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Insertion of 2-Carboxysuccinate and Tricarballylic Acid Fragments into Cyclic-Pseudopeptides: New Antagonists for the Human Tachykinin NK-2 Receptor

Nicholas J. S. Harmat,* Danilo Giannotti, Rossano Nannicini, Enzo Perrotta, Marco Criscuoli, Riccardo Patacchini, Anna-Rita Renzetti, Sandro Giuliani, Maria Altamura and Carlo A. Maggi

Menarini Ricerche S.p.A. Laboratori di Firenze, Via Rismondo 12/A, 50131 Firenze, Italy

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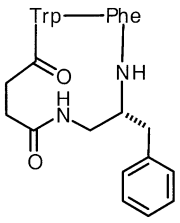
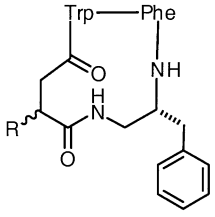
Abstract—A series of cyclic pseudopeptides were synthesized containing the sequence -Trp-Phe-(D)-PheΨCH_2NH-, the terminal ends of which were bound to 2-carboxy succinate or enantiomerically enriched tricarballylic acid to give the final cyclic structures. These two molecules and their subsequent derivatives were screened for h-NK2 receptor binding and functional antagonist activity on the rabbit urinary bladder. © 2002 Published by Elsevier Science Ltd.

The mammalian tachykinins comprise a group of small peptides, referred to as substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) that are located in the central and peripheral nervous systems. They exert a variety of biological responses, such as smooth muscle contraction and relaxation, pain transmission, vasodilation and neurogenic inflammation by interacting with the receptor types NK-1, NK-2 and NK-3.[1,2] NKA induces a biological response by binding to the NK-2 receptor. Our studies have focused on the human NK-2 receptor (h-NK2) which has been identified as a target for the discovery of new drugs for the treatment of inflammatory diseases in the respiratory, gastrointestinal and genitourinary tracts.[3–5]

In the course of our work on the discovery of new small molecules as antagonists for the h-NK2 receptor we recently communicated the SAR on a series of cyclic pseudopeptides which gave the lead MEN 11558, **1** with a potent in vitro h-NK2 receptor binding activity and functional activity.[6] The aim of our work presented here taking **1** as a lead, was to replace the succinoyl moiety with other functionalities without changing the structural characteristics of the monocyclic compounds essential for h-NK2 antagonist activity and yet have compounds with improved potency.



MEN 11558, **1**



R = CO_2H **2**, $CO_2NR'R''$ **8**

= CH_2CO_2H **3**, $CH_2CO_2NR'R''$ **12**

With these premises in mind we decided to prepare a series of analogues of MEN 11558 in which the succinate fragment is replaced by 2-carboxy succinic acid or enantiomerically enriched tricarballylic acid (TCA), giving, respectively, analogue structures **2** and **3**, which were each converted to the amide derivatives **8** and **12**.

Chemistry

The syntheses of both these orthogonally protected 2-carboxy succinic acid[7] and enantiomerically enriched TCA[8] building blocks have been described in earlier communications. It was therefore envisaged to insert the protected 2-carboxy succinic acid and TCA moieties in a regioselective manner into the orginal tripeptide fragment common to **1**, opening the possibility for

*Corresponding author. Fax: +39-055-5680-419; e-mail: chimfarm@menarini.ricerche.it

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subsequent preparation of various derivatives. The synthesis of the first series of compounds is shown in Scheme 1 and starts from the doubly protected tripeptide fragment **4**, which was prepared by analogy to the methods previously described[6] for obtaining this key intermediate.

