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An imidazole based H-Phe-Phe-NH_2 peptidomimetic with anti-allodynic effect in spared nerve injury mice

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ABSTRACT

The dipeptide amide H-Phe-Phe-NH_2 (**1**) that previously was identified as a ligand for the substance P 1–7 (SP_{1-7}) binding site exerts intriguing results in animal models of neuropathic pain after central but not after peripheral administration. The dipeptide **1** is derived from stepwise modifications of the anti-nociceptive heptapeptide SP_{1-7} and the tetrapeptide endomorphin-2 that is also binding to the SP_{1-7} site. We herein report a strong anti-allodynic effect of a new H-Phe-Phe-NH_2 peptidomimetic (**4**) comprising an imidazole ring as a bioisosteric element, in the spare nerve injury (SNI) mice model after peripheral administration. Peptidomimetic **4** was stable in plasma, displayed a fair membrane permeability and a favorable neurotoxic profile. Moreover, the effective dose (ED_{50}) of **4** was superior as compared to gabapentin and morphine that are used in clinic.

The neuropeptide substance P (SP, H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) that acts through the G-protein coupled neurokinin-1 receptor, is an important neurotransmitter and neuromodulator of pain signaling in the nervous system.[1] As other tachykinins, the C-terminal part accounts for the signal transduction at the receptor, whereas the *N*-terminal part of the undecapeptide is important for receptor subtype selectivity.[2] SP coexists in neurons with classic transmitters and is after release subjected to enzymatic cleavage, which results in fragments, some with very diverse bioactivities.[3–5] The *N*-terminal fragment, substance P 1–7 (SP_{1-7}, Fig. 1) has attracted most attention owing to its biological effects that oppose those elicited by the parent peptide with regard to nociception as well as opioid tolerance and withdrawal.[6–9] Our interest in this heptapeptide was reinforced owing to its anti-hyperalgesic and anti-allodynic effects in animal models of neuropathic pain,[10–12] and this prompted us to commence a medicinal chemistry program aimed at converting the heptapeptide into drug-like peptidomimetics.

Neuropathic pain affects 7–10% of the general population and is a complex clinical condition that can arise as a result of several underlying etiologies such as diabetes mellitus, *Herpes zoster* infection, multiple sclerosis (MS) or spinal cord injury (SCI).[13,14] Current first-line treatment includes tricyclic antidepressants (TCA), serotonin and noradrenaline reuptake inhibitors (SNRI) and anticonvulsants like gabapentin and pregabalin, all hampered by CNS side effects.[15] Gabapentin is commonly used in clinic and is in comparison relatively safe despite the high doses needed *vide infra* to combat neuropathic pain.[16] Since opioids are addictive and associated with burdensome side effects like constipation, they are considered only as third line treatment. Morphine is a representative example of opioids used in clinic and is administrated for severe nociceptive pain, which also provides effectiveness in short-term neuropathic pain relief.[17,18] In light of this, safe pharmacological treatments that more efficiently target other sites in the neuropathic pain pathways are of interest as future drug candidates.

Several observations suggest that SP_{1-7} binds to a unique binding site, probably G-protein dependent and distinct from the neurokinin receptors or other receptors associated with pain such as opioid receptors.[19,20] In line with this, the binding is specific, saturable, and reversible. However, it is possible that the heptapeptide elicits its action by interfering with allosteric sites present on other neuropeptide receptors.[3] It is notable that the μ-receptor agonist endomorphin-2 (EM-2,

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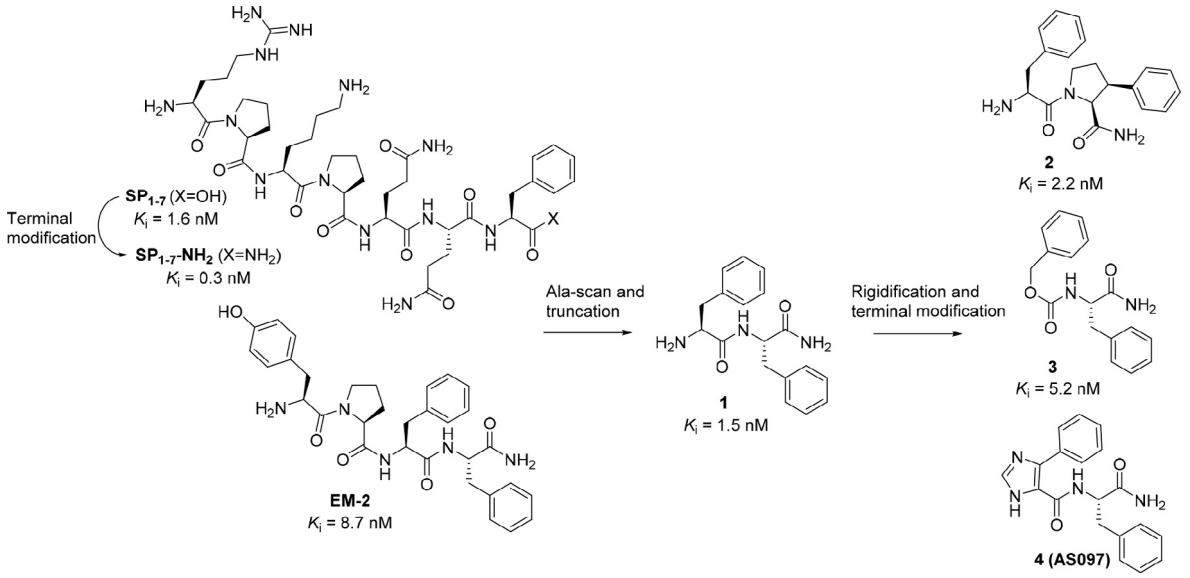

Fig. 1. Lead compound **1** derived from SAR studies of SP_{1-7} and EM-2 along with three rigidified and terminal modified analogues **2**, **3** and **4**. Compounds evaluated in a displacement binding assay using spinal cord membrane from rats and radioactive [^{3}H]SP_{1-7} tracer.

