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Successful Bridging from a Peptide to a Non Peptide Antagonist at the Human Tachykinin NK-2 Receptor

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Abstract—Non peptide products have been found to show nanomolar binding and functional affinities at the human tachykinin NK-2 receptor. The new antagonists do not possess stereogenic centers and their thermal behaviour in solution is featured by a peculiar set of conformational stereoisomers. A macroscopic viewpoint is preferentially adopted to rationalize the obtained results.

The tachykinins form a family of peptide neurotrasmitters which share a common carboxy terminal sequence, Phe-Xxx-Gly-Leu-Met-NH2, and Neurokinin A (NKA, Xxx = Val) is the tachykinin preferentially acting at the NK-2 receptor whose activation in mammals is thought to play a role in the regulation of airways, gut and urinary tract motility.[1,2]

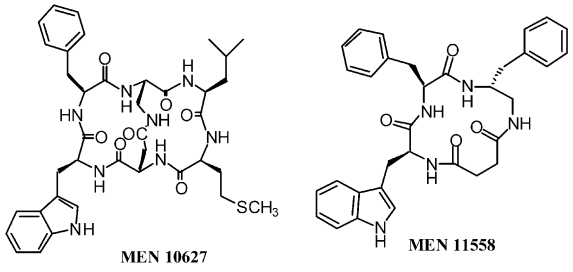

Figure 1. Molecular structure of two potent and selective antagonists at the hNK-2 receptor.

Amongst the known most potent and selective antagonists[3] of NKA at the human NK-2 (hNK-2) receptor, MEN10627, and MEN11558 have been disclosed by our research team (Fig. 1). Particularly, MEN10627 was rationally derived from the structure of the natural agonist,[3a] while MEN11558[3d] stemmed from the insight that only one of the two cycles present in the bicyclic antagonist could be essential for the bioactivity and is characterized by the pseudosymmetric juxtaposition of a second benzyl group in the place of the Leu-Met containing fragment.

Results and Discussion

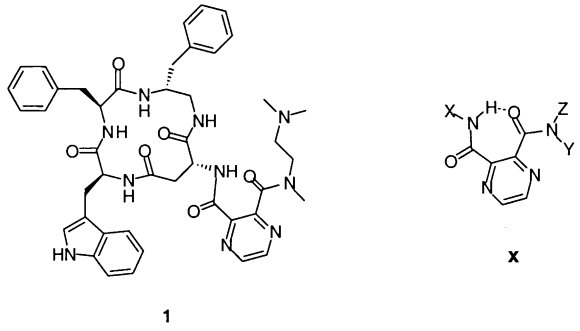

Figure 2. Early rationale to bridge from a peptide to a non peptide antagonist at the hNK-2 receptor.

During our study on MEN 11558 analogues,[4] it was noted that **1** (Fig. 2) retained the potency of the parent compound (Table 1) and it was proposed to use the exocyclic diamide framework to access to a fully non peptide antagonist; on the other hand, a product of general structure **x** could furnish an entry to the use of the benzodiazepine scaffold, the most famous amongst the so-called privileged platforms,[5] because of the possibility of an intramolecular hydrogen bond (Fig. 2).

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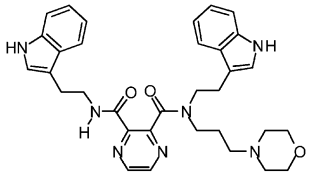
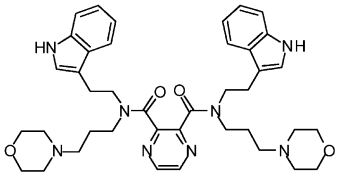
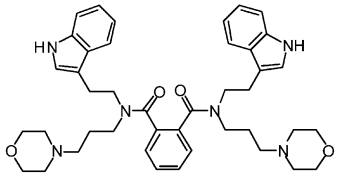
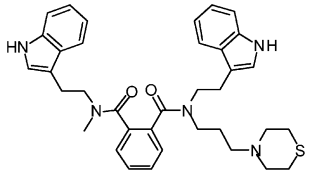
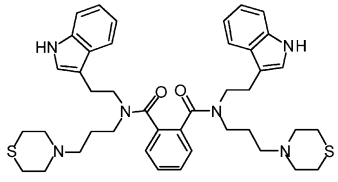
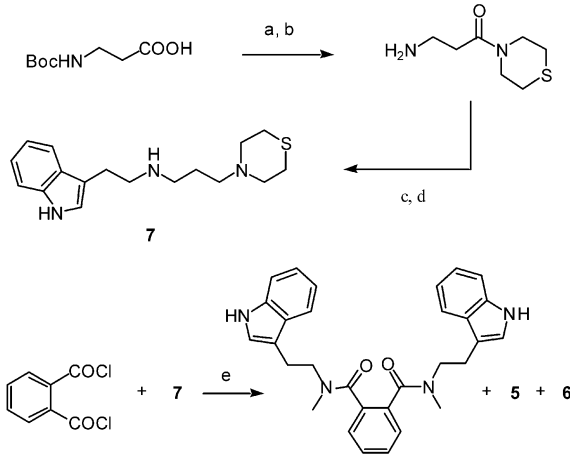
Early biological results, however, showed that the arrangement with two tertiary amides was preferred over that concerning one secondary and one tertiary amide, and eventually the double tertiary amide strategy resulted successful (Table 1), allowing us to obtain the antagonists **5** and **6**, endowed with nanomolar potency at the hNK-2 receptor and featured by a molecular framework inconsistent with those of the other non peptide NK-2 antagonists.[3f–i,6]

