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Endomorphin analogues with balanced affinity for both μ- and δ-opioid receptors

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Abstract

Analogues of endomorphin and tripeptides modified at positions 4 and 3, respectively, with various phenylalanine analogues were synthesized and their affinities for opioid receptors were evaluated. Most of the peptides exhibited potent μ-receptor affinity and selectivity, among them, compound **7** (Dmt-Pro-Tmp-Tmp-NH_2) exhibited potent affinity for both μ- and δ-receptors ($K_i\mu$ = 0.47 nmol/L, $K_i\delta$ = 1.63 nmol/L).

Keywords: Endomorphin; Opioid; Analgesics

Morphine, fentanyl, and pethidine are the most effective and most commonly prescribed analgesics among the analgesics used in clinic, but their prolonged administration is greatly limited due to the side effects of tolerance, dependence, respiratory inhibition, and constipation. Therefore, developing analgesics with less adverse action has long been the target of medicine scientists [1]. The endomorphins (EM), endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2, EM-1) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2, EM-2) are putative endogenous opioids with high affinity and remarkable selectivity for the μ-opioid receptor [2]. Since EM exerts analgesic effect through action on the μ-opioid receptor and express similar pharmacological properties as morphine, they exhibit fewer side effects. Extensive medicinal chemistry investigation has been performed in order to obtain compounds with improved affinity, selectivity, bioactivity, metabolic stability, and to induce a new bioactive profile [3]. For example, we previously reported that substitution of Tyr in the EM-2 sequence with 2′,6′-dimethyl-L-tyrosine (Dmt) greatly increased EM affinity and bioactivity [4] and *N*-allyl-Dmt converted an agonist to a potent antagonist which inhibited alcohol suppression of GABA mediated neurotransmission in the hippocampus [5]. The introduction of alkyl modified Phe analogues, such as 2′,6′-dimethyl-L-phenylalanine (Dmp), 2′,4′,6′-trimethyl-L-phenylalanine (Tmp), and Dmt at the third position of EM-2 resulted in opioids with mixed μ-agonist and δ-antagonist properties, which are expected to show low tolerance and dependence [6]. In keeping with our continuous interests on the investigation of the

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structure–activity relationship of [Dmt^1]EM-2, we report herein the preliminary results of two series of endomorphin analogues, one is modified at the fourth position of [Dmt^1]EM-2 with various Phe analogues (Tmp, Dmp, Dmt, Trp, 1-Nal (1-naphthylalanine), and 2-Nal (2-naphthylalanine)), and the other one consists of the Dmt-Pro-Phe-NH_2 analogues modified at the third position of Phe with the same modified Phe residues.

1. Results and discussion

The unnatural amino acids Dmt [4], Dmp and Tmp [7] were prepared as reported method through [Rh(1,5-COD)(*R*,*R*-DIPAMP)]BF_4 mediated asymmetric catalytic hydrogenation of the corresponding acetamidoacrylate. All the peptides were synthesized by segment condensation method in solution. Peptides **1**–**6** were formed by coupling Boc-Dmt-Pro-Phe-COOH with amides of corresponding amino acids using PyBop as coupling reagent. Peptides **8**–**12** were prepared from the coupling of Boc-Dmt-Pro-COOH with amides of corresponding amino acids, and peptide **7** was prepared by coupling Boc-Dmt-Pro-COOH with Tmp-Tmp-NH_2. The final Boc protecting group was removed with TFA in the presence of anisole, and the resulting peptide was purified by semipreparative RP-HPLC. The identification of the final compounds was verified using MS, and the purity of the peptides was determined by analytical HPLC. The final compounds exhibited greater than 98% purity were used for biological assay. Some analytical data of the final compounds are summarized in Table 1.

