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Novel TIPP (H-Tyr-Tic-Phe-Phe-OH) analogues displaying a wide range of efficacies at the δ opioid receptor. Discovery of two highly potent and selective δ opioid agonists

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Abstract

Analogues of the δ opioid antagonist peptide TIPP (H-Tyr-Tic-Phe-Phe-OH; Tic = 1,2,3,4-tetrahydroisoquinoline3-carboxylic acid) containing various 4′-[*N*-(alkyl or aralkyl)carboxamido]phenylalanine analogues in place of Tyr[1] were synthesized. The compounds showed subnanomolar or low nanomolar δ opioid receptor binding affinity and various efficacy at the δ receptor (antagonism, partial agonism, full agonism) in the [^{35}S]GTPγS binding assay. Two analogues, [1-Ncp[1]]TIPP (1-Ncp = 4′-[*N*-(2-(naphthalene-1-yl)ethyl)carboxamido] phenylalanine) and [2-Ncp[1]]TIPP (2-Ncp = 4′-[*N*-(2-(naphthalene-2-yl)ethyl)carboxamido]phenylalanine), were identified as potent and selective δ opioid agonists.

Keywords

amino acid synthesis; peptide synthesis; opioid peptides; δ opioid agonists; δ partial opioid agonists; δ opioid antagonist

Selective δ opioid receptor agonists are of interest both as pharmacological tools and as potential therapeutic agents (for a review, see ref.[1]). While they have low analgesic efficacy in acute pain models, their potential for the treatment of inflammatory and neuropathic pain has been clearly demonstrated.[2,3] Furthermore, there is evidence to indicate that δ opioid agonists produce anxiolytic and antidepressant effects[4] and that they may be useful as neuroprotective agents as well as for the treatment of addictive disorders.[1] Both peptide and non-peptide δ opioid agonists have been reviewed.[1,5] Examples of highly selective peptide δ opioid agonists are the cyclic opioid peptide analogues H-Tyr-c[D-Pen-Gly-Phe(*p*F)-Pen]Phe-OH[6] and H-Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13)[7], and the linear tripeptide UFP-512.[8]

The tetrapeptide TIPP (H-Tyr-Tic-Phe-Phe-OH) is a highly selective δ opioid antagonist.[9] Recently, we prepared a TIPP analogue containing 4′-[*N*-((4′-

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phenyl)phenethyl)carboxamido]phenylalanine (Bcp, Fig. 1) in place of Tyr^1, [Bcp^1]TIPP, which displayed high δ receptor binding affinity and δ agonist activity in the MVD assay.[10] Here we describe analogues of TIPP in which the Tyr^1 residue was replaced by *p*-carboxamidophenylalanine (Cpa, **1**) and its derivatives containing various substituents at the *p*-carboxamido group, including 4′-[*N*-(hexyl)carboxamido]phenylalanine (Hcp, **2**), 4′-[*N*-(decyl)carboxamido]phenylalanine (Dcap, **3**), 4′-[*N*-((4′-cyclohexyl)phenethyl)carboxamido]phenylalanine (Cpcp, **5**), 4′-[*N*-(2-(naphthalene-1-yl)ethyl)carboxamido]phenylalanine (1-Ncp, **6**), 4′-[*N*-(2-naphthalene-2-yl)ethyl)carboxamido]phenylalanine (2-Ncp, **7**), and 4′-*N*-(2-(indole-3-yl)ethyl)carboxamido]phenylalanine (Tcp, **8**) (Fig. 1). The effects of these various substitutions on binding affinity and efficacy at the δ and μ opioid receptors were determined in binding- and functional assays.