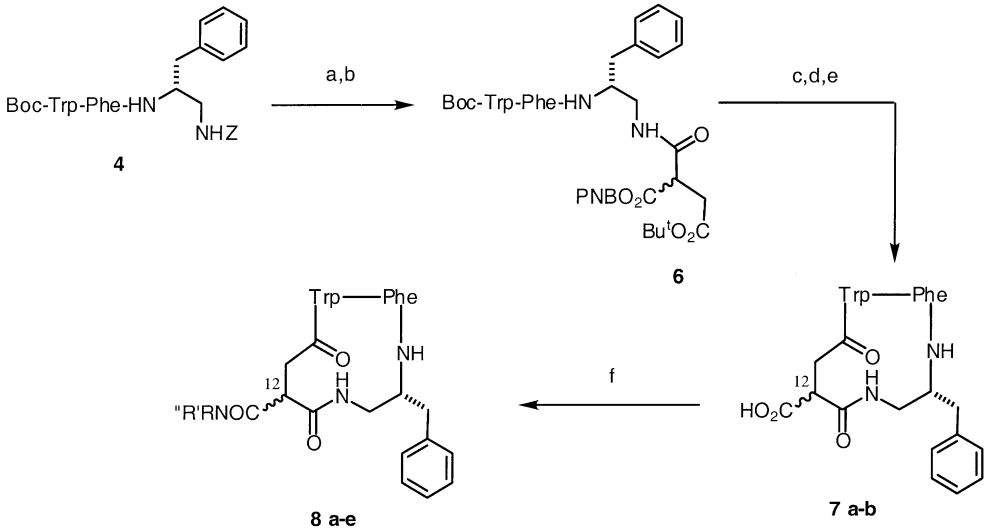
The *Z*-(carbobenzyloxy) protecting group in **4** was removed by hydrogenolysis and the mono-deprotected intermediate then coupled to the orthogonally di-protected carboxy succinic acid derivative **5**,[9] giving the elaborated 2-carboxy succinamide di-ester **6**. In the final steps to the cyclized pseudopeptide products, intermediate **6** was double de-*tert*-butylated with TFA at the N and C termini and the resulting zwitterionic compound cyclized with PyBOP. The final carboxyl protecting group (PNB) was removed by saponification giving **7a**,**b** as a 5.6:1 mixture of epimers which were separable by preparative HPLC.[10] We then proceeded to derivatize the same enriched epimeric mixture **7a**,**b** by coupling this to functionalized hydrophilic amines such as *N*-acetyl glucosamine[11] and 4-piperidinyl piperidine,[12] both of which are known to confer improved water solubility, an important consideration for producing molecules with potential in vivo activity. The resulting derivatives **8a**–**e** were single diastereoisomers, as indicated by HPLC and NMR data with configuration of the chiral C12 ring atom not being definable. In one case **8e**, two epimers were found to be present in a ratio of 5.5:1 and these were separable by HPLC as in the case of the parent compound **7**.

Because of the ambiguity of assignment of the stereochemistry of the 2-carboxy succinate moiety in the final cyclized products **7** and **8**, we turned our attention to the application of the homologous tricarballylic acid (TCA) with the synthesis of this second series of compounds described in Scheme 2. Starting from the same tripeptide intermediate **4**, N-Boc deprotection of the Trp residue and subsequent coupling with enantiomerically enriched orthogonally di-protected (*R*) or (*S*) TCA ester derivatives **9**, gave the single epimeric adduct **10** with defined stereochemistry of the α-CH group in the TCA moiety. The N and C terminus of each separate epimer of **10** was double de-benzylated by hydrogenolysis and cyclized by condensation to give **11**. Final de-*tert*-butylation with TFA and derivatization of the *exo*-cyclic CH_2–carboxyl terminus by condensation with various functionalized amines gave the final target compounds **12a** and **12b** as separate individual stereoisomers with a defined configuration with respect to the C12 ring carbon atom.