The secondary amine **7**, necessary to synthesize **5** and **6**, was obtained as reported in Scheme 1. The final coupling was accomplished by making *o*-phthaloyl dichloride to react with a mixture of *N*-methyl tryptamine and **7** in the presence of triethylamine, this technique being possible because the polarity difference of the three resulting amides permitted a facile chromatographic separation.

Table 1. hNK-2 receptor binding affinity (pK_i) and in vitro functional activity (pA_2) for the reference peptides and the new ligands

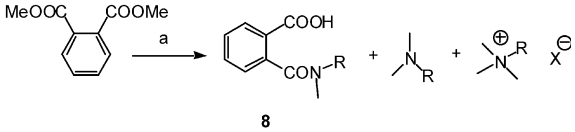
Compd[7]	Structure	pK_i	pA_2[9]
MEN 11558	See Figure 1	8.7	na
1	See Figure 2	8.7	nt
2		6.3	nt
3		6.9	nt
4		7.5	nt
5		8.0	8.0
6		8.4	8.4



Scheme 1. (a) Thiomorpholine, PyBrop, TEA, DCM; (b) HCl, ethyl acetate, Et_2O 29%, as the hydrochloride, two steps; (c) 3-indolylacetic acid, PyBop, TEA, DCM; (d) LAH, THF reflux. 83%, two steps; (e) *N*-methyltryptamine, TEA, DCM, FCC, followed by preparative HPLC for **6**.

In order to make the synthetic route more expeditious, we had also thought to perform a solvent-free double amidation[10] of dimethyl phthalate. Unfortunately, this idea was frustrated by the unexpected results obtained in the simplified experiment (Scheme 2) involving only one secondary amine. Briefly, the disappearance of dimethyl phthalate corresponded to the formation of three new products, identified as phthalamic acid **8**, *N*,*N*′-dimethyltryptamine, and [2-(1*H*-Indol-3-yl)-ethyl]-trimethyl-ammonium salt.[11]

The structural features of the non peptides ligands reported in Table 1 deserve consideration. If at a first glance, compounds **2**–**6** can be imagined as highly flexible molecules, that is not completely true. *o*-Phthaloyl tertiary diamides are known for the discrete pattern of their conformational isomers,[12] a characteristic which has been claimed to be potentially useful for the construction of molecular switches.[13] This peculiarity arises from the 2-fold combination of *cis*/*trans* amide isomerization with the rotation around the C_{Ar}-$CONR_1R_2$ bond. Indeed, the 500 MHz PMR spectra of compounds **3**–**6** are coherent[14] with those expected on the base of literature findings.[12,13] In addition, we report that four isomers are observed in the PMR spectrum of **5**, and that 4 isomers resulted physically isolable to some extent in the case of compound **9** (Fig. 3),[7] an analogue with sub-micromolar binding affinity.[8] The chromatogram of **9**, obtained under our standard, non chiroptical HPLC conditions,[15] consists of four peaks in relative amounts similar to those measured in the 500 MHz



Scheme 2. (a) *N*-methyltryptamine, two molar equivalents, 90–100 °C without solvent. R = (1*H*-Indol-3-yl)-ethyl residue. Seemingly, X = CF_3COO under the conditions of HPLC analysis and chlorine after extraction of the crude with aqueous hydrochloric acid.

PMR by integration of the four *N*-methyl singlets, and a clear separation afforded also by TLC on silica (10% methanol in ethyl acetate as eluent); an observation that allowed us to accomplish a partial separation by FCC and to observe the re-equilibration of the solutes in the collected fractions.[16]

The isomeric nature of the four chromatographic peaks was then proven by LC–MS analysis which showed the same protonated quasi-molecular ions, featured by isotopic patterns in agreement with the presence of four chlorine atoms and superimposable product ion tandem mass spectra.