For the preparation of the various amino acids, Boc-Phe(4′-COOH)-OMe was synthesized by activating the hydroxyl group of Boc-Tyr-OMe as the triflate, followed by carbonylation with carbon monoxide, as described.[11] Boc-Phe-(4′-$CONH_2$)-OMe (Boc-Cpa, **1**)[12] was prepared by reacting Boc-Phe(4′-COOH)-OMe with ammonium chloride using HBTU as coupling agent, followed by hydrolysis with 2N NaOH. The other Boc-protected amino acids (**2, 3, 5–9**)[12] were synthesized by reacting Boc-Phe(4′-COOH)-OMe with the appropriate alkyl- or aralkylamine using HBTU as coupling agent, followed by hydrolysis with 2N NaOH. All amines were commercially available, except for 2-((4′-cyclohexyl)phenyl)ethylamine (**5a**)[12] required for the synthesis of Cpcp-OH. The latter amine was prepared by catalytic hydrogenation of 2-(4′-biphenyl)ethylamine in EtOH using palladium on carbon as catalyst with hydrogen pressure of 140 psig during 45 hours at 95°C. 2-((4′-cyclohexyl)phenyl)ethylamine was obtained as the predominant product (45%) with unreacted 2-(4′-biphenyl)ethylamine (35%) and 2-((4′-phenyl)cyclohexyl)ethylamine (20%) also present. This mixture was used in the synthesis of Boc-Cpcp-OH which was isolated from the reaction product by reversed-phase HPLC.

Peptides were synthesized by the solid-phase method using a Merrifield resin with Boc-protection and DIC/Cl-HOBt or HBTU/DIPEA as coupling agents, and were cleaved from the resin by HF/anisole treatment. The crude products were purified by preparative reversed-phase HPLC and their purity (> 98%) and structural identity were established by TLC, analytical HPLC and ES-MS.[13]

Binding affinities (K_i-values) for μ and δ opioid receptors were determined by displacing, respectively, [^{3}H]DAMGO and [^{3}H]DSLET from rat brain membrane binding sites and κ receptor binding affinities were measured by displacement of [^{3}H]U69,593 from guinea pig brain membrane binding sites, as described.[14] None of the compounds showed significant κ receptor binding affinity ($K_i^{\kappa} > 10$ μM). Opioid agonist potencies (IC_{50}s) or antagonist activities (K_e-values) were determined in the mouse vas deferens (MVD) assay (δ receptor-representative) and in the guinea pig ileum (GPI) assay using previously described protocols.[14] Furthermore, δ receptor agonist potencies and efficacies were determined in the [^{35}S]GTPγS binding assay using HEK293 cells stably expressing the human δ opioid receptor[15] (efficacies were indicated relative to the efficacy of DPDPE [e = 1.0]).

In comparison with the TIPP parent peptide, [Cpa^1]TIPP (**10**) showed comparable δ and μ receptor binding affinities and δ vs. μ selectivity (Table 1), and about 3-fold lower δ antagonist activity in the MVD assay (Table 2). In the [^{35}S]GTPγS binding assay both TIPP and [Cpa^1]TIPP behaved as δ neutral antagonists (e = 0). These data indicate that replacement of Tyr^1 in TIPP with Cpa had little effect on the binding affinity and efficacy at the δ receptor and are in agreement with earlier results indicating that Cpa was also a good surrogate for Tyr^1 in opioid agonist peptides.[16,17] The Hcp^1-analogue (**11**) displayed about

2.5-fold higher δ receptor binding affinity than **10** and retained high δ vs. μ receptor selectivity. Peptide **11** was a δ full agonist in the MVD assay with subnanomolar potency and a weak partial agonist in the GPI assay. It showed δ partial agonist activity in the [^{35}S]GTPγS binding assay (e = 0.486) with an ED_{50} of 0.103 nM. In comparison with **11**, the [Dcap^{1}]analogue (**12**) displayed somewhat diminished δ receptor binding affinity and lower δ vs. μ selectivity in the binding assays, 4-fold lower δ agonist potency in the MVD assay, and slightly decreased δ partial agonist activity (ED_{50} = 0.239 nM) with comparable efficacy (e = 0.527) in the [^{35}S]GTPγS binding assay.

