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Aminomethylthiophene-2-carboxylic Acids as Dipeptide Mimetic in New Growth Hormone Secretagogues

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Abstract 3-Aminomethylbenzoic acid is a well established dipeptide mimetic. Herein, aminomethylthiophene-2-carboxylic acids[1)] have been synthesized as analogues of 3-aminomethylbenzoic acid. Their use as a dipeptide-mimetic at the *N*-terminal of novel growth hormone secretagogues is described. © 1997 Elsevier Science Ltd.

Introduction

The discovery, that the hexapeptide His-*D*-Trp-Ala-Trp-*D*-Phe-Lys-NH_2 (GHRP-6)[2)] releases growth hormone by a distinct mechanism, which is different from that of growth hormone releasing hormone (GHRH)[3)], has resulted in a number of research programs, which lead to other peptidic growth hormone releasers, such as *D*-Ala-*D*-2-Nal-Ala-Trp-*D*-Phe-Lys-NH_2 (GHRP-2)[4)] or non-peptidic releasers such as L-692,429[5)] or MK-0677[6)]. The aim is to develop an orally available drug, that might advantageously substitute the direct administration of growth hormone in a number of therapeutical indications.

Discussion

During our screening for growth hormone secretagogues, we identified **1**[7)] as lead compound. The utilization of the well established dipeptide mimetic 3-aminomethylbenzoic acid[8)] at the *N*-terminal gave an equipotent compound **2**. We decided to focus on similar thiophene analogues[1)] and their ability to serve as dipeptide-mimetic.

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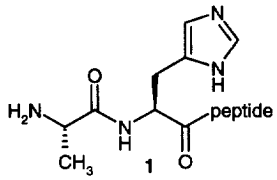
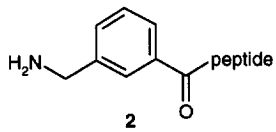
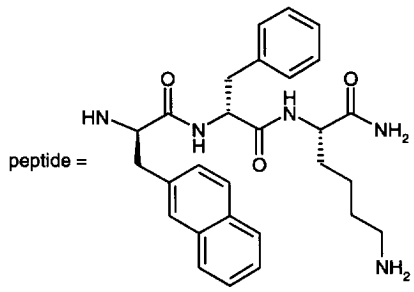
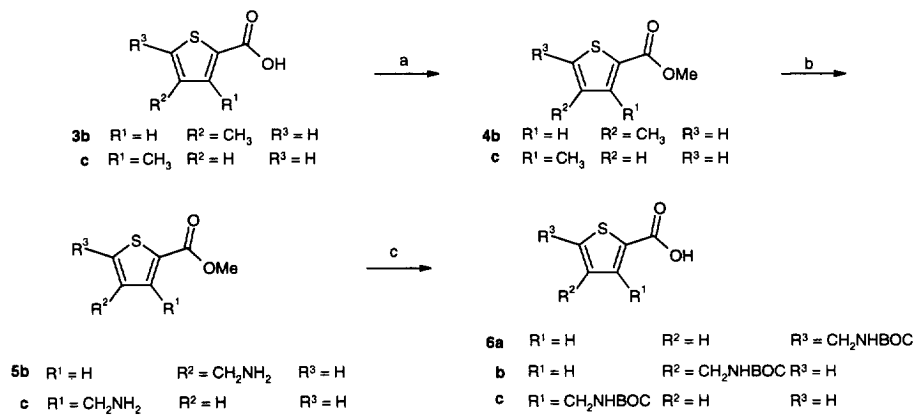






Therefore the BOC-protected 5-aminomethyl-2-carboxylic acid (**6a**)[8] and its isomers **6b** and **6c** were synthesized and incorporated into peptides of type **1**. Starting with methylthiophene-2-carboxylic acids **3b**[9] and **3c**[10], the corresponding esters **4b** and **4c** were prepared. NBS-bromination followed by treatment with ammonia in DMF furnished the aminoesters **5b** and **5c**[11]. A one-pot saponification and BOC-protection afforded the protected amino acids **6b** and **6c**[12].

