Letters in Drug Design & Discovery, 2004, *1*, 285-288

Amide Mono Thioxylated Derivatives of MEN 10627

M. Altamura, G. Balacco, A. Giolitti, A. Guidi*, R. Patacchini, A. R. Renzetti, A. Triolo and C. A. Maggi

Menarini Ricerche S.p.A. Via dei Sette Santi 3, 50131 Florence, Italy

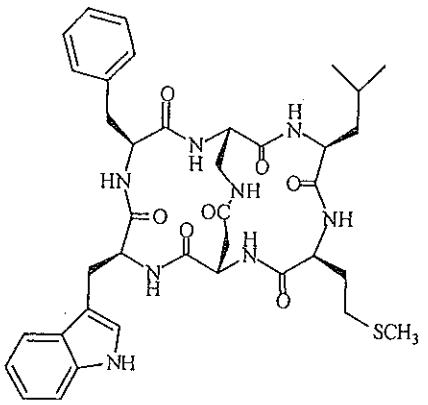
Received April 01, 2004: Accepted April 22, 2004

Abstract: The thioxylation of the potent Neurokinin A antagonist MEN 10627 by Lawesson's reagent showed to be potentially useful for the post-synthetic manipulation of the related family of structurally peculiar bicyclic hexapeptides, whose synthesis in combinatorial manner has been recently described.

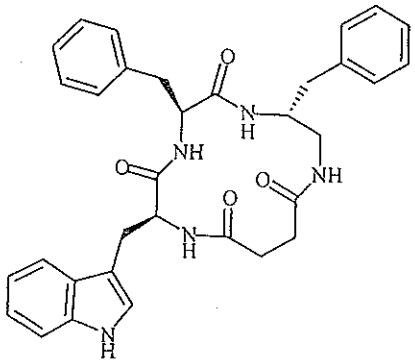
Keywords: Tachykinins, thiopeptides, thioxopeptides, Lawesson's reagent.

INTRODUCTION

The homodetic bicyclic peptide *cyclo*[(Met[1]-Asp[2]-Trp[3]-Phe[4]-Dpr[5]-Leu[6]) *cyclo*(2β-5β)], MEN 10627, (Fig. 1), has been one of the first highly potent and selective peptide antagonists of Neurokinin A (His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2, NKA) at the NK-2 receptor of mammalian tachykinins to be discovered [1]. Although its physico-chemical properties discouraged clinical investigation, it was used as reference compound to obtain a water soluble congener, Nepadutant, which is presently undergoing clinical trials [2] and to derive a new family of potent monocyclic peptide antagonists, exemplified in Fig. (1) by compound (1) [3].



MEN 10627



compound (1)

Fig. (1).

Aside from that, the structural features shared by MEN 10627 with other known congeners of general formula *cyclo*[(Aa$_1$[1]-Asp[2]-Aa$_2$[3]-Aa$_3$[4]-Dpr[5]-Aa$_4$[6]) *cyclo*(2β-5β)] have attracted our interest. The title compound is characterised, both in solution and in the solid state, by a type I and type II β-turn, with Trp-Phe and Leu-Met as corner residues, respectively [4]. The same arrangement is displayed in solution by Nepadutant [5] and, in both states, by the pseudosymmetric analogue MEN 10698 [6]. The possibility that the same kind of molecular arrangement could be common to many other members of the family prompted us to start a programme aimed at their synthesis in combinatorial manner [7] with the ultimate purpose to use the pool, a set of peptide privileged structures [8], towards different biological targets.

In connection with this project, we disclose here the results of a study, made on MEN 10627 as model compound, having the object to synthesise derivatives capable to discriminate which turn is more involved in the recognition process. In principle, the fact that two β-turns are displayed on the same molecule, albeit promising for screening purposes, could be a source of confusion since a single β-turn is often the site of sychnologic recognition [9]. Actually, at the time of MEN 10627 discovery, it was thought that the fragment Leu-Met, reminiscent of the natural agonist primary structure, should be crucial for high affinity [10]. Conversely, it was later found that the replacement of this entire fragment with a benzyl group, opportunely juxtaposed on the remaining monocycle, as in compound (1), is conservative of both the type I β-turn and high potency at the targeted receptor [3].

