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N-terminal guanidinylation of TIPP (Tyr-Tic-Phe-Phe) peptides results in major changes of the opioid activity profile

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ABSTRACT

Derivatives of peptides of the TIPP (Tyr-Tic-Phe-Phe; Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) family containing a guanidino (Guan) function in place of the N-terminal amino group were synthesized in an effort to improve their blood–brain barrier permeability. Unexpectedly, N-terminal amidination significantly altered the in vitro opioid activity profiles. Guan-analogues of TIPP-related δ opioid antagonists showed δ partial agonist or mixed δ partial agonist/μ partial agonist activity. Guanidinylation of the mixed μ agonist/δ antagonists H-Dmt-Tic-Phe-Phe-NH_2 (DIPP-NH_2) and H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (DIPP-NH_2[Ψ]) converted them to mixed μ agonist/δ agonists. A docking study revealed distinct positioning of DIPP-NH_2 and Guan-DIPP-NH_2 in the δ receptor binding site. Lys^3-analogues of DIPP-NH_2 and DIPP-NH_2[Ψ] (guanidinylated or non-guanidinylated) turned out to be mixed μ/κ agonists with δ antagonist-, δ partial agonist- or δ full agonist activity. Compounds with some of the observed mixed opioid activity profiles have therapeutic potential as analgesics with reduced side effects or for treatment of cocaine addiction.

The tetrapeptides H-Tyr-Tic-Phe-Phe-OH (TIPP) (**1a**), H-Dmt-Tic-Phe-Phe-OH (DIPP) (**2a**) and H-Tyr-TicΨ[CH_2NH]Phe-Phe-OH (TIPP[Ψ] (**3a**) are highly selective δ opioid antagonists with low nanomolar or subnanomolar δ opioid receptor binding affinity.[1–3] The TIPP-derived tetrapeptide amides H-Dmt-Tic-Phe-Phe-NH_2 (DIPP-NH_2) (**4a**) and H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (DIPP-NH_2[Ψ]) (**5a**) act as agonists at the μ opioid receptor and as antagonists at the δ receptor. On the basis of a well established pharmacological rationale,[4,5] compounds with such a mixed μ agonist/δ antagonist profile are expected to be analgesics with low propensity to produce analgesic tolerance and physical dependence. Indeed, DIPP-NH_2[Ψ] given i.c.v. produced a potent analgesic effect in the rat tail flick test and upon chronic administration induced less analgesic tolerance than morphine and no physical dependence.[6] Both δ opioid antagonists and mixed μ agonist/δ antagonists of the TIPP peptide family proved to be valuable tools in opioid receptor research and proof-of-concept studies in the opioid field. However, TIPP peptides do not effectively cross the BBB.

Cationization of proteins and peptides by guanidine (Guan) addition has been shown to improve metabolic stability and BBB permeability.[7,8] N-terminal amidination of a μ-selective dermorphin-derived tetrapeptide did not result in a significant change in μ receptor binding affinity and high selectivity for μ versus δ and κ receptors was retained.[9] The resulting compound produced centrally mediated analgesia with oral and sc administration, indicating its ability to cross the BBB. N-terminally amidinated endorphin-1 analogues displayed somewhat reduced μ receptor binding affinity as compared to their respective non-amidinated parent peptides, were still μ receptor-selective and showed enhanced BBB permeability.[10]

Here we describe the syntheses and in vitro opioid activity profiles of the N-terminally amidinated TIPP antagonists **1a**, **2a** and **3a**: Guan-Tyr-Tic-Phe-Phe-OH (**1**), Guan-Dmt-Tic-Phe-Phe-OH (**2**) and Guan-Tyr-TicΨ[CH_2NH]Phe-Phe-OH (**3**), and of the mixed μ agonist/δ antagonists **4a** and **5a**: Guan-Dmt-Tic-Phe-Phe-NH_2 (**4**) and Guan-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (**5**). The novel Lys^3-containing peptides H-Dmt-Tic-Lys-Phe-NH_2 (**6a**) and H-Dmt-TicΨ[CH_2NH]Lys-Phe-NH_2 (**7a**), and their respective guanidinylated analogues Guan-Dmt-Tic-Lys-Phe-NH_2 (**6**) and Guan-Dmt-TicΨ[CH_2NH]Lys-Phe-NH_2 (**7**) were also synthesized and

Abbreviations: BBB, blood–brain barrier; Boc, *tert*-butyloxycarbonyl; Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; DAMGO, H-Tyr-D-Ala-Gly-Phe(NMe)-Gly-ol; DIC, 1,3-diisopropylcarbodiimide; DIPP, H-Dmt-Tic-Phe-Phe-OH; DIPP-NH_2, H-Dmt-Tic-Phe-Phe-NH_2; DIPP-NH_2[Ψ], H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2; Dmt, 2′,6′-dimethyltyrosine; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; ES-MS, electrospray mass spectrometry; GPI, guinea pig ileum; Guan, guanidino; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HPLC, high performance liquid chromatography; MVD, mouse vas deferens; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIPP, H-Tyr-Tic-Phe-Phe-OH; TIPP[Ψ], H-Tyr-TicΨ[CH_2NH]Phe-Phe-OH; U69,593, (5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide.

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pharmacologically characterized. Finally, we describe the synthesis and in vitro opioid activity profile of the guanidinylated dipeptide opioid δ antagonist H-Dmt-Tic-OH[11] (**8a**): Guan-Dmt-Tic-OH (**8**).