As previously reported,[10] [Bcp^{1}]TIPP (**13**) was a potent δ full agonist in the MVD assay; however, it was now characterized as a δ partial agonist (e = 0.678) in the [^{35}S]GTPγS binding assay with an overall *in vitro* activity profile similar to that of **12**. The results obtained with peptides **11–13** in the [^{35}S]GTPγS binding assay indicate that they all behave as δ partial agonists with regard to G protein activation. The fact that these compounds show δ full agonist behavior in the MVD assay could be due to the presence of spare δ receptors in the vas preparation or to a different signal transduction mechanism.

The Cpcp^{1}-analogue (**14**) showed diminished δ vs. μ receptor binding selectivity and somewhat reduced δ full agonist potency in the MVD assay. It was essentially a δ full agonist (e = 0.933) with an ED_{50} of 1.69 nM in the [^{35}S]GTPγS binding assay. [1-Ncp^{1}]TIPP (**15**) (1-NIPP) was a potent and selective δ opioid agonist with an IC_{50} of 0.533 nM in the MVD assay and with δ full agonist properties (e = 0.894) in the [^{35}S]GTPγS binding assay. Similarly, [2-Ncp^{1}]TIPP (**16**) (2-NIPP) displayed high δ receptor binding affinity (K_i^{δ} = 1.01 nM), high δ agonist potency in the MVD assay (IC_{50} = 0.950 nM) and δ full agonist potency (ED_{50} = 0.475 nM, e = 0.989) in the [^{35}S]GTPγS binding assay. In a direct comparison with the standard δ opioid agonist DPDPE, 1-NIPP and 2-NIPP show 5–8 fold higher δ receptor binding affinity, comparable δ vs. μ receptor selectivity (K_i^{μ}/K_i^{δ} = 250–520 vs. K_i^{μ}/K_i^{δ} for DPDPE), 2–4-fold higher δ agonist potency in the MVD assay and 7–14-fold higher δ agonist potency in the [^{35}S]GTPγS binding assay. Finally, replacement of the naphthyl moiety in **15** with an indole moiety resulted in a compound, [Tcp^{1}]TIPP (**17**) with somewhat diminished δ agonist potency in the MVD assay and reduced δ partial agonist activity in the [^{35}S]GTPγS binding assay.

In conclusion, all compounds described here displayed high δ opioid receptor binding affinity, δ antagonist or δ full agonist activity in the MVD assay and various efficacy, ranging from δ antagonism to δ partial agonism to δ full agonism, in the [^{35}S]GTPγS binding assay. Two of the compounds, 1-NIPP and 2-NIPP, turned out to be potent δ full agonists in both functional assays. The δ partial agonist vs. δ full agonist behavior of the compounds in G protein activation can be explained with two different receptor activation models. Assuming that the receptor exists in only two functional conformational states, inactive and active, the partial agonists may simply induce a smaller fraction of receptors to undergo the transition to the active state than do the full agonists. The more likely explanation is that the partial agonists induce a receptor conformation distinct from that induced by the full agonists. It has been convincingly demonstrated that β_2-adrenergic receptor partial agonists and full agonists induce distinct receptor conformations.[18,19] In the case of the δ opioid partial agonists and full agonists described here, distinct δ receptor conformations could be induced through diverse interactions of the various large lipophilic substituents at the 1-position residue of these peptides with hydrophobic residues of an accessory receptor binding site. Because these structurally flexible peptides contain 4–6 aromatic rings they may have unique ability to selectively induce or stabilize a number of distinct receptor conformations through diverse hydrophobic interactions with the numerous aromatic and aliphatic residues present in the receptor binding site.[10] For this reason, they need to be further examined in various assay systems for possible functional selectivity.