The μ- and δ-opioid receptor affinities of the synthesized peptides were determined by competitive displacement assay using brain P_2 synaptosomal membranes prepared from Sprague–Dawley rats [5,6], [^{3}H]DAMGO and [^{3}H]deltorphin-II were used for labeling μ- and δ-receptor, respectively (Table 2). EM-2 is one of the opioids isolated from mammalian brain that shows a high affinity and selectivity for μ-opioid receptor ($K_i\mu$ = 1.33 nmol/L, $K_i\delta$ = 6085 nmol/L, $K_i\delta/K_i\mu$ = 4575) [2]. While substitution of the first amino acid Tyr with Dmt, [Dmt^1]EM-2, improves the affinity of EM-2 for both μ- and δ-receptor, it greatly enhanced its affinity for δ-receptor ($K_i\mu$ = 0.26 nmol/L, $K_i\delta$ = 99.2 nmol/L, $K_i\delta/K_i\mu$ = 382) [4]. Previously, we reported that introduction of alkyl substituted Phe analogue Tmp and Dmp, and Dmt to the third position of [Dmt^1]EM-2 to give Dmt-Pro-[Tmp/Dmp/Dmt]-Phe-NH_2 with slightly improved affinities for μ-receptor and with profoundly enhanced affinity for δ-receptor, for example, [Dmt^1,Tmp^3]EM-2 has $K_i\mu$ = 0.18 nmol/L and $K_i\delta$ = 1.83 nmol/L [6]. In contrast, when the same amino acids Tmp, Dmp, Dmt, and other aromatic amino acids, such as Trp, 1-Nal, and 2-Nal, were introduced to the fourth position of [Dmt^1]EM-2 resulted in compounds **1**–**6**, respectively. Their affinities for the μ-receptor, in general, were nearly unchanged (**1**–**5**, $K_i\mu$ = 0.28–0.37 nmol/L) except **6** that decreased by threefold compared with the parent compound [Dmt^1]EM-2 ($K_i\mu$ = 0.28 nmol/L).

In regard to the δ-receptor, increased lipophilicity at the forth position amino acid (**1**–**6**) enhanced opioid's affinity for δ-receptor. Of Phe surrogates, Tmp (**2**), Trp (**4**), 2-Nal (**6**) had the most potent affinity ($K_i\delta$ = 23.2–25.4 nmol/L).

Table 1
Analytical data of endomorphin-2 analogues.

Compd.	Peptide	TLC	TOF mass [M+1]		HPLC (min)
		R_f[a]	Calcd.	Found	t_R[b]
1	H-Dmt-Pro-Phe-Dmp-NH_2	0.83	627	628	11.01
2	H-Dmt-Pro-Phe-Tmp-NH_2	0.85	641	642	11.68
3	H-Dmt-Pro-Phe-Dmt-NH_2	0.81	643	644	9.57
4	H-Dmt-Pro-Phe-Trp-NH_2	0.78	638	639	12.67
5	H-Dmt-Pro-Phe-1-Nal-NH_2	0.82	649	650	13.72
6	H-Dmt-Pro-Tmp-2-Nal-NH_2	0.82	649	650	13.74
7	H-Dmt-Pro-Tmp-Tmp-NH_2	0.85	683	684	12.75
8	H-Dmt-Pro-Dmp-NH_2	0.76	480	481	9.50
9	H-Dmt-Pro-Tmp-NH_2	0.74	494	495	10.05
10	H-Dmt-Pro-Trp-NH_2	0.72	491	492	9.24
11	H-Dmt-Pro-1-Nal-NH_2	0.75	502	503	10.12
12	H-Dmt-Pro-2-Nal-NH_2	0.75	502	503	10.30

[a] Solvent: *n*-BuOH/AcOH/H_2O = 4:1:1.
[b] HPLC elution on a Cosmosil C_{18} column (4.6 mm × 250 mm, 5 μm) using the solvent system of 0.05% (v/v) TFA in water (A) and 0.05% (v/v) TFA in CH_3CN (B) and a linear gradient of 10–90% solvent B over 20 min at a flow rate of 1.2 mL/min.

Table 2
Receptor affinity of endomorphin-2 analogues.

Compd.	Peptide	$K_i\mu$ nmol/L ± SE (n)[a]	$K_i\delta$ nmol/L ± SE (n)[b]	$K_i\mu/K_i\delta$
1	H-Dmt-Pro-Phe-Dmp-NH_2	0.31 ± 0.07 (3)	40.0 ± 4.4 (4)	129
2	H-Dmt-Pro-Phe-Tmp-NH_2	0.37 ± 0.02 (3)	24.5 ± 2.3 (3)	66
3	H-Dmt-Pro-Phe-Dmt-NH_2	0.28 ± 0.02 (3)	87.2 ± 6.7 (4)	311
4	H-Dmt-Pro-Phe-Trp-NH_2	0.34 ± 0.03 (3)	25.4 ± 3.2 (3)	59
5	H-Dmt-Pro-Phe-1-Nal-NH_2	0.28 ± 0.02 (3)	51.7 ± 6.3 (4)	185
6	H-Dmt-Pro-Phe-2-Nal-NH_2	0.81 ± 0.15 (4)	23.2 ± 2.4 (3)	29
7	H-Dmt-Pro-Tmp-Tmp-NH_2	0.47 ± 0.07 (3)	1.63 ± 0.2 (4)	3.5
	H-Tyr-Pro-Phe-Phe-NH_2 (EM-2)[c]	1.33	6085	4575
	H-Dmt-Pro-Phe-Phe-NH_2 ([Dmt^1]EM-2)[c]	0.26	99.2	382
	H-Dmt-Pro-Tmp-Phe-NH_2 ([Dmt^1,Tmp^3]EM-2)[c]	0.18	1.83	10
8	H-Dmt-Pro-Dmp-NH_2	0.15 ± 0.03 (4)	33.0 ± 7.1 (4)	220
9	H-Dmt-Pro-Tmp-NH_2	0.13 ± 0.03 (4)	13.1 ± 1.7 (4)	101
10	H-Dmt-Pro-Trp-NH_2	0.21 ± 0.02 (3)	66.4 ± 7.1 (3)	316
11	H-Dmt-Pro-1-Nal-NH_2	0.70 ± 0.08 (4)	120.8 ± 10.2 (4)	173
12	H-Dmt-Pro-2-Nal-NH_2	16.9 ± 3.1 (4)	68.3 ± 9.9 (4)	4
	H-Dmt-Pro-Phe-NH_2[d]	0.12	53.2	170

