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Biphalin analogs containing β³-*homo*-amino acids at the 4,4′ positions: Synthesis and opioid activity profiles

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ABSTRACT

Biphalin, a synthetic opioid octapeptide with a palindromic sequence has high analgesic activity. Biphalin displays a strong affinity for μ and δ-opioid receptors, and a significant to κ-receptor. The paper reports the synthesis of novel analogs of biphalin containing β³-*homo*-amino acid residues at the 4,4′ positions and a hydrazine or 1,2-phenylenediamine linker. The potency and selectivity of the peptides were evaluated by a competitive receptor-binding assay in rat brain homogenate using [³H]DAMGO (a μ ligand) and [³H]DELT (a δ ligand). Analogs with β³-*h*-*p*-NO_2Phe in positions 4 and 4′ are the most active compounds. Selectivity depends on the degree of freedom between the two pharmacophore moieties. Analogs with a hydrazine linker show noticeable binding selectivity to μ receptors ($IC_{50}{}^{\mu}$ = 0.72 nM; $IC_{50}{}^{\delta}$ = 4.66 nM), while the peptides with a 1,2-phenylenediamine linker show slight δ selectivity ($IC_{50}{}^{\mu}$ = 10.97 nM; $IC_{50}{}^{\delta}$ = 1.99 nM). Tyr-D-Ala-Gly-β³-*h*-*p*-NO_2PheNHNH-β³-*h*-*p*-NO_2Phe (1) and (Tyr-D-Ala-Gly-β³-*h*-*p*-NO_2PheNH)$_2$ (2) produced greater antinociceptive effect compared to morphine after i.t. administration.

1. Introduction

Since the discovery of enkephalins in 1975 [12] thousands of opioid analogs have been synthesized to understand the relationship between their conformation and bioactivity, and to improve their pharmacological profile. Leu-enkephalin and Met-enkephalin are involved in many physiological processes, their roles in behavior, neuroendocrinology and pain transmission being well documented [15,32]. The use of enkephalins in pain treatment has been limited by the lack of metabolic stability and bioavailability [2]. Several chemical approaches, such as the incorporation of D-amino acids, unusual amino acids [1], cyclic moieties [9] or cyclization [33] of peptides have resulted in more stable enkephalin analogs. Among the numerous strategies of modification, the substitution of proteinogenic amino acids with β-amino acids represents an interesting possibility [14]. In the process of searching for new opioid analgesics with two active elements in one molecule, a dimeric enkephalin analog – biphalin, (Fig. 1), was synthesized [21].

Biphalin has been found to exhibit unique properties as it is 257 and 6.7 times more potent than morphine (a reference μ-agonist) and etorphine (a ultrapotent opioid agonist), respectively, in eliciting antinociception when administrated intracerebroventricularly [11]. Suzuki and coworkers demonstrated lower risk of developing physical dependence on biphalin following chronic biphalin infusion in rats, in contrast to morphine [38]. Furthermore, Singh and coworkers reported that biphalin and its analogs [Tyr-D-Ala-Gly-Phe-NH-NH-Phe(p-Cl)-H] stimulate human T cell proliferation, natural killer (NK) cell cytotoxicity *in vitro* and interleukin-2 (IL-2) production [23].

The minimal fragment necessary to express equal affinities and the same biological activity profile as biphalin is the analog Tyr-D-Ala-Gly-Phe-NH-NH-Phe [22]. Biphalin analogs cover the modification of the 4,4′ residues [19,25] and hydrazine linkers [29,31]. Cyclic analogs containing a disulphide bridge have also been synthesized [26,28]. Analogs containing non-hydrazine linkers (1,4-phenylenediamine, 1,2-phenylenediamine or piperazine) have shown good binding affinity and bioactivity, which are comparable to biphalin or higher [6].

In recent years, much attention has been focused on various β-amino acids and β-peptides. Investigations include the synthesis of α,β-hybrids and β-peptides [5]. The substitution of α-amino acids with their β-isomers in biologically active peptides may result in

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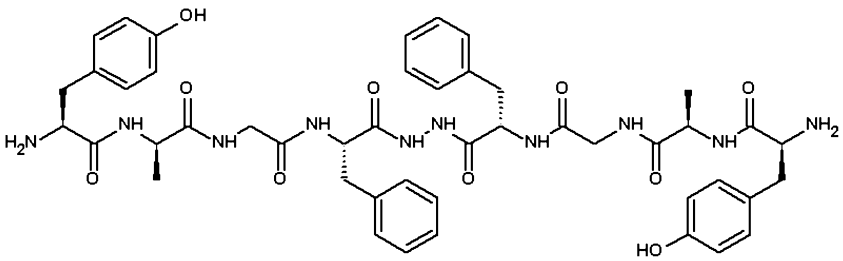


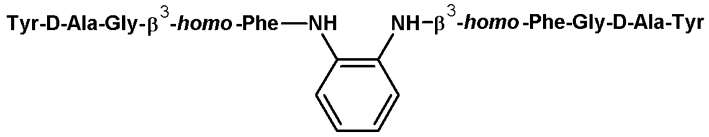
Fig. 1. Biphalin.