Acknowledgments

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Abbreviations

Boc	*tert*-butyloxycarbonyl
Cl-HOBt	6-chloro-1-hydroxybenzotriazole
DAMGO	H-Tyr-D-Ala-Gly-Phe(NMe)-Gly-ol
DIC	1,3-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DPDPE	H-Tyr-c[D-Pen-Gly-Phe-D-Pen]OH
DSLET	H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH
ES-MS	electrospray mass spectrometry
GPI	guinea pig ileum
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HPLC	high performance liquid chromatography
MVD	mouse vas deferens, Pen, penicillamine
Tic	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid
TIPP	H-Tyr-Tic-Phe-Phe-OH
U69	593, (5α,7α,8β-(—)-*N*-methyl-N-[7-(1pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide

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7.40-7.32 (d, 2H, *J* = 7.8 Hz), 4.46-4.36 (m, 1H), 3.27-3.21 (m, 1H), 3.02-2.95 (m, 1H), 1.41-1.37 (s, 8H), 1.36-1.34 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.9, 171.1, 156.6, 142.0, 132.1, 129.4, 127.6, 79.4, 54.8, 37.3, 27.5; HRMS (EI) *m/e* calcd for $C_{15}H_{20}N_2NaO_5$ $[M+Na]^+$ 331.1270, found 331.1023.2. $[\alpha]_D^{20}$ −5.8° (c 1, DMSO); mp 152–153° C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.64-12.50 (br s, 1H), 8.38-8.32 (t, 1H, *J* = 5.5 Hz), 7.77-7.71 (d, 2H, *J* = 7.8 Hz), 7.34-7.29 (d, 2H, *J* = 8.0 Hz), 7.14-7.10 (d, 1H, *J* = 8.3 Hz), 4.15-4.08 (m, 1H), 3.24-3.20 (m, 2H), 3.09-3.03 (m, 1H), 2.90-2.83 (m,1H), 1.54-1.46 (m, 2H), 1.33-1.30 (s, 8H), 1.30-1.23 (m 7H), 0.89-0.84 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.1, 166.5, 156.1, 141.9, 133.5, 129.6, 127.7, 78.8, 55.6, 39.8, 36.9, 31.7, 29.8, 28.8, 26.8, 22.8, 14.6; HRMS (EI) *m/e* calcd for $C_{21}H_{32}O_5N_2Na$ $[M+Na]^+$ 415.2209, found 415.2201.3. $[\alpha]_D^{20}$ −3.2° (c 1, DMSO); mp 136–137° C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.74-12.34 (br s, 1H), 8.36-8.32 (t, 1H, *J* 5.6 Hz), 7.77-7.72 (d, 2H, *J* = 8.1 Hz), 7.34-7.28 (d, 2H, *J = 8.1 Hz*), 7.14-7.08 (d, 1H, *J* = 8.3 Hz), 4.15-4.08 (m, 1H), 3.26-3.19 (m, 2H), 3.09-3.03 (m, 1H), 2.90-2.83 (m, 1H), 1.54-1.46 (m, 2H), 1.34-1.30 (s, 8H), 1.29-1.21 (m, 15H), 0.88-0.83 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.2, 166.5, 156.1, 141.9, 133.5, 129.6, 127.7, 78.8, 55.6, 39.8, 36.9, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 28.8, 27.2, 22.8, 14.7; HRMS (EI) *m/e* calcd for $C_{25}H_{40}O_5N_2Na$ $[M+Na]^+$ 471.2835, obsd 471.2823.5. $[\alpha]_D^{20}$ +12.2° (c 1, MeOH); mp 206–208° C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.75-12.50 (br s, 1H), 8.53-8.47 (t, 1H, *J* = 5.5 Hz), 7.77-7.72 (d, 2H, *J* = 8.0 Hz), 7.36-7.29 (d,2H, *J* = 8.0 Hz), 7.17-7.11 (m,5H), 4.18-4.09 (m,1H), 3.48-3.41 (m, 2H), 3.10-3.04 (m, 1H), 2.91-2.85 (m, 1H), 2.81-2.73 (m, 2H), 2.49-2.40 (m, 1H), 1.80-1.66 (m, 6H), 1.40-1.34 (m, 4H), 1.33-1.30 (s, 8H), 1.27-1.24 (s, 1H); ^{13}C NMR 125 MHz, DMSO-d_6) δ 175.4, 166.6, 146.0, 142.2, 137.6, 133.0, 129.6, 129.2, 127.7, 127.3, 78.8, 55.6, 45.0, 44.1, 36.9, 35.4, 34.7, 26.3, 25.5; HRMS (EI) *m/e* calcd for $C_{29}H_{38}N_2O_5Na$ $[M+Na]^+$ 517.2678, obsd 517.3116. 