (75−85%).

Table 2. Affinities of the Test Compounds for the Tachykinin NK-2 Receptor Evaluated in Binding Experiments (pK_i values) and Functional in Vitro Experiments (pK_B values)



compd	R	pK_i [a]	pK_B [a]
14c	-CH_2(Ph)	8.7 ± 0.3	7.6 ± 0.09
14d	-CH_2(cyclohexyl)	6.5 ± 0.1	6.5 ± 0.01
14e	-CH_2CH_2(Ph)	8.0 ± 0.07	6.5 ± 0.09
14f	-CH_2(2-naphthyl)	8.1 ± 0.25	7.0 ± 0.01
14g	-CH_2[(4-OMe)Ph]	8.0 ± 0.15	7.1 ± 0.01
14h	-CH_2[(4-CF_3)Ph]	7.7 ± 0.1	7.1 ± 0.01
14i	-CH_2[(3,4-diF)Ph]	8.8 ± 0.1	7.8 ± 0.02
14j	-CH_2[(3,4-diCl)Ph]	10.0 ± 0.17	8.1 ± 0.03
MEN 10627		9.2 ± 0.09	8.1 ± 0.1
MEN 11420		8.6 ± 0.08	8.7 ± 0.07

[a] See corresponding footnote in Table 1.

in vitro functional test[18] of the novel NK-2 receptor antagonists are presented in Tables 1 and 2.[19]

Table 1 shows that compound **14c** is the best NK-2 antagonist of this series, while no significant difference exists between our lead compound **5a** and the other benzyl derivatives. It is noteworthy that introduction of the benzyl group at the C-8 position with (*R*)-configuration increased binding affinity about 1000-fold with respect to **5a** and led to the discovery of the cyclic pseudopeptide **14c** that possesses the same nanomolar potency ($pK_i = 8.7 \pm 0.3$) as MEN 11420 ($pK_i = 8.6 \pm 0.08$) and is slightly less potent than MEN 10627 (Table 2).

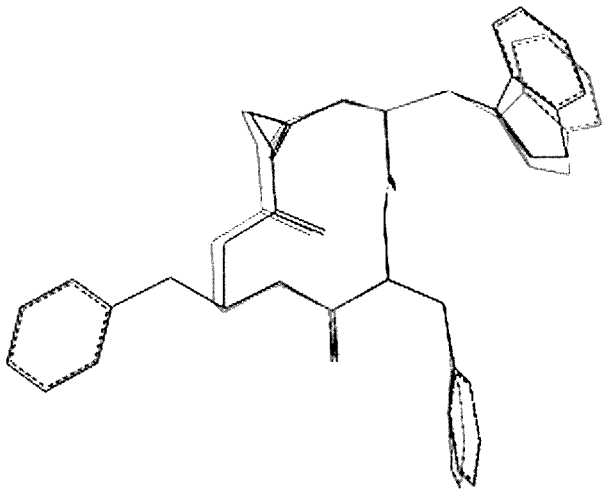


Figure 2. Superposition of **5a** (light) and **14c** (dark).

The NK-2 binding affinity and functional activity are enantioselective properties of this chemical series, the C-8 (*R*)-stereoisomer **14c** being about 3 orders of magnitude more potent than the C-8 (*S*)-compound **14b** in binding and 2 orders of magnitude more potent in functional activity. The selective antagonist activity of **14c** for the tachykinin receptors was tested in guinea-pig isolated ileum bioassay,[20] and the resulting values: NK-1 $pK_B = 5.7 \pm 0.1$, NK-2 $pK_B = 7.8 \pm 0.06$, NK-3 $pK_B = 6.1 \pm 0.08$, convinced us to consider compound **14c** as the structural lead (designated MEN 11558) for a new class of compounds with potent NK-2 receptor antagonist activities.

Figure 2 shows the comparison between **14c** and **5a**. The good overlap indicates that the introduction of a benzyl group in position 8 with the (*R*)-configuration does not change the β-turn conformation of the monocyclic structure. This observation suggests that the increase in activity observed in **14c** is probably due to favorable interaction of the benzyl group with the NK-2 receptor rather than to a conformational constraint effect.

To understand the role of the lipophilic substituent in position 8 and to optimize the binding affinity, we prepared other C-8 derivatives with the (*R*)-configuration (Table 2). Initial replacement of the benzyl moiety by the cyclohexylmethyl group dramatically decreased the pK_i and pK_B values of the resulting compound **14d**. For this reason we used aromatic residues such as phenylethyl by which **14e** was obtained. However it also showed a reduction in affinity (pK_i) and a poor pK_B value. A slighty better result was observed for compound **14f** where the benzyl moiety was substituted with a 2-naphthylmethyl residue.