[a] Versus [^{3}H]DAMGO.
[b] Versus [^{3}H]deltorphin-II. n is the number of independent repetitions conducted for each analogue using five to eight doses of peptide.
[c] Data are taken from Ref. [6].
[d] Data are taken from Ref. [7], the $K_i\delta$ versus [^{3}H]DPDPE.

Compared with [Dmt^1, Dmp^4]EM-2 (**1**) and [Dmt^1, Tmp^4]EM-2 (**2**), the additional hydroxyl group at the side chain of the fourth amino acid of **3** ([Dmt^1, Dmt^4]EM-2) was detrimental to binding, indicating that decreased lipophilicity at this position is unfavorable to binding to δ-receptor. Compound **6** showed onefold greater δ-receptor affinity than compound **5**, indicating that the orientation of the aromatic group also affects peptide binding to δ-receptor. On the whole, modification at position 4 slightly decreases affinity for μ-receptor and slightly improves affinity for δ-receptor. Compound **7** containing two Tmp residues at 3 and 4 positions exhibited the synergic effects of the substitution position 3 on δ-receptor activity; the change at position 4 (compound **7**) revealed high affinity for both μ- and δ-receptor ($K_i\mu$ = 0.47 nmol/L, $K_i\delta$ = 1.63 nmol/L, $K_i\delta/K_i\mu$ = 3.5), with a more balanced receptor selectivity than [Dmt^1]EM-2 ($K_i\delta/K_i\mu$ = 10).

Research on the structure of endomorphins indicated that the Tyr^1 and either Phe^3/Trp^3 or Phe^4 in opioid peptides are important topological elements that interact with opioid receptors [8]. Research also showed that deletion of the forth position's amino acid of [Dmt^1]EM-2 to give Dmt-Pro-Phe-NH_2 maintained high binding affinity for both μ- and δ-receptors [9]. Substitution of the Phe^3 of the Dmt-Pro-Phe-NH_2 with Tmp, Dmp, Trp, 1-Nal, and 2-Nal resulted in peptides **8**–**12**, respectively. The binding assay indicated that all the new opioids except **12** retained high affinity for μ-receptor. Of these analogues, compound **9** had the highest affinity ($K_i\mu$ = 0.13 nmol/L). Relative to the δ-receptor, **8** and **9** showed enhanced affinity, and in agreement with the first series, Tmp containing compound **9** contained the most potent affinity for the δ-receptor. The results also indicated that 1-Nal (**9**) is unfavorable to adopt the binding pocket of δ-receptor and showed decreased affinity.

In conclusion, both position 4 of [Dmt^1]EM-2 and position 3 of Dmt-Pro-Phe-NH_2 were well tolerated by substitution with various aromatic amino acids, of which Tmp is the best for both positions in its interaction with the μ- and δ-binding pockets. Pharmacological and biochemical evidence reveals that μ- and δ-opioid receptors readily form heterodimers [10], and either δ-receptor agonists or antagonists can facilitate internalization of morphine-binding μ-receptor, thereby decreasing morphine-induced tolerance and dependence [11]. This, therefore, becomes the rationale in opioid research focused on the development of compounds with mixed and dual opioid receptor bioactivities [1]. Among the newly synthesized peptides, Tmp at positions 3 and 4 (compound **7**) exhibited potent and balanced affinities for both μ- and δ-receptors, and preliminary functional assay indicated that compound **7** is a potent μ-receptor agonist, therefore, this compound is of special interesting in screening analgesics with low tolerance and dependence. Further detailed functional and pharmacological evaluations are undergoing.

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