5a. A small quantity of 5a was purified by HPLC for analytical characterization: mp 118–120° C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.24-7.17 (m, 4), 3.19-3.13 (t, 2H, *J* = 7.5 Hz), 2.96-2.89 (t, 2H, *J* = 7.5 Hz), 2.56-2.46 (m, 1H), 1.91-1.74 (m, 5H), 1.51-1.39 (m, 5H), 1.38-1.26 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 147.2, 133.9, 128.6, 128.5, 127.3, 127.2, 44.4, 40.8, 34.5, 33.0, 26.8, 26.1; HRMS (EI) *m/e* calcd for $C_{14}H_{22}N$ $[M+H]^+$ 204.1752, found 204.1747.6. $[\alpha]_D^{20}$ −15.5° (c 1, DMSO); mp138–140° C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.84-2.38 (br s, 1H), 8.68-8.61 (t, 1H, *J* = 5.6 Hz), 8.31-8.25 (d, 1H, *J* = 8.3 Hz), 7.96-7.90 (d, 1H, *J* = 8.0 Hz), 7.82-7.79 (d, 1H, *J* = 8.3 Hz), 7.79-7.73 (d, 2H, *J* = 8.3 Hz), 7.62-7.56 (m, 1H), 7.56-7.50 (m, 1H), 7.46-7.38 (m, 1H), 7.38-7.30 (d, 2H, *J* = 8.3 Hz), 7.17-7.10 (d, 1H, *J* = 8.5 Hz), 4.18-4.10 (m, 1H), 3.63-3.55 (m, 2H), 3.35-3.28 (m, 2H), 3.10-3.04 (m, 1H), 2.91-2.85 (m,1H), 1.38-1.33 (s, 8H),1.28-1.25 (s 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.2, 166.8, 156.4, 142.1, 136.2, 134.2, 132.4, 129.6, 127.7, 127.6, 126.4, 124.5, 78.8, 55.8, 41.0, 36.5, 33.3, 28.8; HRMS (EI) *m/e* calcd for $C_{27}H_{30}O_5N_2Na$ $[M+Na]^+$ 485.2052, found 485.2038.7. $[\alpha]_D^{20}$ −5.5° (c 1, DMSO); mp 226–227° C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.80-12.40 (br s, 1H), 8.56-8.50 (t, 1H, *J* = 5.6 Hz), 7.89-7.82 (m, 3H), 7.76-7.70 (m, 3H), 7.50-7.40 (m, 3H), 7.34-7.28 (d, 2H, *J* = 8.0 Hz), 7.16-7.11 (d, 1H, *J* = 8.3 Hz), 4.15-4.08 (m, 1H), 3.62-3.56 (m, 2H), 3.09-3.04 (m, 1H), 3.03-2.98 (m, 2H), 2.89-2.82 (m, 1H), 1.33-1.28 (s, 8H), 1.27-1.22 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.1, 166.7, 156.1, 142.0, 137.9, 133.8, 133.4, 132.4, 129.6, 128.4, 128.2, 128.1, 128.0, 127.7, 127.4, 126.7, 126.0, 78.8, 55.5, 41.4, 36.9, 35.9, 28.8; HRMS (E/I) *m/e* calcd for $C_{27}H_{30}N_2O_5Na$ $[M+Na]^+$ 485.2052, found 485.2038.8. $[\alpha]_D^{20}$ −5.8° (c 1, DMSO); mp 118–120° C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77-12.58 (br s, 1H), 10.83-10.78 (s, 1H), 8.57-8.52 (t, 1H, *J* = 5.6 Hz), 7.79-7.74 (d, 2H, *J* = 8.1 Hz), 7.62-7.58 (d, 1H, *J* = 7.8 Hz), 7.35-7.30 (m, 3H), 7.18-7.16 (s, 1H), 7.16-7.12 (d, 1H, *J* = 8.5 Hz); 7.09-7.04 (m, 1H), 7.00-6.96 (m, 1H), 4.15-4.08 (m, 1H), 3.56-3.49 (m, 2H), 3.10-3.04 (m, 1H), 2.97-2.90 (m, 2H), 2.90-2.84 (m, 1H), 1.33-1.30 (s, 8H), 1.28-1.25 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.1, 166.6, 156.1, 142.0, 137.0, 133.5, 129.7, 128.0, 127.7, 123.2, 121.6, 118.9, 112.6, 78.8, 56.6, 45.0, 36.9, 28.8, 25.9; HRMS (E/I) *m/e* calcd for $C_{25}H_{30}O_5N_3$ $[M+H]^+$ 452.2185, found 452.2178.

