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Highly Potent Growth Hormone Secretagogues: Hybrids of NN703 and Ipamorelin

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Abstract—A series of NN703 analogues with lysine mimetics combined with naphthyl- or biphenylalanine in the core has been prepared and tested in vitro in a rat pituitary cell based assay and subsequently in vivo in pigs in a single dose at 50 nmol/kg. Re-introduction of certain pharmacophores in the C-terminal of NN703, which were originally removed during optimisation for oral bioavailability, led to unexpectedly potent compounds in vitro as well as in vivo. © 2001 Elsevier Science Ltd. All rights reserved.

The field of growth hormone secretagogues (GHSs) has continued to expand since the discovery by Momany and Bowers in the 1970s that certain derivatives of enkephalines specifically were capable of releasing growth hormone from the pituitary gland.[1] Since an endogenous ligand for the GHS receptor,[2] Ghrelin,[3] was discovered only recently, these small peptides have served as lead compounds in efforts to develop orally active GHSs[4] in several groups. Among the resulting development candidates were the highly potent pentapeptide, ipamorelin,[5] and the modified tripeptide, NN703,[6] which is a somewhat less potent but orally bioavailable compound derived from ipamorelin (Fig. 1).

Most of the SAR work in the GHS field has been guided by an in vitro assay based on measurement of GH release from isolated pituitary cells from rats. It became clear during the progress of our project that another assay was needed in order to certify whether particular structural changes on very potent compounds constituted genuine SAR progress. Certain compounds that were approximately equally potent in the rat pituitary assay turned out to exhibit extreme differences in potency in vivo in either rats, dogs or pigs. Often, this difference could not be accounted for by differences in PK parameters. We settled for an additional assay where a single dose was given intraveneously to pigs and followed by measurement of the subsequently induced GH peak. The dose was chosen based on full dose response curves of three reference compounds which were ipamorelin, NN703 and MK-677 (Fig. 2).[7] As can be seen from Table 1, ipamorelin and NN703 are equipotent while MK-677 is slightly more potent in the rat pituitary assay, but not significantly. But moving to an

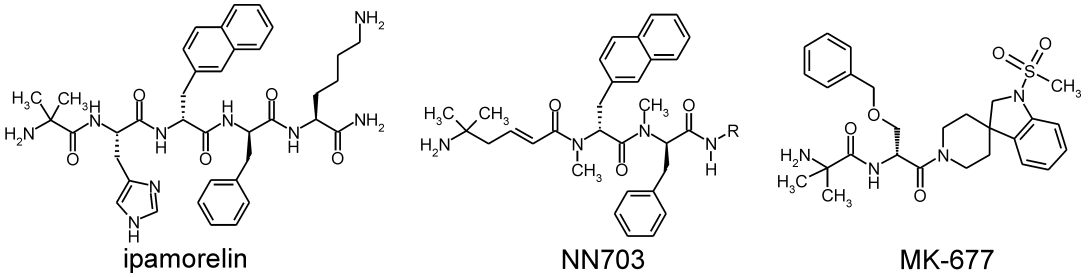

Figure 1.

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in vivo situation in pigs it can be estimated from Fig. 2 that ipamorelin has an impressive ED_{50} of 2.3 ± 0.03 nmol/kg whereas the corresponding parameters for NN703 and MK-677 are 153 ± 36 nmol/kg and 84 ± 5 ng/mL. This means that in vivo ipamorelin is about 70 times more potent than NN703, and such a large potency discrepancy seemed unlikely to be caused by pharmacokinetic differences only. MK-677 is slightly more efficacious than ipamorelin and NN703.

Although NN703 is sufficiently potent for clinical purposes we were puzzled about the discrepancy between the rat pituitary assay and the in vivo situation. This led us to modify our screening plan to include a single dose intraveneously at 50 nmol/kg to pigs ($n = 6$) in order to identify the additional pharmacophoric group that made ipamorelin highly potent in vivo, although we realized that we could jeopardize our previous strategy ('rule of five')[8] to obtain oral bioavailability. Taking the observed differences in efficacy into account, we made the assumption that compounds that could induce a GH release above 50 ng/mL at a dose of 50 nmol/kg were likely to constitute significant improvements in potency compared to NN703.

