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Design, Synthesis and Biological Activity of Carbohydrate-Containing Peptidomimetics as New Ligands for the Human Tachykinin NK-2 Receptor

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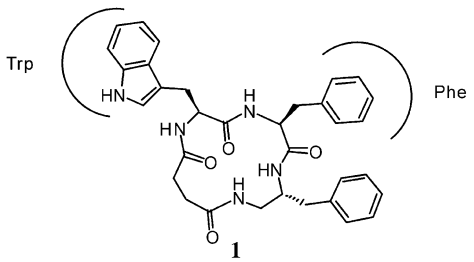
Abstract—Enantiopure cycloadducts between glycals and alkyl or aryl heterodienes were selected as small, rigid, nonpeptide molecules able to superimpose to the structure of the cyclopeptide tachykinin NK-2 antagonist **1**. The presence of three aromatic groups in the pyranose ring resulted essential for NK-2 affinity, while an increase in activity was shown by the corresponding sulfoxides.

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 system 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 tract.[1] Two different potent and selective NK-2 antagonists, the nonpeptide compound saredutant[2] and the glycosylated bicyclic hexapeptide nepadutant or cyclo{[Asn(β-D-GlcNAc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)},[3] are currently in phase II clinical trials.

Structural studies on MEN 10627, the parent hexapeptide from which nepadutant was derived, showed the presence of a type I and a type II β-turn, with Trp-Phe and Leu-Met as corner residues, respectively.[4,5] In addition, site-directed mutagenesis studies on labelled nepadutant suggested a primary role of Trp-Phe moiety in the binding interactions with the tachykinin NK-2 receptor.[6,7]

On this background, a chemical programme was undertaken to search for lower molecular weight compounds which maintained the same potency and selectivity of MEN 10627 and nepadutant. Our first approach was directed to the simplification of the bicyclic structure of these peptide antagonists to obtain monocyclic derivatives: in fact, the novel pseudopeptide **1** not only maintained nanomolar affinity at the human NK-2 receptor but its structure was superimposable to that of nepadutant, with the indole and phenyl moieties of tryptophan and phenylalanine, respectively, occupying a corresponding region in the space.[8]



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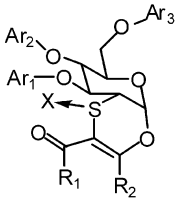
In order to overcome some of the drawbacks of the peptide compounds (e.g., their low absorption after oral administration), we turned our attention to small, rigid, nonpeptide scaffolds able to address in the correct region of the space the structural aromatic moieties stated essential for the affinity at the NK-2 receptor. The validity of this kind of approach has been demonstrated in several cases by the Hirschmann's group,[9,10] through the use of a glucose scaffold as a peptidomimetic to obtain nonpeptide somatostatin analogues and NK-1 antagonists.

Monosaccharides provide enantiomerically pure, rigid molecular systems that show high combinatorial density and exhibit numerous, alternatively addressable and eligible stereogenic centers. In pyranose rings, indeed, the five sites of attachment present two possible defined spatial orientations each.[11,12] The model compounds we chose are enantiopure cycloadducts between protected or partially protected glycals and alkyl or aryl heterodienes that allow to rely on all the advantages mentioned above. Moreover the dienic part of the cycloadducts offers two more sites for introducing chemical diversity (vide infra).

In the preparation of a model series of compounds we decided to use the phenyl group as the simplest aromatic moiety, to avoid synthetic difficulties due to the need to selectively protect the NH on the indole group in **1**. The target compounds designed were gluco derivatives bearing from one to five aromatic groups in different positions of the carbohydrate scaffold (see Table 1) and presenting ester or ketone moieties in the 1′ and 3′ positions (see Table 1). In addition, selective oxidation at the sulfur atom was performed on several derivatives to obtain the corresponding sulfoxides and sulfones.

Table 1. Structures and binding affinities (K_i, μM) of compounds **15**–**28** at the human NK-2 receptor transfected in CHO cells[a]



Compd	Ar_1	Ar_2	Ar_3	R_1	R_2	X	K_i (μM)
15	Bn	Bn	Bn	OPh	Me	—	>10
16	Bn	Bn	Bn	OPh	Me	O	0.40
17	Bn	Bn	Bn	OEt	Me	—	12.60
18	Bn	Bn	Bn	OEt	Me	O	0.25
19	H	Bn	Bn	OEt	Me	—	12.60
20	H	Bn	Bn	OEt	Me	O	0.79
21	Bn	H	Bn	OEt	Me	O	>10
22	Bn	Bn	H	OEt	Me	—	4
23	Bn	Bn	H	OEt	Me	O	4
24	H	Bn	H	OEt	Me	—	>10
25	H	Bn	H	OEt	Me	O	>10
26	Bn	Bn	Bn	Ph	Ph	—	>10
27	Bn	Bn	Bn	Ph	Ph	O	0.79
28	Bn	Bn	Bn	Ph	Ph	O_2	5.01

[a]K_i values were determined in competition experiments by using [^{125}I]NKA as radioligand. The K_i values, calculated using EBDA and LIGAND programs[22] in sequence, represent the mean value determined in two to six experiments, each performed in duplicate.