13. Analytical data of peptides. TLC in the following systems (all v/v): (I) *n*-BuOH/AcOH/H_2O (4:1:1), (II) *n*-BuOH/pyridine/AcOH/H_2O (15:10:3:12); analytical reversed-phase HPLC performed on a Vydac column (10×250 mm) with a linear gradient of 45–95% MeOH in 0.1% TFA over 50 min ($K' = (t_r\text{-}t_m)/t_m$); ES-MS (*m/e*). 10. R_f 0.571 (I), R_f 0.768 (II); K' 1.72; ES-MS *m/e* 662. 11. R_f 0.457 (I), R_f 0.840 (II); K' 4.65; ES-MS *m/e* 746. 12. R_f 0.493 (I), R_f 0.852 (II); K' 6.17, ES-MS *m/e* 802. 14. R_f 0.593 (I), R_f 0.841 (II); K' 4.97; ES-MS *m/e* 848. 15. R_f 0.709,

R_f 0.860 (II); K' 4.86; ES-MS *m/e* 816. 16. R_f 0.463 (I), R_f 0.846 (II); K' 4.77; ES-MS *m/e* 816. 17. R_f 0.743 (I), R_f 0.797 (II), K' 3.74; ES-MS *m/e* 805.

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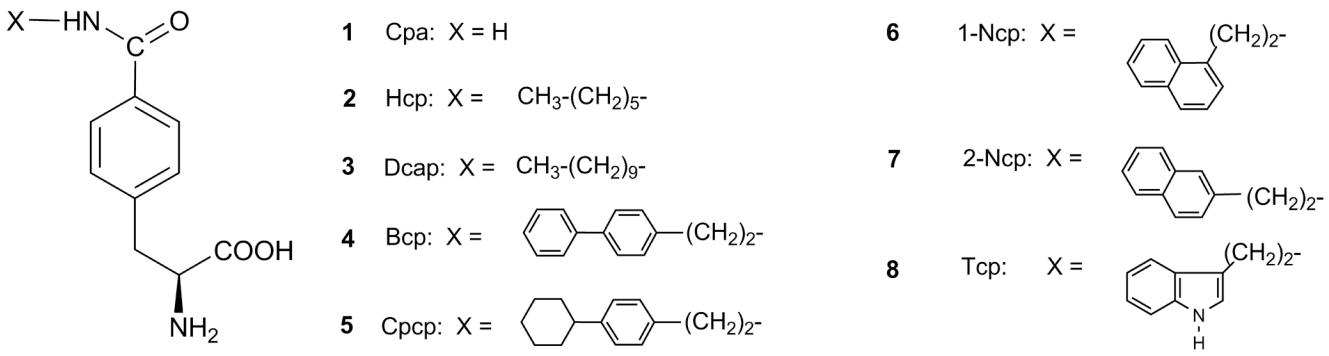

Figure 1.
Structures of Phe(4′-CONHX) amino acids.