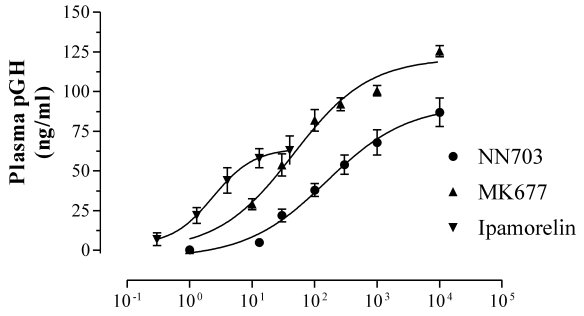
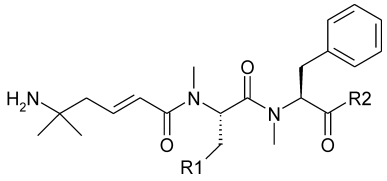


Figure 2. The release of GH from pigs after administration of various doses of ipamorelin, NN703 and MK677. The *x*-axis (doses) is logarithmic and given in mg/kg.

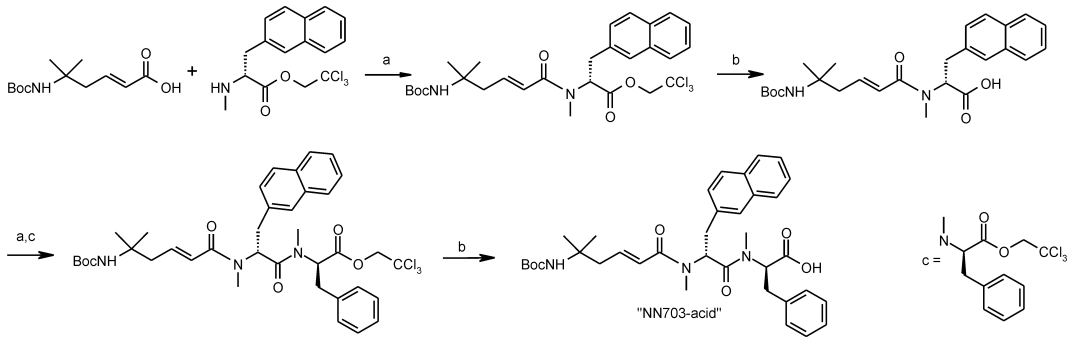
A selection of the compounds that resulted from this program is presented in Table 1. Some compounds (**1**, **9**–**15**) were prepared in solution using Boc-chemistry and HOAt/EDAC as coupling reagents. In the cases where derivatives of lysine were incorporated, the side chain was protected with Cbz during synthesis. In contrast, compounds **2**–**5** were made by solid-phase synthesis from the commercially available diamines anchored to a chlorotrityl resin and using Fmoc/HOAt/EDAC strategy. We also employed an alternative N to C approach in which we were able to prepare an 'NN703-acid'-fragment that allowed easy derivatisation of the C-terminus as depicted in Scheme 1.

The assembly of this fragment went well and in good yield using trichloroethylesters as C-terminal protection and with very little racemization (in contrast to an initial methyl ester strategy). However, the coupling of 'NN703-acid' to various amines was less reliable and often caused poor yields and side products. Compounds **6**–**8** were prepared in a different way. Mono-Boc-protected diamines were coupled stepwise using Troc-protected *N*-methyl amino acids. After removal of the C-terminal Boc-group we had access to a fragment with Troc-protection in the N-terminus that could be derivatized with acetic anhydride (i.e., **6**), pyrazolylamidine (i.e., **7**) and ethylisocyanate (i.e., **8**) and submitted to Troc-removal with Zn.

Table 1.