A preliminary comparison between the reference compound **1** and a representative of this new series (then synthesized as **18**) is shown in Figure 1. The three aromatic residues of **18**, as resulting from 100 ns of molecular dynamics (MMFF in Tripos Sybyl 6.6, at 300 K) and geometry optimization (MOPAC, AM1 hamiltonian), appear to be well superimposable to those of **1**.

The synthesis of compounds **15**, **17**, **19**, **22**, **24**, **26** was performed following the chemo-, regio- and stereoselective inverse electron-demand [4+2] cycloaddition between glycals and α,α′-dioxothiones, as previously reported by some of us.[13,14] The tri-*O*-benzylglucal **2**, the di-*O*-benzylglucal **3**–**5** and the mono-benzylglucal **6** were prepared using known procedures[15] and were reacted with the 'in situ' generated, highly reactive electronpoor dienic α,α′-dioxothiones **7**–**9** as reported in Scheme 1.[17]

Sulfoxides **16**, **18**, **20**, **21**, **23**, **25**, **27**, and sulfone **28** were synthesized treating the parent cycloadducts in dichloromethane with 1.0 and 2.5 equivalents of *meta*-chloroperbenzoic acid, respectively.[18] As expected,[19] sulfoxides were obtained as diasteromerically pure compounds. No trace of the other epimer at sulfoxide sulfur was ever detected (^{1}H NMR analysis of the crude).

Binding affinities (K_i, μM) of compounds **15**–**28** at the human NK-2 receptor transfected in CHO cells were determined in competition experiments by using [^{125}I]NKA as radioligand[3] and are reported in Table 1.

The presence of three aromatic groups in the pyranose ring Ar_1–Ar_3=Bn resulted essential to obtain at least micromolar affinity at the NK-2 receptor, as the removal of any of these groups uniformly decreased activity. Surprisingly, compound **20** (Ar_1=H, Ar_2=Ar_3=Bn) showed an interesting affinity which can likely be rationalized considering the presence of the S→O bond on the same side of the molecule; this might represent another binding site and positively effect the affinity at the receptor, making the lack of a third Bn

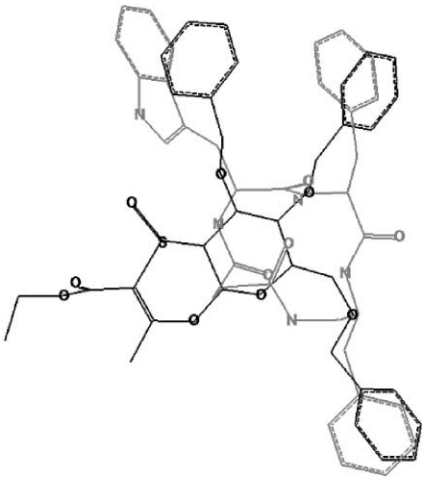
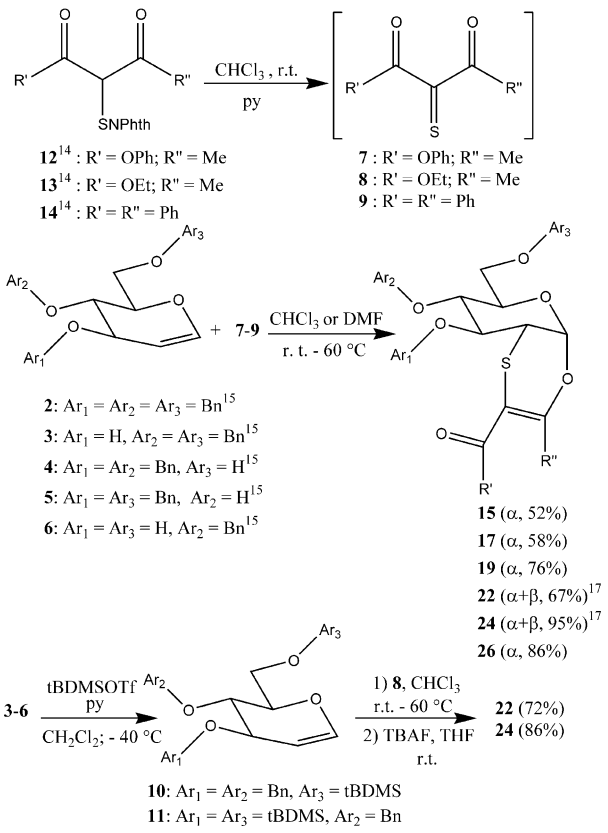


Figure 1. Superposition of the structures of the reference compound **1** and **18** as resulting from molecular dynamics (see text for details).



