



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

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### ABSTRACT

Analogues of the  $\delta$  opioid antagonist peptide TIPP (H-Tyr-Tic-Phe-Phe-OH; Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) containing various 4'-[N-(alkyl or aralkyl)carboxamido]phenylalanine analogues in place of Tyr<sup>1</sup> were synthesized. The compounds showed subnanomolar or low nanomolar  $\delta$  opioid receptor binding affinity and various efficacy at the  $\delta$  receptor (antagonism, partial agonism, full agonism) in the [<sup>35</sup>S]GTP $\gamma$ S binding assay. Two analogues, [1-Ncp<sup>1</sup>]TIPP (1-Ncp = 4'-[N-(2-(naphthalene-1-yl)ethyl)carboxamido]phenylalanine) and [2-Ncp<sup>1</sup>]TIPP (2-Ncp = 4'-[N-(2-(naphthalene-2-yl)ethyl)carboxamido]phenylalanine), were identified as potent and selective  $\delta$  opioid agonists.

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Selective  $\delta$  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.<sup>2,3</sup> Furthermore, there is evidence to indicate that  $\delta$  opioid agonists produce anxiolytic and antidepressant effects<sup>4</sup> and that they may be useful as neuroprotective agents as well as for the treatment of addictive disorders.<sup>1</sup> Both peptide and non-peptide  $\delta$  opioid agonists have been reviewed.<sup>1,5</sup> Examples of highly selective peptide  $\delta$  opioid agonists are the cyclic opioid peptide analogues H-Tyr-c[D-Pen-Gly-Phe-(pF)-Pen]Phe-OH<sup>6</sup> and H-Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13)<sup>7</sup>, and the linear tripeptide UFP-512.<sup>8</sup>

**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, (5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ -)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide.

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The tetrapeptide TIPP (H-Tyr-Tic-Phe-Phe-OH) is a highly selective  $\delta$  opioid antagonist.<sup>9</sup> Recently, we prepared a TIPP analogue containing 4'-[N-((4'-phenyl)phenethyl)carboxamido]phenylalanine (Bcp, Fig. 1) in place of Tyr<sup>1</sup>, [Bcp<sup>1</sup>]TIPP, which displayed high  $\delta$  receptor binding affinity and  $\delta$  agonist activity in the MVD assay.<sup>10</sup> Here we describe analogues of TIPP in which the Tyr<sup>1</sup> 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  $\delta$  and  $\mu$  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.<sup>11</sup> Boc-Phe-(4'-CONH<sub>2</sub>)-OMe (Boc-Cpa, 1)<sup>12</sup> was prepared by reacting Boc-Phe(4'-COOH)-OMe with ammonium chloride using HBTU as coupling agent, followed by hydrolysis with 2 N NaOH. The other Boc-protected amino acids (2, 3, 5–9)<sup>12</sup> were synthesized by reacting Boc-Phe(4'-COOH)-OMe with the appropriate alkyl- or aralkylamine using HBTU as coupling agent,

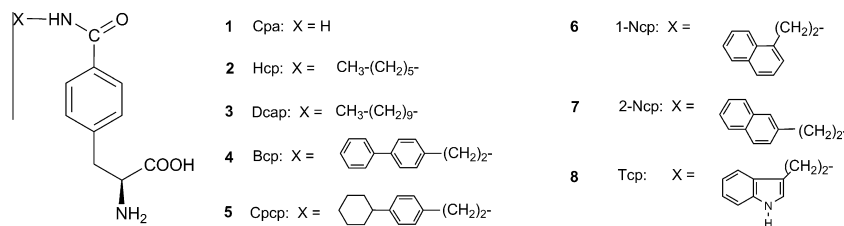


Figure 1. Structures of Phe(4'-CONHX) amino acids.

followed by hydrolysis with 2 N NaOH. All amines were commercially available, except for 2-((4'-cyclohexyl)phenyl)ethylamine (**5a**)<sup>12</sup> 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 h 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.<sup>13</sup>

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

In comparison with the TIPP parent peptide, [Cpa<sup>1</sup>]TIPP (**10**) showed comparable  $\delta$  and  $\mu$  receptor binding affinities and  $\delta$  versus  $\mu$  selectivity (Table 1), and about threefold lower  $\delta$  antagonist activity in the MVD assay (Table 2). In the [<sup>35</sup>S]GTP $\gamma$ S binding assay both TIPP and [Cpa<sup>1</sup>]TIPP behaved as  $\delta$  neutral antagonists ( $e = 0$ , Table 3). These data indicate that replacement of Tyr<sup>1</sup> in TIPP with Cpa had little effect on the binding affinity and efficacy at the  $\delta$  receptor and are in agreement with earlier results indicating that

