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Original article

Hybrid peptides endomorphin-2/DAMGO: Design, synthesis and biological evaluation

Adriano Mollica [a,*], Roberto Costante [a], Azzurra Stefanucci [a], Francesco Pinnen [a], Grazia Luisi [a], Stefano Pieretti [b], Anna Borsodi [c], Engin Bojnik [c], Sándor Benyhe [c]

[a] Dipartimento di Farmacia, Università degli Studi "G. d'Annunzio" di Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy
[b] Istituto superiore di Sanità, Dipartimento del Farmaco, Viale Regina Elena 299, 00161 Rome, Italy
[c] Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Temesvari krt 62, 6726 Szeged, Hungary

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ABSTRACT

Endomorphin-2 [Tyr-Pro-Phe-Phe-NH_2] and DAMGO [Tyr-D-Ala-Gly-(*N*–Me)Phe-Gly-ol] are natural (EM2) and synthetic (DAMGO) opioid peptides both selective for μ opioid receptor with high analgesic activity. In this work we report synthesis, *in vitro* and *in vivo* biological evaluation of five new hybrid EM2/DAMGO analogues, with the aim to obtain compounds with high affinity at μ-opioid receptor, high activity in animal nociception tests (hot plate and tail flick) and improved enzymatic stability. Double *N*-methylation on both Phe residues and C-terminal ethanolamide led to analogue **6e**, which possesses a good *in vitro* μ affinity (K_i^{μ} = 34 nM), combined with a remarkable *in vivo* antinociceptive activity.

1. Introduction

Morphine and other opioid drugs are the most used analgesics for the relief of moderate and severe pain [1]. However, prolonged use of these compounds leads to a well-known range of side-effects, including tolerance, physical dependence and respiratory depression [2,3].

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2, EM1) and Endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2, EM2) are endogenous μ-selective opioid peptides originally isolated from bovine brain [4] and subsequently from human brain cortex [5] by Zadina and Hackler. Endomorphins (EMs) present a unique *N*-terminal sequence Tyr-Pro-Trp/Phe structurally related to opioid peptide morphiceptin [6,7], different from the typical *N*-terminal sequence Tyr-Gly-Gly-Phe of other opioid peptides, such as enkephalins and dynorphins [8]. This structural features deeply influence the activity, hence EMs label μ opioid receptor (MOR) with a high affinity and selectivity compared to a poor affinity for δ and κ receptors [2,9–12]. Endomorphins exhibit potent *in vivo* antinociceptive activity, which duration depends on the species, pain tests applied, and administration route [13–16]. In contrast to other opioids, including morphine, EMs could also be effective in treatment of neuropathic pain [17,18].

According to the *message/address* concept [19], endomorphins' structure can be divided into two parts: the *N*-terminal sequence Tyr-Pro-Trp/Phe, which represents the *message* sequence and provides the correct conformation in the receptor binding and the Phe^4-NH_2 moiety, which is the *address* sequence which contributes to peptide stability and selectivity [20–22]; the Pro^2 residue acts as stereochemical spacer, inducing the correct orientation of the other residues necessary for receptor–ligand interaction [23–26]. The Tyr^1-Pro^2 amide bond exists in an equilibrium mixture of *cis*/*trans* conformations [27]; the isomerization around this bond seems to be a crucial factor to control π–π interaction between the three aromatic rings, determining the preferred EM conformation [23,28,29]. Keller et al. demonstrated that the bioactive conformation is *cis* [30], after estimation of *cis*/*trans* EMs ratios in various conditions [20–23,25].

* Corresponding author. Tel.: +39 08713554476; fax: +39 08713554470.
E-mail address: a.mollica@unich.it (A. Mollica).

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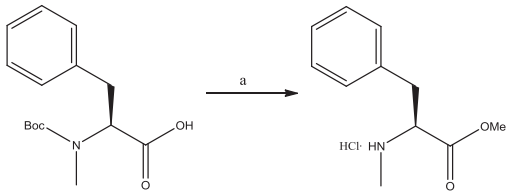
Endomorphin-related ligands show favorable profiles in terms of analgesia and tolerance/dependence, and seem to produce less respiratory depression than other agonists [31], nevertheless the susceptibility to enzymatic degradation [32,33] and a limited uptake in the central nervous system of opioid peptides strongly reduces a possible therapeutic application [34].

To obtain more stable and efficacious μ selective agonists, several chemical modifications of EMs were performed, and a large number of products were synthesized [16,35–46].

DAMGO [Tyr-D-Ala-Gly-(*N*-Me)Phe-Gly-ol] is a highly μ selective opioid peptide, synthetized for the first time in 1980 [47]. Its structural features are different from those of endomorphins being more similar to enkephalins, in fact Tyr^1 is conserved, $D\text{-}Ala^2$ was introduced in place of Gly, while *N*-methyl Phe in position 3 and the *C*-terminus ethanolamine represent the distinctive features. In *mouse writhing nociception test*, DAMGO was more than 100-fold potent than morphine [47]. In addition, DAMGO displays an increased enzymatic stability compared to other opioid peptides. These characteristics make DAMGO excellent as radiolabeled ligand for μ receptors for *in vitro* binding assays, used as a standard [48,49].