Scheme 1.

group at C-3 unessential. On the other hand, the presence of one or two aromatic groups on the oxathiinic moiety does not seem to have any effect on the affinity as shown by the comparison between **18** and **27**. Given the strong difference in bulkiness, it likely appears that these residues are accommodated in a steric-free region of the receptor, probably towards the extracellular side. On the other hand **20**, which bears only two aromatic moieties, still shows a submicromolar affinity to the NK-2 receptor. This is probably index of a different binding mode for this compound.

An increase in activity was repeatedly shown going from the sulfides to the corresponding sulfoxides (see **15** vs **16**, **17** vs **18**, **26** vs **27**). This seems to be an interesting point, not completely unexpected considering that one of the most studied nonpeptide antagonists, the Glaxo sulfoxide compound GR 159697[20] was reported to have increased NK-2 affinity (one order of magnitude) respect to the parent sulfide. However, further oxidation to the sulfone **28** only led to a decrease in activity.

Compounds of Table 1 showed antagonist activity in functional experiments on isolated endothelium-deprived rabbit pulmonary artery preparations when evaluated against a contractile concentration–response curve to NKA ($pK_B = 6.0$ for **18**).[21]

In conclusion, we have shown that a glucose-oxathiinic scaffold, suitably substituted with simple aromatic moieties, can be used to obtain nonpeptide micromolar ligands for the human tachykinin NK-2 receptor. Further work, aimed to improve affinity through the insertion on the scaffold of more complex aromatic or heteroaromatic moieties (such as indole in **1**), will be reported in due time.

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15. The tribenzylglucal **2** and the dibenzylglucals **3** and **5** were prepared treating a suspension of NaH in DMF with D-(+)glucal. After 30 min stirring at rt, the mixture was cooled to 0 °C and an excess of benzyl bromide was added. The reaction, complete after 24 h stirring at rt, was quenched with H_2O and the mixture extracted with CH_2Cl_2. Flash column chromatography afforded **2** (25%), **3** (20%), **5** (16%) and small amounts of monobenzylated glucals, easily separable from tri- and di-benzylated derivatives. The monobenzylglucal **6** and the dibenzylglucal **4** were obtained from 3,6-*O*-di *tert*butyldimethylsilylglucal and 6-*O*-*tert*butyldimethylsilylglucal[16] as starting material respectively, following the above reported procedure.
16. For the silylation of glycals, see: Capozzi, G.; Falciani, C.; Menichetti, S.; Nativi, C.; Raffaelli, B. *Chem. Eur. J.* **1999**, *6*, 1748.

17. Cycloadditions of di-benzylglucal **5** and mono-benzylglucal **6** with α,α′-dioxotiones afforded the α-glycosides **22** and **24**, respectively (see Scheme 1 and Table 1), with amounts (13%, **22** and 89%, **24**) of the corresponding β isomers. The temporary protection of the free hydroxy groups of **5** and **6** as *tert*butyldimethylsilyl ethers **10** and **11** (see Scheme 1) and their cycloaddition with dioxothiones allowed to completely avoid the undesired formation of the β isomer.
18. Synthesis of compound **18**: A solution of 105 mg (0.18 mmol) of **17** in CH_2Cl_2 (3 mL) was cooled to −15 °C and treated with a solution of 20 mg (0.18 mmol) of *m*-CPBA in CH_2Cl_2 (2 mL). After 1 h, the mixture was washed with a 10% soln of $Na_2S_2O_3$ and $NaHCO_3$ (satd soln). The crude (282 mg) was purified by flash column chromatography on silica gel (eluant hexane/EtOAc 3/2) to give 94 mg (87%) of **18** as white solid. Mp 108–110 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37 (t, 3H), 2.47 (s, 3H), 3.22–3.40 (m, 2H), 3.72–4.06 (m, 4H), 4.32 (q, 2H), 4.50–4.86 (m, 6H), 5.91 (bs, 1H), 7.16–7.38 (m, 15H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 21.8, 58.2, 61.5, 67.7, 73.5, 74.3, 75.3, 75.4, 75.6, 78.0, 92.1 (C-1), 107.3, 127.8, 127.9, 128.0, 128.2, 128.4, 128.5, 136.6, 137.3, 137.5, 164.0, 171.0. For the synthesis of sulfoxides, see also: Capozzi, G.; Fratini, P.; Menichetti, S.; Nativi, C. *Tetrahedron Lett*. **1995**, *36*, 5089.
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