Cpa was also a good surrogate for Tyr<sup>1</sup> in opioid agonist peptides.<sup>16,17</sup> The Hcp<sup>1</sup>-analogue (**11**) displayed about 2.5-fold higher  $\delta$  receptor binding affinity than **10** and retained high  $\delta$  versus  $\mu$  receptor selectivity. Peptide **11** was a  $\delta$  full agonist in the MVD assay with subnanomolar potency and a weak partial agonist in the GPI assay. It showed  $\delta$  partial agonist activity in the [<sup>35</sup>S]GTP $\gamma$ S binding assay ( $e = 0.486$ ) with an  $\text{ED}_{50}$  of 0.103 nM. In comparison with **11**, the [Dcap<sup>1</sup>]analogue (**12**) displayed somewhat diminished  $\delta$  receptor binding affinity and lower  $\delta$  versus  $\mu$  selectivity in the binding assays, fourfold lower  $\delta$  agonist potency in the MVD assay, and slightly decreased  $\delta$  partial agonist activity ( $\text{ED}_{50} = 0.239 \text{ nM}$ ) with comparable efficacy ( $e = 0.527$ ) in the [<sup>35</sup>S]GTP $\gamma$ S binding assay.

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

The Cpcp<sup>1</sup>-analogue (**14**) showed diminished  $\delta$  versus  $\mu$  receptor binding selectivity and somewhat reduced  $\delta$  full agonist potency in the MVD assay. It was essentially a  $\delta$  full agonist ( $e = 0.933$ ) with an  $\text{ED}_{50}$  of 1.69 nM in the [<sup>35</sup>S]GTP $\gamma$ S binding assay. [1-Ncp<sup>1</sup>]TIPP (**15**) (1-NIPP) was a potent and selective  $\delta$  opioid agonist with an  $\text{IC}_{50}$  of 0.533 nM in the MVD assay and with  $\delta$  full agonist properties ( $e = 0.894$ ) in the [<sup>35</sup>S]GTP $\gamma$ S binding assay. Similarly, [2-Ncp<sup>1</sup>]TIPP (**16**) (2-NIPP) displayed high  $\delta$  receptor binding affinity ( $K_i^{\delta} = 1.01 \text{ nM}$ ), high  $\delta$  agonist potency in the MVD assay ( $\text{IC}_{50} = 0.950 \text{ nM}$ ) and  $\delta$  full agonist potency ( $\text{ED}_{50} = 0.475 \text{ nM}$ ,  $e = 0.989$ ) in the [<sup>35</sup>S]GTP $\gamma$ S binding assay. In a direct comparison with the standard  $\delta$  opioid agonist DPDPE, 1-NIPP and 2-NIPP show 5–8 fold higher  $\delta$  receptor binding affinity, comparable  $\delta$  versus  $\mu$  receptor selectivity ( $K_i^{\mu}/K_i^{\delta} = 250\text{--}520$  versus ( $K_i^{\mu}/K_i^{\delta} = 336$  for DPDPE), 2–4-fold higher  $\delta$  agonist potency in the MVD assay and 7–14-fold higher  $\delta$  agonist potency in the [<sup>35</sup>S]GTP $\gamma$ S binding assay. Finally, replacement of the naphthyl moiety in **15** with an indole moiety resulted in a compound, [Tcp<sup>1</sup>]TIPP (**17**) with somewhat diminished  $\delta$  agonist potency in the MVD assay and reduced  $\delta$  partial agonist activity in the [<sup>35</sup>S]GTP $\gamma$ S binding assay.

In conclusion, all compounds described here displayed high  $\delta$  opioid receptor binding affinity,  $\delta$  antagonist or  $\delta$  full agonist activity in the MVD assay and various efficacy, ranging from  $\delta$  antagonism to  $\delta$  partial agonism to  $\delta$  full agonism, in the [<sup>35</sup>S]GTP $\gamma$ S binding assay. Two of the compounds, 1-NIPP and 2-NIPP, turned out to be potent  $\delta$  full agonists in both functional assays. The  $\delta$  partial agonist vs.  $\delta$  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

Table 1  
Receptor binding affinities of TIPP analogues

Compound	$K_i^{\delta a}$ (nM)	$K_i^{\mu a}$ (nM)	$K_i^{\mu}/K_i^{\delta}$
<b>10</b> H-Cpa-Tic-Phe-Phe-OH	0.978 ± 0.195	2190 ± 170	2240
<b>11</b> H-Hcp-Tic-Phe-Phe-OH	0.369 ± 0.028	507 ± 17	1370
<b>12</b> H-Dcap-Tic-Phe-Phe-OH	1.54 ± 0.25	174 ± 40	113
<b>13</b> H-Bcp-Tic-Phe-Phe-OH <sup>b</sup>	0.605 ± 0.058	87.7 ± 8.0	145
<b>14</b> H-Cpcp-Tic-Phe-Phe-OH	2.72 ± 0.40	47.6 ± 17.3	17.5
<b>15</b> H-1-Ncp-Tic-Phe-Phe-OH	1.65 ± 0.31	850 ± 190	515
<b>16</b> H-2-Ncp-Tic-Phe-Phe-OH	1.01 ± 0.10	249 ± 32	247
<b>17</b> 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

<sup>a</sup> Mean of 3 determinations ± SEM. Data taken from Ref. 10.

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