Assuming that the aromatic ring and the interatomic distance between its centroid and the C-8 carbon of the core structure can play a key role on the activity of our compounds, we used substituted benzyl derivatives to obtain compounds **14g**−**j**. The benzyl group substituted at the *para* position with electron-withdrawing groups, e.g. trifluoromethyl (**14h**), or the electron-donating methoxy group (**14g**) did not show any improvement in

Table 3. K_d Values for the Two Antagonists MEN 11420 and **14c** Against [^{125}I]NKA (NKA) and [^{3}H]SR 48968 (SR) on the Wild-Type and Four Point-Mutated Human NK-2 Receptors (see text)

	wild-type		Tyr206Ala		Tyr206Phe		Tyr266Phe		Phe270Ala	
	NKA	SR	NKA	SR	NKA	SR	NKA	SR	NKA	SR
MEN 11420	2.1	4.4		>1000	3.1	1.9	26	48		291
14c	2.7	10		1960	3.6	1.3	34	48		208

activity if compared to **14c**. The substitution of positions 3 and 4 of the benzyl group by halogen atoms was more satisfactory: in fact the 3,4-difluoro derivative **14i** retains the activity of **14c** and the 3,4-dichloro derivative **14j** showed an improvement in the activity values: $pK_i = 10 \pm 0.17$ and $pK_B = 8.1 \pm 0.03$.

On the basis of these results, we hypothesized that while the benzyl moieties in compounds **14c,i,j** produce a new interaction with a previously unexplored hydrophobic pocket of the NK-2 receptor, the Trp-Phe moiety of our new class of cyclic compounds interacts with the NK-2 receptor similarly as in MEN 11420.[10] To confirm this, we tested the binding affinity of **14c** toward a series of point-mutated human NK-2 receptors transfected in CHO cells that were previously identified as relevant for the interaction with Trp-Phe in MEN 11420.[10] The results, reported in Table 3, agree with this hypothesis adding experimental support to our initial assumption. These compounds possess pK_i and pK_B values comparable to those of MEN 10627 and MEN 11420 with the advantage of lower molecular weight and fewer stereogenic centers, two factors that confer to this type of molecule an interesting opportunity for further development.

Ongoing studies are evaluating the potential therapeutic utility of compounds in this series, and new derivatives related to MEN 11558 will be reported in due time.

Acknowledgment. We thank Dr. Antonio Triolo and Dr. Giuseppe Balacco (Menarini Ricerche S.p.A.) for mass spectra and NMR spectroscopic determinations. This work was supported in part by MURST (IMI Grant No. 63217).

Supporting Information Available: MS and ^{1}H NMR spectra for compounds **14c**,**i**,**j**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Binding affinity for the human NK-2 receptor transfected in CHO cells was determined in competition experiments using [^{125}I]NKA as radioligand (see ref 6). The pK_i values, calculated using EBDA and LIGAND programs (Munson, P. J.; Rodband. *Anal. Biochem.* **1980**, *107*, 220−239) in sequence, represent the mean value determined from 2−6 experiments, each performed in duplicate.

(18) Functional studies were performed in the rabbit isolated pulmonary artery, set up as described previously: Patacchini, R.; et al. *Br. J. Pharmacol.* **1991**, *104*, 91. The pK_B values represent the average of 3−8 independent determinations.

(19) The purity of final compounds was found to be ≥95% by HPLC.

(20) The selectivity of compound **14c** was assayed on the guinea-pig isolated ileum, set up for recording NK-1 or NK-3 receptor-mediated responses (Patacchini, R.; Maggi, C. A. Tachykinin Receptor Assays. In *Current Protocols in Pharmacology*; Enna, S., Ferkany, M., Williams, J., Kenakin, T., Porsolt, R., Sullivan, J., Eds.; J. Wiley and Sons: New York, 1998; Chapter 4, units 4.10, 4.10.1−4.10.26). The data obtained in the former bioassay were compared to the affinity of compound **14c** shown at the NK-2 receptor of the guinea-pig isolated bronchus set up as described previously: Maggi, C. A.; et al. *Eur. J. Pharmacol.* **1991**, *197*, 167−174. The pK_B values represent the average of 3−8 independent determinations.

JM0010217