Fig. 1), but not EM-1, exhibited high affinity for the SP_{1-7} binding site.[20,21]

In our ongoing medicinal chemistry program, we aimed at developing pharmacological tools for in depth studies of the SP_{1-7} system but also employing SP_{1-7} as starting point in a design process with the long term goal to discover new chemical entities as potential new therapeutics useful for treatment of neuropathic pain. Important anchor points of SP_{1-7} and EM-2 were identified through a structure activity relationship (SAR) study.[22,23] The extensive SAR study revealed that a C-terminal primary amide is improving the binding affinity and, hence, resulted in the identification of SP_{1-7}-NH_2 with a K_i of 0.3 nM (Fig. 1). *N*-terminal truncation of SP_{1-7}-NH_2 and EM-2 along with an alanine scan led to the remarkable discovery of the dipeptide lead compound **1** (H-Phe-Phe-NH_2) with equipotent affinity (K_i = 1.5 nM) as the SP_{1-7} itself (Fig. 1).

A pharmacophore hypothesis was proposed for **1** where (*S,S*) configuration of the side-chains, primary amine in the N-terminal and primary amide in the C-terminal were essential features for binding.[24] The *in vivo* effect of **1** was further evaluated in a diabetic neuropathy model induced by streptozotocin (STZ). Following central (intrathecal, i.t.) administration **1** produced potent anti-allodynic and anti-hyperalgesic effect at a 0.5–4 pmol dosage range.[25] However, when evaluated in mice suffering from spared nerve injury (SNI) after peripheral (intraperitoneal, i.p.) administration of a dose at 185 nmol/kg, the dipeptide amide **1** failed to reach any distinct anti-allodynic effect.[12] This observation is probably due to low brain exposure since **1** displayed poor drug-like properties such as poor plasma stability, high hepatic metabolism and low permeability and stability over Caco-2 cells.[24]

With the aim to develop drug-like analogues to **1** acting as the neuropeptide SP_{1-7}, compound **2** and **3** were designed (Fig. 1).[24,26] Both compounds exhibited retained binding *in vitro* along with improved metabolic stability and permeability. When evaluated *in vivo* in the SNI model, the rigidified compound **2** displayed a strong anti-allodynic effect. The effect, however, was short-lasting with a peak of effect after 30 min, which may be attributed to low brain exposure due to high blood-brain barrier (BBB) efflux as indicated *in vitro* by Caco-2 cells measurements.[12] The carbamate **3** on the other hand, displayed no *in vitro* efflux over the Caco-2 monolayer and was shown to enter the CNS according to an *in vivo* infusion study but failed to exhibit any anti-allodynic effect in the SNI mice, possibly as a results of fast hydrolysis of the carbamate functionality in plasma.[12,26]

Herein, as an alternative to the primary amine in **2** and the carbamate in **3**, compound **4** was designed and prepared, comprising an C5 phenyl substituted imidazole carboxamide in the *N*-terminal (Fig. 1).[27] This phenylalanine isostere match both the side chain phenyl and contains nitrogen atoms that can act as hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and sites for positive charges, depending on tautomeric state and pH. The assessment of *in vitro* plasma stability and permeability as well as its *in vitro* cytotoxic profile in primary neuronal cell cultures of the new imidazole based H-Phe-Phe-NH_2 peptidomimetic **4** is presented. Furthermore, the *in vivo* anti-allodynic potential of the compound in an SNI model of neuropathic pain after peripheral administration (i.p.) was assessed. Moreover, to confront its action with the SP_{1-7}-NH_2 peptide and with gabapentin and morphine used in clinic the activity of the four compounds were compared in the SNI model.

The introduction of the *ortho*-substituted imidazole moiety in **4** complies with a suitable atom-to-atom match to the *N*-terminal phenylalanine residue in **1** (Fig. 2). In order to investigate if **1** and **4** can obtain similar spatial arrangement of their potential pharmacophore groups in low energy conformations, a pharmacophore group alignment analysis was performed using Phase.[28] Conformational analysis of **1** and **4** resulted in 69 and 76 conformations, respectively, within 21 kJ mol^{-1} of the lowest energy conformation found, which were included in the pharmacophore group alignment analysis. Requirement of matching all the mutual potential pharmacophore groups identified by Phase (three HBD, two HBA, and two aromatic rings), produced six clusters of pharmacophore group match hypotheses. The match with the lowest energy conformations (ΔE = 3.6 kJ mol^{-1} and 7.8 kJ mol^{-1} from the lowest energy conformation found for **4** and **1**, respectively) is presented in Fig. 2 and shows a very good overlap of the mutual structural features that can be of importance for target-ligand interactions. Taken together, the analysis show that compounds **4** and **1** indeed can obtain a similar spatial arrangement of their potential pharmacophore groups in low energy conformations. Albeit the rigidified **4** should not be able to cover all spatial arrangements of the more flexible **1**, the analysis supports that the *ortho*-substituted imidazole can act as a suitable mimic of the *N*-terminal phenylalanine residue in **1.**

The synthesis of **4** was performed adopting a previously described method[27] and is outlined in Scheme 1. The Pd-catalyzed C5 arylation of 1-benzyl-1*H*-imidazole was conducted under microwave irradiation for 1 h at 160 °C using bromobenzene, $Pd(OAc)_2$, $P(2\text{-furyl})_3$, pivalic acid