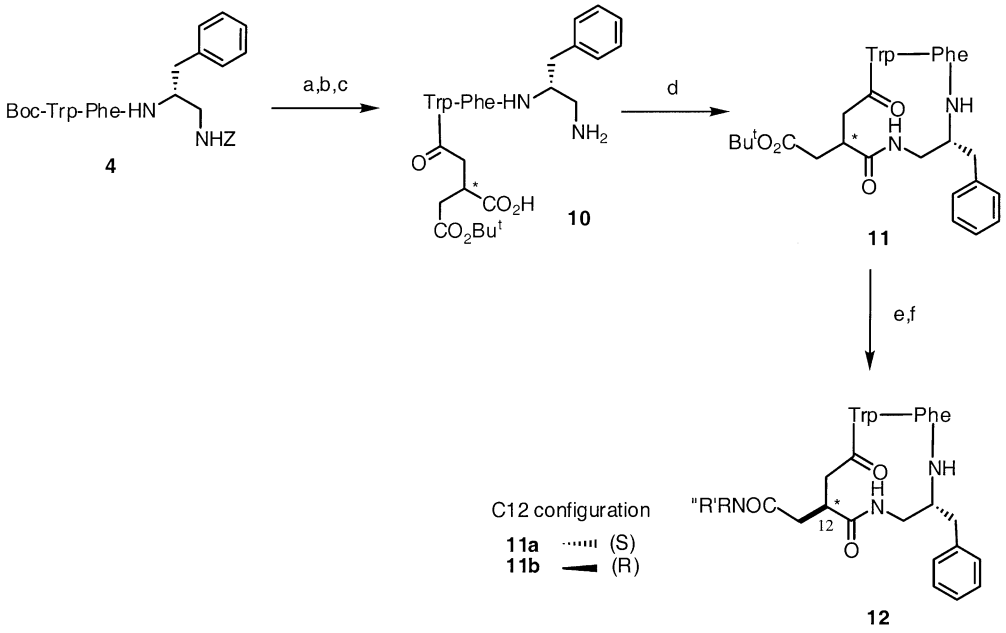
Results and Discussion

The cyclo-pseudopeptide target molecules for both the 2-carboxysuccinate series (**8a**–**e**) and the tricarballylic acid (TCA) series (**12a**–**e**)[13] were evaluated for their in vitro binding affinity to the human NK-2 receptor (pK_i values)[14] and were also screened for their functional activity (pA_2 values) on isolated rabbit urinary bladder (RUB) tissue.[15] The results of both these series of new cyclo-pseudopeptide derivatives were compared against our in vitro lead compound MEN 11558, **1**.

The results for the first series with insertion of 2-carboxysuccinic acid in the cyclopeptide framework are presented in Table 1. For the derivatization of the free carboxyl group in with various polar functionalised amines, we chose as a starting point the amino sugar *N*-acetyl glucosamine. This substituent is present also in the form of attachment to a pendant carboxyl group in the complex bicyclic peptide NK2 antagonist nepadutant (MEN 11420) which is presently in clinical trials.[12] The attachment of this sugar group to **7** gave the derivative **8a**, which was approximately equipotent with **1** in terms in receptor binding affinity and functional activity. We then searched for simpler hydroxylated or ring oxygenated amine derivants the structural features of which could mimic the sugar moiety yet give molecules of lower molecular weight.



Scheme 1. (a) H_2, Pd/C MeOH/DMF; (b) $Bu^tO_2CCH_2CH(CO_2PNB)CO_2H$ (**5**), EDC.HCI/HOBt/DMF; (c) TFA/CH_2CL_2 (15%, v:v); (d) PyBOP/DIPEA in DMF; (e) K_2CO_3/*i*-PrOH/H_2O; (f) R′R″N H, EDC.HCI/HOBt/DMF.



Scheme 2. (a) TFA/CH_2Cl_2 (15% v:v); (b) HO_2C $CH^*_2CH(CH_2CO_2Bu^t)CO_2Bn$, (**9**), (*R*) or (*S*), EDC; HCl/HOBt in DMF; (c) H_2, Pd/C MeOH/DMF; (d) PyBOP/D IPEA in DMF; (e) TFA, 80% in CH_2Cl_2; (f) ″R′RNH, EDC·HCl/HOBt/DMF.