In this work we report synthesis, *in vitro* and *in vivo* biological evaluation of five new hybrid EM2/DAMGO analogues (Fig. 1). The aim of this project is to combine the distinctive features of EMs and DAMGO in order to obtain compounds with a remarkable affinity at μ-opoid receptor, high activity in animal nociception tests (hot plate and tail flick), together with an improved resistance toward enzymatic degradation due to the presence of *N*-methylated residues as previously reported [50–52]. In this paper the typical EM Pro^2 residues have been maintained, as well as the DAMGO *C*-terminal ethanolamine. *N*-methylation was performed on the Phe^3 and Phe^4 residues, with the exception for compound **6d**, in which Sarcosine (*N*–Me Gly) was used in place of Phe^3.



1a

Scheme 1. Synthesis of intermediate **1a**. Reagents and conditions: (a) $SOCl_2$, MeOH.

2. Chemistry

All products were synthesized in solution phase [53]. For compounds **6a** and **6e**, HCl·(*N*–Me)Phe-OMe was obtained starting from commercially available Boc-(*N*–Me)Phe-OH by treatment with $SOCl_2$ in MeOH for 3 h at r.t. (Scheme 1) [54]. Coupling reactions were performed using EDC/HOBt/DIPEA in DMF, with the exception of compound **6e**, for which Bop-Cl/NMM in DMF were used in the first two couplings. Deprotection of N^{α}-*tert*-butyloxycarbonyl group was performed using 1:1 TFA/CH_2Cl_2 mixture for 1 h, under N_2 atmosphere (Scheme 2).

3. Results and discussion

The *in vitro* biological evaluation of novel EM2-DAMGO analogues **6a**–**e** was performed as previously described [55–57] and results are shown in Table 1. The binding affinity of the hybrid peptides **6a**–**e** toward rat δ- and rat μ-opioid receptors was determined by competitive binding against [^{3}H]Ile^{5,6}deltorphin-II [58] and [^{3}H]DAMGO [49] respectively. For functional characterization

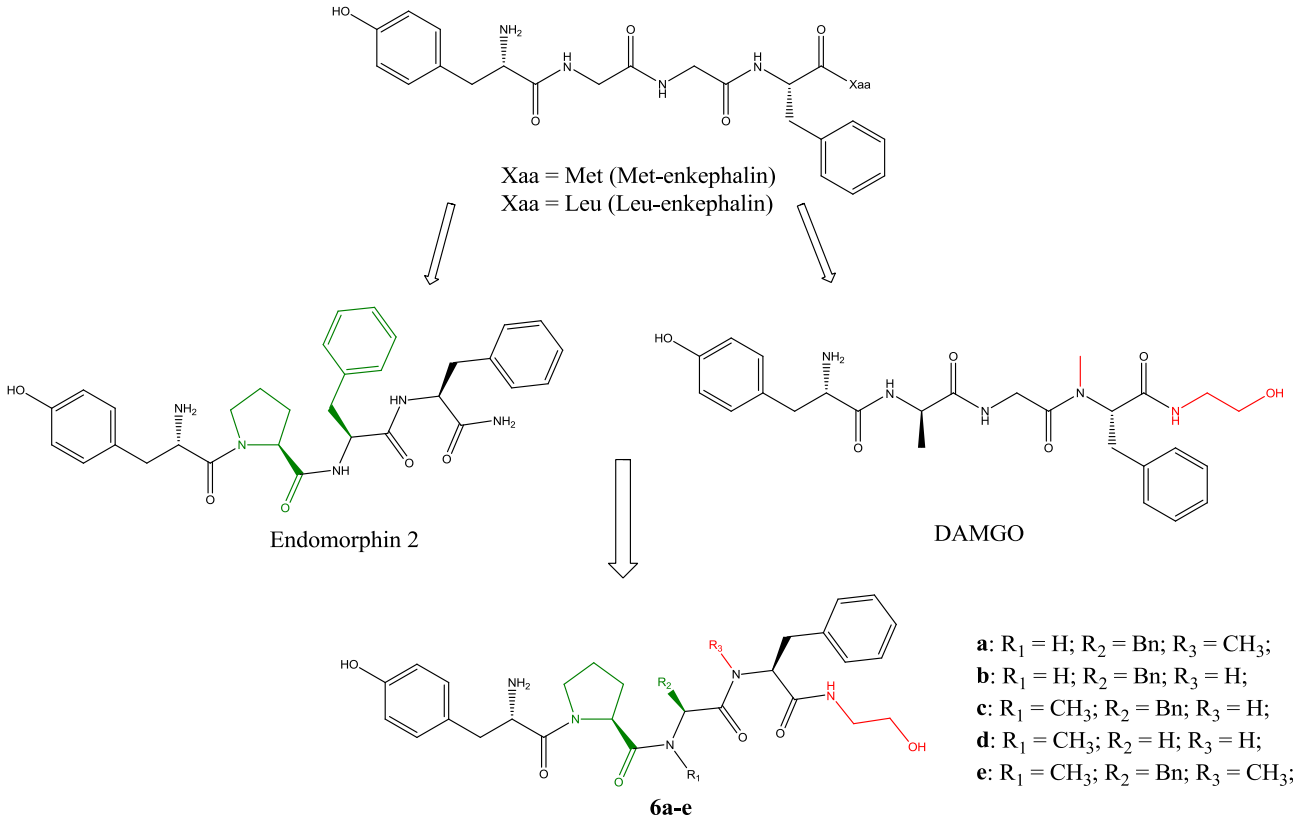


Fig. 1. Structures of Met/Leu-enkephalins, Endomorphin-2, DAMGO and analogues **6a**–**e**.