	R1	R2	Rat pit EC_{50} (nM)	Rat pit efficacy E_{max} (% of GHRP-6)	Pig GH_{max} (ng/mL) at 50 nmol/kg
NN703	—	—	2.7	98	22
Ipamorelin	—	—	1.3	85	54
MK677	—	—	0.6	115	60
1	Napthyl	Lys–NH_2	1.8	105	76
2	Biphenyl	$HN(CH_2)_2NH_2$	5.0	90	62
3	Biphenyl	$HN(CH_2)_3NH_2$	6.0	130	25
4	Biphenyl	$HN(CH_2)_4NH_2$	1.0	110	91
5	Biphenyl	$HN(CH_2)_6NH_2$	1.0	130	80
6	Napthyl	$HN(CH_2)_5NHAc$	10	110	26
7	Napthyl	$HN(CH_2)_5NHC(=N)NH_2$	17	140	49
8	Napthyl	$HN(CH_2)_5NHCONEt$	14	95	8
9	Napthyl	$CH_3N(CH_2)_3CONHCH_3$	30	100	32
10	Napthyl	$CH_3N(CH_2)_3CON(CH_3)_2$	21	105	21
11	Napthyl	$CH_3N(CH_2)_3N(CH_3)_2$	2.0	105	45
12	Biphenyl	$CH_3N(CH_2)_3N(CH_3)_2$	4.0	95	50
13	Napthyl	Lys–$N(CH_3)_2$	0.3	130	105
14	Biphenyl	Lys–$N(CH_3)_2$	0.15	110	116
15	Napthyl	$CH_3NCH_2CON(CH_3)_2$	10	105	48



Scheme 1. (a) HOAt, EDAC, DIEA, DCM; (b) activated Zn powder, THF, aqueous phosphate buffer pH 4.5.

There are three major differences between ipamorelin and NN703. These are: (i) the Aib-His residue in the N-terminal has been substituted with the dipeptide mimetic 5-methyl-5-amino-2-hexenoic acid; (ii) NN703 is *N*-methylated in contrast to ipamorelin; and (iii) ipamorelin has a C-terminal lysine amide that NN703 lacks. One of the first derivatives we made was adding this C-terminal to NN703 obtaining the compound **1**. This compound was almost equipotent to ipamorelin and NN703 in the in vitro rat assay but released more GH at 50 nmol/kg in the pig model than the two others. This indicated that the focus should be put on the C-terminal and we decided to reduce the lysine further. Compounds **2**–**5** contain a basic moiety as in the lysine and indicate that a rather long spacer is optimal although an ethylene spacer is quite good too. This series was carried out with biphenylalanine instead of 2-napthylalanine as previous work has shown that the two amino acids can be used arbitrarily.[8,9] Next we investigated the need for a basic moiety in the C-terminus. Compounds **6** (amide), **8** (urea), and **9**–**10** (reversed amides) are no better than NN703 whereas a basic guanidine (i.e., **7**) seems to improve potency. Compounds **11**–**12** introduced fully *N*-methylated but still basic C-terminals and these are indeed moderate improvements to NN703. Meanwhile, *N*-methylation of the Lys-amide of **1** resulted surprisingly in a major boost in potency, which was reflected in the in vitro rat assay as well as the pig assay. Thus compounds **13** and **14** were 300 and 150 pM, respectively, in the in vitro rat assay and to our knowledge the most potent GHSs ever made. Likewise in vivo, these two compounds are at least twice as efficacious at 50 nmol/kg as ipamorelin. Structurally, it is interesting and highly surprising to note that the polar dipeptide Aib-His moiety in ipamorelin can easily be replaced by the dipeptidomimetic 5-methyl-5-amino-2-hexenoic acid and with a few additional methylations obtain a dramatic increase in potency. Removing the lysine side chain leaving a glycine amide as in **15** caused a 4–5-fold loss of potency on the in vitro rat assay compared to NN703 but this compound was good in the in vivo assay, indicating the pitfall in having too low in vitro cut-off levels for in vivo screening. Quite often, differences in the in vivo activity between in vitro equipotent compounds can be explained by different pharmacokinetic parameters (i.e., lower volume of distribution results in higher plasma concentration which again may result in better potency) and in this case it is likely that the improved in vivo potency of ipamorelin compared to NN703 is partly due to a lower volume of distribution, simply caused by the higher polarity of ipamorelin. Whether the differences in the in vivo activity of this series are caused by pharmacokinetic parameters or simply better affinity to the involved receptor in vivo was outside the scope of this study to explain. Since this reported series was part of an extensive program,[8–11] which did lead to improved NN703 analogues, only a few of the compounds in the series we report here were evaluated for oral bioavailability and none of them showed sufficient oral bioavailability (cut-off limit: fpo $>20\%$) to continue in our program. In conclusion, we believe we have shown that with minor modifications it is possible to improve the in vivo potency of NN703 dramatically without necessarily improving the in vitro potency. In addition, we have shown with the hybrid between ipamorelin and NN703, namely the highly in vivo potent compound **1**, that the N-terminal 5-methyl-5-amino-2-hexenoic acid can fully replace the Aib-His moiety.

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