Tyr-D-Ala-Gly-β^3-*homo*-p-NO$_2$-Phe-NH-NH-β^3-*homo*-p-NO$_2$-Phe

1

Tyr-D-Ala-Gly-β^3-*homo*-p-NO$_2$-Phe-NH-NH-β^3-*homo*-p-NO$_2$-Phe-Gly-D-Ala-Tyr

2



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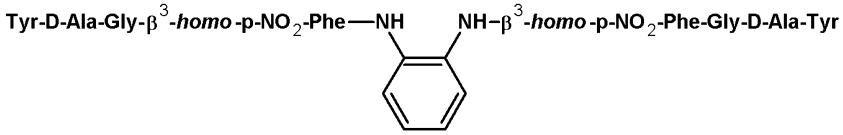

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Fig. 2. Structure of biphalin analogs containing β^3-*homo*-amino acids.

increased enzymatic stability [10] and also in a strong influence on peptide conformation [13]. Previously, we described the conformational and metabolic consequences of the substitution of Phe with β^3-*homo*-Phe in positions 3 or 4 and *N*-Me-β^3-*h*-Phe or β^3-*h*-Tic in position 3 of TAPP [36].

In this paper, we present the synthesis and the effects on receptor binding of new α,β-hybrids of biphalin, modified in positions 4,4′ with β^3-*homo*-amino acids containing hydrazine and 1,2-phenylenediamine linkers (**1**–**4**) (Fig. 2). Investigations of the influence of amino acid residues in positions 4,4′ on the potency and selectivity of biphalin have shown that di(*p*-nitrophenylalanine)biphalin is a more active and δ-selective analog than biphalin [25].

Previously, we synthesized an analog containing β^3-*homo*-tyrosine in positions 1, and β^3-*homo*-phenylalanine in positions 4,4′ as well as two β^3-*homo*-amino acid residues in positions 1,1′ and 3,3′ (β^3-*h*-Tyr-D-Ala-β^3-*h*-Phe-NH-NH-β^3-*h*-Phe-D-Ala-β^3-*h*-Tyr), without a glycine residue to keep a similar distance between aromatic rings [8]. Analogs modified with β^3-*homo*-amino acids were published recently by Mollica et al. [30].

2. Material and methods

Peptide synthesis

Protected *N*-Boc-α-amino acid derivatives were purchased from Fluka AG. *N*-Protected β^3-*homo*-amino acids were synthesized using the procedures reported in the literature, with enantiomerically pure *N*-protected α-amino acids as starting materials. Optically pure isomeric Boc-β^3-*homo*-amino acids were prepared in two-step *Arndt–Eistert* homologation of *N*-protected amino acids [16,20,34]. *p*-Nitrophenylalanine was obtained *via* nitration of phenylalanine [4].

Analogs **1–4**, containing 1,2-phenylenediamine or hydrazine bridge, were synthesized using a convergent synthetic strategy (Scheme 1).

The tripeptide precursor was obtained by stepwise elongation starting from glycine methyl ester (Scheme 1a). The Boc group was removed by 4 N HCl/AcOEt and the TBTU method was used throughout to give Boc-Tyr(Boc)-D-Ala-Gly-OMe. The methyl ester group was hydrolyzed with 1 N NaOH. The *N*-protected bridged dimers were synthesized by one-pot cross-coupling reaction of hydrazine or 1,2-phenylenediamine (1 equiv), Boc-β^3-*h*-Phe(R) (R = H or NO$_2$) (2 equiv), and HOBt•H$_2$O/EDC/TEA in DMF. Cross coupling reactions were conducted between unprotected bridged dimers (1 equiv) and Boc-Tyr(Boc)-D-Ala-Gly-OH (2 equiv for analogs **2**–**4** or 1 equiv for **1**) to yield the corresponding *N,O*-Boc protected biphalin analogs.

The Boc-protected peptides (**1**–**4**) were deprotected with 90% TFA/H$_2$O and purified on a preparative RP-HPLC column. The purity of the final TFA salts of analogs **1**–**4** was assessed by analytical RP-HPLC and FAB-MS.

Receptor binding

New α/β-peptides, analogs of biphalin were tested for μ and δ-opioid receptors affinity. Receptor binding assays were performed as described previously. Rat membrane preparation followed the procedure described by Misicka et al. [24]. The radioligand receptor binding protocol was based on a study performed by Fichna