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Table 1

Receptor binding affinities of TIPP analogues

Compound	K_i^{δ} [nM][a]	K_i^{μ} [nM][a]	K_i^{μ}/K_i^{δ}
10 H-Cpa-Tic-Phe-Phe-OH	0.978 ± 0.195	2190 ± 170	2240
11 H-Hcp-Tic-Phe-Phe-OH	0.369 ± 0.028	507 ± 17	1370
12 H-Dcap-Tic-Phe-Phe-OH	1.54 ± 0.25	174 ± 40	113
13 H-Bcp-Tic-Phe-Phe-OH[b]	0.605 ± 0.058	87.7 ± 8.0	145
14 H-Cpcp-Tic-Phe-Phe-OH	2.72 ± 0.40	47.6 ± 17.3	17.5
15 H-1-Ncp-Tic-Phe-Phe-OH	1.65 ± 0.31	850 ± 190	515
16 H-2-Ncp-Tic-Phe-Phe-OH	1.01 ± 0.10	249 ± 32	247
17 H-Tcp-Tic-Phe-Phe-OH	1.84 ± 0.28	3600 ± 1250	1960
H-Tyr-Tic-Phe-Phe-OH	1.22 −± 0.07	1720 ± 50	1410
DPDPE	8.39 ± 0.70	2820 ± 0.7	336

[a]Mean of 3 determinations ± SEM. Data taken from ref.[10]

Table 2

Opioid activities of TIPP analogues in the MVD and GPI assays[a]

Compound	MVD		GPI	
	IC_{50} [nM]	K_e^{δ} [nM][b]	IC_{50} [nM]	K_e^{μ} [nM][c]
10 H-Cpa-Tic-Phe-Phe-OH		18.3 ± 2.1		6030 ± 1600
11 H-Hcp-Tic-Phe-Phe-OH	0.392 ± 0.051		875 ± 171 (IC_{30})[d]	
12 H-Dcap-Tic-Phe-Phe-OH	1.54 ± 0.25		730 ± 109 (IC_{30})[d]	
13 H-Bcp-Tic-Phe-Phe-OH[e]	3.42 ± 0.36		223 ± 37 (IC_{40})[d]	
14 H-Cpcp-Tic-Phe-Phe-OH	6.20 ± 0.40		23.4 ± 5.1 (IC_{35})[d]	
15 H-1-Ncp-Tic-Phe-Phe-OH	0.533 ± 0.047		inactive at 10 μM	
16 H-2-Ncp-Tic-Phe-Phe-OH	0.950 ± 0.142		P.A.[d]	734 ± 17
17 H-Tcp-Tic-Phe-Phe-OH	10.1 ± 1.4		P.A.[d]	
H-Tyr-Tic-Phe-Phe-OH		4.80 ± 0.20	> 10000 (inactive)	
DPDPE	2.02 ± 1.6		6340 ± 410	

[a] Mean of 3 determinations ± SEM.

[b] Determined against DPDPE.

[c] Determined against TAPP.

[d] Partial agonist.

[e] Data taken from ref.[10]

Table 3

[^{35}S]GTPS binding assay of TIPP analogues

Compound	ED_{50} [nM][a]	e[a]
10 H-Cpa-Tic-Phe-Phe-OH	antagonist	0
11 H-Hcp-Tic-Phe-Phe-OH	0.103 ± 0.012	0.486 ± 0.060
12 H-Dcap-Tic-Phe-Phe-OH	0.239 ± 0.023	0.527 ± 0.080
13 H-Bcp-Tic-Phe-Phe-OH	0.413 ± 0.015	0.678 ± 0.076
14 H-Cpcp-Tic-Phe-Phe-OH	1.69 ± 0.25	0.933 ± 0.026
15 H-1-Ncp-Tic-Phe-Phe-OH	1.03 ± 0.05	0.894 ± 0.009
16 H-2-Ncp-Tic-Phe-Phe-OH	0.475 ± 0.064	0.989 ± 0.44
17 H-Tcp-Tic-Phe-Phe-OH	7.08 ± 2.19	0.643 ± 0.051
H-Tyr-Tic-Phe-Phe-OH	antagonist	0
DPDPE	6.81 ± 0.68	1

[a]Mean of 3–6 determinations ± SEM.