Therefore, we looked for a synthetic tool capable to furnish, smoothly and efficiently from the pre-synthesised peptide, at least a couple of derivatives modified at the

*Address correspondence to this author at the Menarini Ricerche S.p.A. Via dei Sette Santi 3, 50131, Florence, Italy; E-mail: aguidi@menarini-ricerche.it

1570-1808/04 $45.00+.00

corresponding positions of each β-turn. In reason of the need of potential wide applicability, we chose to act on the peptide backbone and decided to study the thioxylation of preformed MEN 10627. The replacement CONH/CSNH by a commercially available thioxylating agent constitutes still today one of the few routine methods for direct modification on a peptide backbone [11].

RESULTS

Aware of the possibility to obtain complex and intractable mixtures of mono and poly thioxylated derivatives, first we studied the feasibility of the idea by means of the sole HPLC-MS and UV monitoring of the reaction outcome. Since the use of Lawesson's reagent, LR [12], in boiling acetonitrile resulted to be encouraging at this stage and gave us a good deal of information, we did not use other thioxylating reagents and conditions.

The reaction appeared to be progressive and the degree of thioxylation could be fairly kept under control acting on the amount of used LR. In certain runs, we used up to 10 molar equivalents overall excess of LR, by consecutive portion-wise additions, and could observe that the various products clustered, as RP-HPLC retention times, in dependence of their thioxylation degree, the less thioxylated being eluted faster. The mono thioxylation step (protonated quasi-molecular ion at m/z = 777), the most interesting to our concerns, seemed to involve 3 chromatographic peaks, the relative abundance of the fast eluted of which was valuable only at the very early stage of the process.

After having labelled the putative mono thioxylated products as compounds (2), (3) and (4), in adherence to their order of RP-HPLC elution, mass spectrometry permitted also to achieve further structural information by the comparison with the behaviour of the parent compound.

The fragmentation of MEN 10627 protonated quasi-molecular ion, (Fig. 2), is characterised by the fragments at m/z = 428 and at 517 indicating the losses of the dipeptides Trp-Phe and Leu-Met, respectively, as well as by the losses of unchanged amino acids, namely Leu (fragment at m/z 648), Met (m/z 630), Phe (m/z 614), and Trp (m/z 575). Further, MS^3 experiments reveal that both ions at m/z = 428 and 517 loose carbon monoxide. Concerning compound (3), the fragmentation pathway 517-489 is still present, but the pathway 428-400 is absent. Concerning compound (4), the opposite behaviour is shown. In addition, these compounds do not show the losses of the single amino acid, rather, formally, those of methyl mercaptan (m/z = 729) and ethyl methyl sulphide (m/z = 701) for compound (3), those of indole (m/z = 660) and quinoline (m/z = 648) for compound (4). The mass spectral data of compound (2) are also characteristic. The MS-MS spectrum of the protonated quasi-molecular ion reveals the neutral losses of intact amino acids Leu (fragment at m/z 664), Met (m/z 646), Phe (m/z 630), and Trp (m/z 591) and the fragments at m/z = 533 and 444, which correspond to the fragments at m/z = 517 and 428 observed for MEN 10627 to which 16 Daltons have been added. In addition, the appropriate MS^3 experiments reveal that the loss of carbon monoxide is active for both ions (fragments at m/z = 505 from 533 and at m/z = 416 from 444).

In summing up these preliminary observations, we concluded that the process could possess a certain degree of selectivity and could be interesting to our purposes. Moreover, the mass spectrometry investigation supported the hypothesis that compound (2) bore the sulphur atom at the amide bridge; compounds (3) and (4) bore the sulphur atom at one of the two carboxy groups of the fragments Leu-Met or Trp-Phe, respectively.

As a consequence of that, we chose reaction conditions, namely 1 molar equivalent of LR and 0.5 hours in boiling CH_3CN, putatively maximising the relative abundance of compounds (3) and (4), and decided to operate with two consecutive preparative HPLC runs for their isolation. The first run was useful to separate the mixture of compounds (3) and (4) from unreacted starting material, from the very small