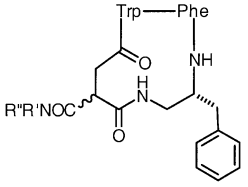
On this basis, the derivatives **8b**–**8d** were prepared and from these four products the morpholine derivative **8c** resulted in the best binding affinity for the NK-2 receptor and functional activity, both superior to that of **1**. The derivitization of compound **7** with 4-piperidinyl piperidine gave two isolable epimers **8e** and **8f**, both which had markedly superior binding affinity and functional activity to **1**.

The results for receptor binding affinity and functional activity for the second series with insertion of either single enantiomer of tricarballylic acid (TCA) into the cyclopeptide framework, are presented in Table 2.

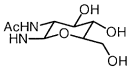
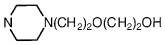
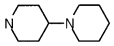
Attachment of the *N*-acetylglucosamine to the pendant CH_2–carboxyl group of either epimer gave derivatives **12a** or **12b** which both maintained the receptor binding and functional activity compared to the lead **1**. This first result also indicated that the activity was independent of the configuration of the chiral TCA moiety inserted into the cylopeptide structure. This was confirmed in the example with the 4-piperidinyl piperidine derivatives **12f** and **12g**. Since the (*S*) enantiomer of TCA was more readily available we produced further derivatives **12c**–**12e** both of which maintained a comparable activity with the lead compound **1**, with the exception of the morpholine derivative **12d** which showed pK_i and pA_2 ≥ 9. However, the improvements in the binding activity are more limited in this second series when compared to the 2-carboxysuccinate derivatives of Table 1.

In conclusion, we have described the application of orthogonally protected tricarboxylic acid building blocks that have been previously reported by us, in the synthesis of target molecules of interest as potential NK-2 receptor antagonists. Replacement of the succinoyl fragment with either 2-carboxy succinnate or enantiomerically enriched (*R*)- or (*S*)-TCA gave cyclic peptide analogues with a derivatizable pendant carboxyl group. The 2-carboxysuccinate analogues showed more marked improvements in binding and functional activity compared to **1**, notably the morpholine derivative **8c** and the basic 4-piperidinyl piperidine epimeric derivatives **8e** and **8f**. The TCA derivatives overall maintained activity compared to the lead **1**, with however the morpholine **12d** derivative showing increased binding

Table 1. h-NK2 receptor binding activity (pK_i) and in vitro functional activity (pA_2) for the 2-carboxy succinate series of derivatives **8a**–**f**

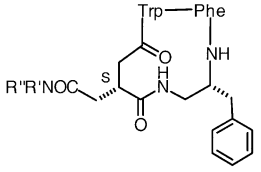


8a-f

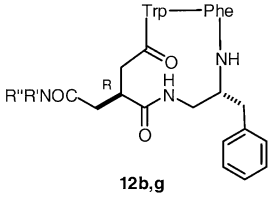
Compd	R′R″NH	pK_i	pA_2 (RUB)
8a		8.6	8.4
8b		8.4	8.5
8c		9.9	9.2
8d		8.8	9.0
8e[a]		10.3	9.1
8f[a]		9.5	9.1
MEN 11558, **1**	–	8.7	8.3

[a]Two epimers formed and separated.

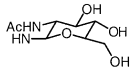
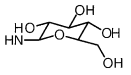
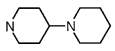
Table 2. h-NK2 receptor binding activity (pK_i) and in vitro functional activity (pA_2) for the tricarballylic acid series of derivatives **12a**,**c–f** and **12b**,**g**



12 a,c-f



12b,g

Compd	R′R″NH	pK_i	pA_2 (RUB)
12a		8.1	8.2
12b		8.2	8.1
12c		8.0	8.2
12d		9.2	9.0
12e		8.2	8.5
12f		8.8	8.6
12g		8.3	8.4
MEN 11558, **1**	–	8.7	8.3

affinity and an improvement in in-vitro functional activity compared to MEN 11558. No apparent variation in activity with the chirality of the TCA unit was observed in these analogues of **1**. A selection of these compounds have been taken for further in-depth studies regarding in vivo activity.

Acknowledgements

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