Scheme 1

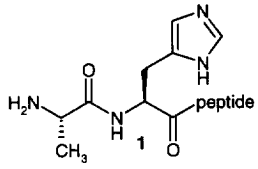
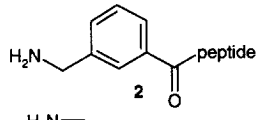
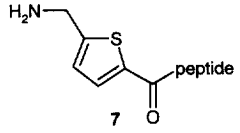
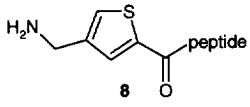
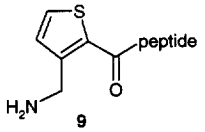
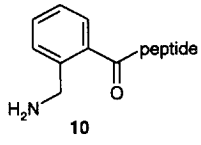


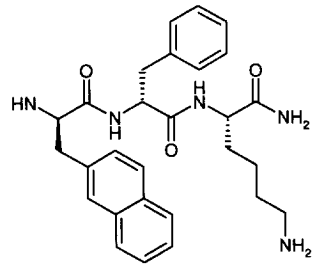
Reagents: a) CH_3OH, H_2SO_4, reflux; b) i) NBS, dibenzoylperoxide, CCl_4, reflux; ii) NH_3, DMF; c) 2 eq. NaOH, $(BOC)_2O$, dioxane/water

The amino acids **6a** - **c** were incorporated into the peptide by standard peptide synthesis on *Rink*-resin, and cleaved with trifluoroacetic acid[13]. The resulting peptides were tested in a functional *in vitro* rat pituitary assay[14,15]. The test results are shown in Table 1 as mean values

of two experiments. The best dipeptide mimetics were 3-aminomethylbenozic acid and its 2,3-substituted thiophene analogue **6c**, leading to peptides **2** and **9**, respectively.

Table 1

	EC_{50} [nM]
 **1**	6
 **2**	7
 **7**	210
 **8**	675
 **9**	27
 **10**	85

peptide = 

Conclusion

In growth hormone secretagogues of type **1**, the *N*-terminal Ala-His-moiety can be replaced by the known dipeptide-mimetic 3-aminomethylbenzoic acid without loss of activity. When the thiophene-analogue **6c** is used at the *N*-terminal, the resulting peptide **9** shows very similar activity. Surprisingly, **6a** and **6b** as *N*-terminal cause a sharp drop in activity of the peptides **7** and **8**, even though the orientation of substituents on the thiophene-rings is similar to that of 3-aminomethylbenzoic acid. Peptide **10**, with the *ortho*-substituted 2-aminomethylbenzoic

acid at the *N*-terminal, shows decreased activity, compared to **2**. From this one can conclude that, despite different orientation of substituents, 3-aminomethylbenzoic acid and 3-aminomethylthiophene-2-carboxylic acid (**6c**) are dipeptide mimetics at the *N*-terminal of growth hormone secretagogues. The low activities of **7** and **8** may be caused by different conformation of the amino acids **6a** or **6c** at the *N*-terminal due to enhanced sterical demand by the sulfur atom. Another hypothesis is hydrogen-bonding of the sulfur atom to groups at the binding site, that does not occur with benzene and results in a different orientation of the thiophene moiety.

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12) ^{1}H-NMR (DMSO d_6): **6b**: δ 1.40 (s, 9 H); 4.10 (d, 2 H); 7.40 (t, 1 H); 7.56 (s, 1 H); 7.61 (s, 1 H); 13.05 (br, 1 H). MS: calc: 257.07218, found: 257.071944.
^{1}H-NMR (DMSO d_6): **6c**: δ 1.40 (s, 9 H); 4.43 (d, 2 H); 7.05 (d, 1 H); 7.37 (t, 1 H); 7.75 (d, 1 H); 13.05 (br, 1 H). MS: 257.07215, found: 257.071864.

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