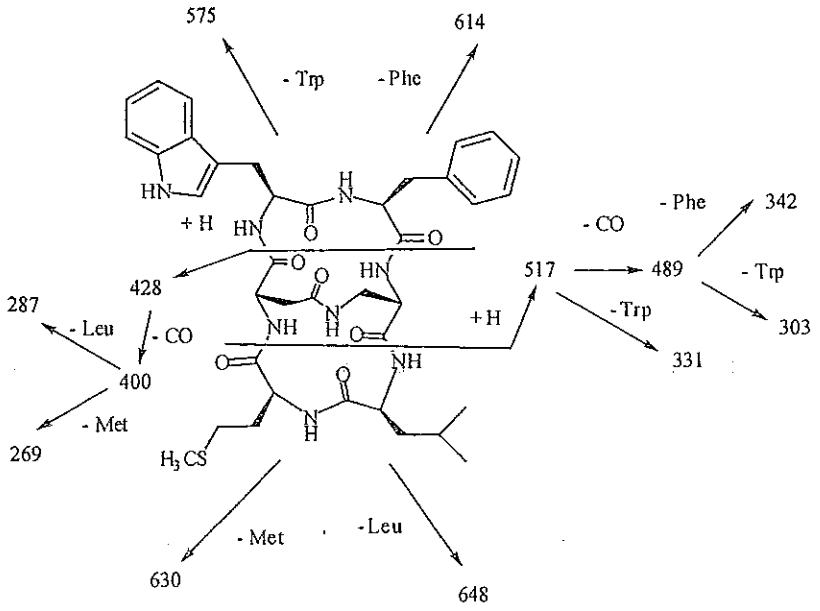
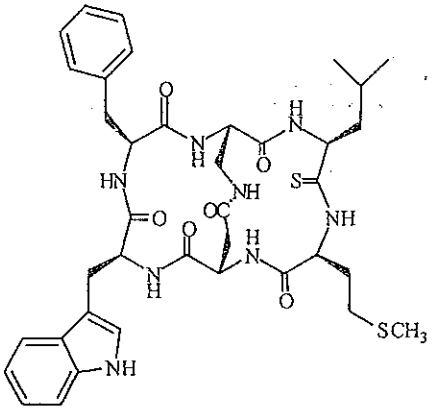
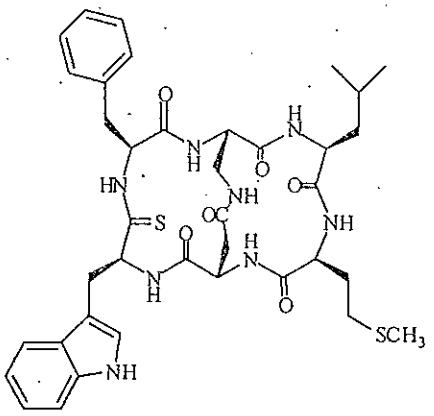


Fig. (2).



Compound (3)



Compound (4)

Fig. (3).

amount of compound (2) and from compounds of higher degree of thioxylation. The second run permitted to isolate samples of both products suitable for a binding affinity test (MEN 10627 content, as well as cross contamination, < 0.1% at 220 nm). Compounds (3) and (4) were thus isolated in 15% and 6% yield, respectively, on the 0.1 mmole scale of starting material.

The NMR spectra (500 MHz) of compounds (3) and (4) in deuterated acetonitrile at 300 K confirmed the homogeneity of the isolated material and allowed us to discard one of the two alternatives proposed by mass spectrometry, after comparison with MEN 10627 spectrum. Most of the chemical shifts change little in going from MEN 10627 to compounds (3) and (4). The greatest changes (1.31 and 1.43 ppm) refer to the Met NH in compound (3) and to the Phe NH in compound (4). Thus, in agreement with previous literature [11], we concluded that compound (3) bore the sulphur atom at Leu and compound (4) at Trp, (Fig. 3).

Compounds (3) and (4) were eventually evaluated for their ability to inhibit the binding of [^{125}I]NKA to human tachykinin NK-2 receptor. The results are reported in Table 1 as p*Ki* in comparison with the performances of MEN 10627 and compound (1) in the same test [3].

Table 1. Inhibition of Binding of [^{125}I]NKA to hNK-2 Receptor

Compds	pK_D[a]	p*Ki*[a]
NKA	8.6 ± 0.7	
MEN 10627		9.2 ± 0.1
1		8.7 ± 0.3[b]
3		8.9 ± 0.1
4		7.6 ± 0.2

[a]mean p*Ki* and pK_D values ± s.e.m. (n = 2-4).
[b]Taken from ref. [3].