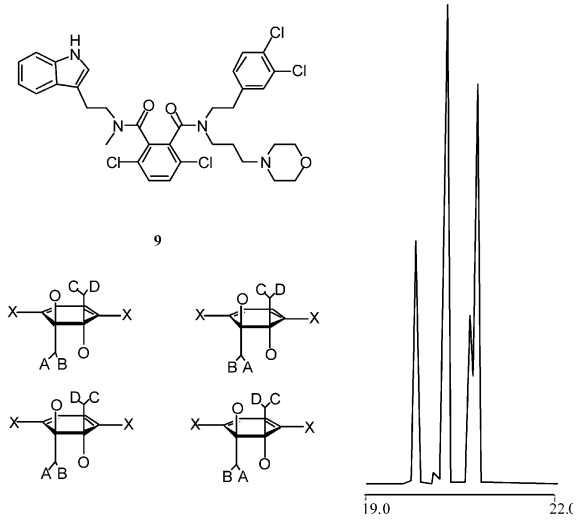


Figure 3. Structure of compound **9**, reproduction of its HPLC chromatogram at 280 nm and schematic representation of the 4 putative conformational isomers observed in the chromatogram. The four *syn* arrangements have not been taken into consideration because they have been found much less stable in the congeners described in literature.[12,13]

Apparently, the structural features of the known *o*-phthaloyl tertiary diamides, which also compounds **3**–**6** and **9** show, are an obstacle both to infer guesses on the binding mode and to put into practice an investigation strategy based, as often occurs, on the imaginary active conformer. Thus, since the number of conjecturable active conformers[17] is at least 16 for compound **5**, and at least 10 for compound **6**,[14] it is likely that here, the desire to uncover the conformation preferred by hNK-2 receptor, by the investigation of restricted analogues, could result a synthetic sisyphean toil. On the other hand, if speculations on ligand–receptor interactions, as well as those on the receptor-bound conformation are neglected at all, as we did when we decided to overlook the cyclopeptide moiety in **1**, to concentrate on the redundant pyrazine (redundant, in the sense of binding affinity), it is easy to observe that the pseudosymmetry introduced into MEN11558 by the added benzyl group, has been conserved in the structures of **5** and **6**.[18]

Conclusions

In this report we have disclosed two novel antagonists of NKA at the human NK-2 receptor. Work is in progress aiming at their complete pharmacological evaluation, with particular attention to their selectivity profile. The results of this investigation will be reported in due time.

Acknowledgements

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6. Curiously, *N*,*N*,*N*′,*N*′-tetraethylphthalamide, the compound which can be considered the structural progenitor of products **3**–**6**, is a known drug. See: The *Merck Index*, 13th ed., ref 9278; p 1642. Merck & Co.: Whitehouse Station, 2001. Anyway, it does not bind to the hNK-2 receptor up to micromolar concentrations.[7,8]

7. All final compounds, **1** included, were characterized by ^{1}H NMR, mass spectrometry and had a purity by analytical HPLC of $\geq$95%.
8. Binding affinity for the hNK-2 receptor transfected in CHO cells were determined in competition experiments using [^{125}I]NKA as the radioligand. The pK_i values were calculated according to the procedure described by: Munson, P. J. *Anal. Biochem*. **1980**, *107*, 220.
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14. Compounds **3**, **4** and **6**. 10 different conformational isomers computed in a chiral environment by combining the two kinds of thermal motion. That is two achiral *syn* isomers, 2×2 dissymmetric (C_2) isomers, 2×2 asymmetric isomers (C_1), one of which *syn* and one *anti*. Expected isomers[12,13] on the room temperature NMR timescale: 2×2 dissymmetric isomers and 2×1 asymmetric *anti* isomer. Observed isomers in the PMR spectra, without the addition of external chiral substances: 3: Noteworthy, in one of compound **3** isomers, the two pyrazinic protons resonate as an AB system ($\delta = 8.76$ and 8.79 ppm, $J = 2.6$ Hz).

Compound **5**: 16 different conformational isomers computed in a chiral environment by combining the two kinds of thermal motion. That is: 2×4 *anti* asymmetric isomers and 2×4 *syn* asymmetric isomers. Expected isomers[12,13] on the room temperature NMR timescale: 2×4 *anti* isomers. Observed isomers in the PMR spectrum, without the addition of external chiral substances: 4. The spectroscopic behaviour in the presence of a chiral shift reagent will be reported elsewhere. For an investigation on the stereomutation processes in compounds of very similar geometry, see: (a) Dell' Erba, C.; Gasparrini, F.; Grilli, S.; Lunazzi, L.; Mazzanti, A.; Novi, M.; Pierini, M. Tavani, C.; Villani, C. *J. Org. Chem*. **2002**, *67*, 1663.
15. Luna C8(2) 5 μm, 25 cm length, 4.6 mm internal diameter column. Mobile phase: A water, B acetonitrile, both containing 0.1% trifluoroacetic acid. Gradient conditions: from 20 to 80% B in 20 min, at a flow rate of 1 mL/min.
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