Noteworthy, the p*Ki* difference between compounds (3) and (4) has the expected sign and indicates, for the first time, that the same modification at the corresponding positions of the two moieties of MEN 10627, discriminates them. Also the p*Ki* difference between MEN 10627 and compound (4) is noteworthy and prompts to speculate on the binding mode of the unmodified antagonist. Although, we were not able to grow crystals of compound (4) suitable for X-rays analysis, it is unlikely that the observed p*Ki* difference descends from conformational changes occurring when passing from MEN 10627 to compound (4). In fact, the computational analyses of the two products, by means of the Simulated Annealing technique [10] give superimposable results and the two NOESY spectra show the same cross-peaks, an indication of a similar backbone conformation. So, taking into consideration that thioxoamides are known to be less prone than amides to act as hydrogen bond acceptors [11], we think that a Trp-Phe amide involving hydrogen bond could contribute to stabilise the complex between MEN 10627 and human tachykinin NK-2 receptor.

CONCLUSION

In this study, we have shown that it is possible to modify a compound of general formula *cyclo*[(Aa_1^1-Asp^2-Aa_2^3-Aa_3^4-Dpr^5-Aa_4^6) *cyclo*(2β-5β)] at corresponding positions of the two structural moieties which it consists of, as well as that the way to operate can be relatively simple on the 0.1 mmole scale. The overall result does not discourage the application of the method to other members of the same family of peptide-privileged structures. The result of this extension of the work will be described in due time.

REFERENCES

[1] Pavone, V.; Pedone, C.; Quartara, L.; Renzetti, A. R.; Giachetti, A. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1489.
[2] Lördal, M.; Navalesi, G.; Theodorsson, E.; Maggi, C.A.; Hellström, P.M. *Br. J. Pharmacol.* **2001**, *134*; 215.
[3] Giannotti, D.; Perrotta, E.; Di Bugno, C.; Nannicini, R.; Harmat, N. J. S.; Giolitti, A.; Patacchini, R.; Renzetti, A. R.; Rotondaro, L.; Giuliani, S.; Altamura, M.; Maggi, C. A. *J. Med. Chem.* **2000**, *43*, 4041.
[4] Pavone, V.; Lombardi, A.; Nastri, F.; Saviano, M.; Maglio, O.; D' Auria, G.; Quartara, L.; Maggi, C. A.; Pedone, C. *J. Chem. Soc. Perkin Trans. II* **1995**, 987.
[5] Weißhoff, H.; Nagel, T.; Hänsicke, A.; Zschunke, A.; Mügge, C. *FEBS Lett.* **2001**, *491*, 299.

] Lombardi, A.; D' Auria, G.; Saviano, M.; Maglio, O.; Nastri, F.; Quartara, L.; Pedone, C.; Pavone, V. *Biopolymers* **1996**, *40*, 505.
] Teixido, M.; Altamura, M.; Quartara, L.; Giolitti, A.; Maggi, C. A.; Giralt, E.; Albericio, F. *J. Comb. Chem.* **2003**, *5*,760.
] Horton, D. A.; Bourne, G. T.; Smythe, M. L. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 415.
] Farmer, P. S.; Ariens, E. J.; *Trends Pharmacol. Sc.* **1982**, *4*, 362.
0] Giolitti, A.; Maggi, C. A. *J. Comput. Aid. Mol. Des.* **1994**, *8*, 341.
1] For examples of thioxylations of cyclic peptides see: (a) Bock, M. G.; DiPardo, R. M.; Williams, P. D.; Pettibone, D. J.; Clineschmidt, B. V.; Ball, R. G.; Veber, D. F.; Freidinger, R. M. *J. Med. Chem.* 1990, *33*, 2321. (b) Seebach, D.; Ko, S. Y.; Kessler, H.; Köck, M.; Reggelin, M.; Schmieder, P.; Walkinshaw, M. D.; Bölsterli, J. J.; Bevec, D. *Helv. Chim. Acta* **1991**, *74*, 1953. (c) Kessler, H.; Geyer, A.; Matter, H.; Köck, M. Int. *J. Peptide Protein Res.* **1992**, *40*, 25. (d) Hitotsuyanagi, Y.; Suzuki, J.; Matsumoto, Y.; Takeya, K.; Itokawa, H. *J. Chem. Soc. Perkin Trans. 1* **1994**, 1887. (e) Morita, H.; Nagashima, S.; Takeya, K.; Itokawa, H. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2327. (f) Morita, H.; Yun, Y. S.; Takeya, K.; Itokawa, H.; Shirota, O. *Bioorg. Med. Chem.* **1997**, *5*, 631.
[12] Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 223.