Peptides **1**–**7** were synthesized by the manual solid-phase method using a Boc-Phe resin for peptides **1**–**3** and a *p*-methylbenzhydrylamine resin for peptides **4**, **5**, **6**, **6a**, **7** and **7a** with Boc-protection and DIC/Cl-HOBt as coupling agents. To introduce the reduced peptide bond between the Tic[2] and Phe[3] or Tic[2] and Lys[3] residues in peptides **3**, **5**, **7** and **7a**, a reductive alkylation reaction[12] between 2-Boc-1,2,3,4-tetrahydroisoquinoline-3-aldehyde[13] and the α-amino group of the resin-bound H-Phe-Phe- or H-Lys-Phe dipeptide segments was performed. Amidination on the resin was performed using the reagent *N*,*N*′-bis-(2-chloro-benzyloxycarbonyl)-1*H*-1-pyrazole-1-carboxamidine.[14] Peptides were cleaved from the resin by HF/anisole treatment. The guanidinylated dipeptide Guan-Dmt-Tic-OH (**8**) was prepared in solution by coupling Boc-Dmt-OH with H-Tic-OMe using HBTU as coupling agent. The reagent 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine[15] was used for N-terminal guanidinylation. Subsequent NaOH hydrolysis of the methyl ester and Boc deprotection with TFA afforded the target product. Crude products were purified by reversed-phase HPLC and their purity (>98%) and structural identity were established by TLC, analytical HPLC and ES-MS.

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 opioid receptor binding affinities were measured by displacement of [^{3}H]U69,593 from guinea pig brain membrane binding sites, as described.[16] Opioid agonist potencies (IC_{50} values) or antagonist activities (K_e values) were determined in the mouse vas deferens (MVD) assay (δ receptor-representative) or in the guinea pig ileum (GPI) assay (μ and κ receptor-representative) using previously described protocols.[16]

Guanidinylation of the δ antagonist TIPP (**1a**) resulted in a compound (**1**) with δ receptor binding affinity (K_i^{δ} = 2.29 nM) similar to that of the TIPP parent (K_i^{δ} = 1.22 nM) (Table 1). In comparison with the δ antagonists DIPP (**2a**) and TIPP[Ψ] (**3a**), the respective guanidinylated peptides Guan-Dmt-Tic-Phe-Phe-OH (**2**) and Guan-Tyr-TicΨ[CH_2NH]Phe-Phe-OH (**3**) retained similar subnanomolar δ receptor binding affinities (K_i^{δ} = 0.146 nM and 0.968 nM, respectively). Like their parent peptides, the guanidinylated peptides **1**, **2** and **3** showed high δ receptor binding selectivity with weak binding affinities for μ opioid receptors (K_i^{μ} = 126–875 nM) and very weak affinity for κ receptors (K_i^{κ} > 2000 nM). Whereas peptides **1a**, **2a** and **3a** were potent δ antagonists in the MVD assay with K_e values in the 0.2–4.8 nM range, the guanidinylated peptides **1**, **2** and **3** showed δ partial agonist behavior (Table 2). For peptide **2** an IC_{35} of 1.57 nM could be determined based on 70% maximal inhibition of the electrically evoked contractions of the vas produced by this compound. Peptides **1** and **3** showed lower maximal inhibitions to the extent of 33% and 50%, respectively, which did not permit the determination of accurate IC values.

Guanidinylation of the mixed μ agonist/δ antagonist H-Dmt-Tic-Phe-Phe-NH_2 (**4a**) had unexpected effects on the in vitro opioid activity profile. While Guan-Dmt-Tic-Phe-Phe-NH_2 (**4**) retained very high δ and μ receptor binding affinities (K_i^{δ} = 0.146 nM, K_i^{μ} = 0.518 nM), it showed significant κ receptor binding affinity (K_i^{κ} = 35 nM), in contrast to the low κ affinity of the non-guanidinylated peptide (K_i^{κ} > 1 μM). Surprisingly, this compound turned out to be a potent δ full agonist in the MVD assay (IC_{50} = 1.72 nM). The effect was naloxone-reversible (K_e = 0.308 ± 0.57 nM), indicating that it was mediated by opioid receptors. As expected on the basis of its high μ receptor binding affinity, this compound also showed high μ agonist potency in the GPI assay (IC_{50} = 8.09 nM). Guan-Dmt-Tic-Phe-Phe-NH_2 thus represents a potent, balanced μ agonist/δ agonist.

A study of flexible docking of compounds **4a** and **4** to the δ opioid receptor was performed using Mosberg's models of the receptor in the inactive and activated state.[17] The mixed μ agonist/δ antagonist **4a** and the mixed μ agonist/δ agonist **4** were docked to the inactive and the activated form of the δ receptor, respectively (Fig. 1). In general, a comparison of the ligand–receptor interactions of **4a** bound to the inactive receptor form with those of **4** bound to the activated form revealed that most of the interactions involved the same lipophilic receptor residues, including Tyr[129], Phe[133], Val[217], Phe[218], Ile[277], Val[281], Leu[200], Trp[284], Leu[299] and Met[199]. Both the N-terminal amino group of **4a** and the N-terminal guanidino group of **4** were engaged in an electrostatic interaction (salt bridges) with Asp[128] in the third transmembrane helix of the receptor. However, due to the steric bulk of the guanidino group, peptide **4** was shifted relative to the position of peptide **4a** (average rms deviation = 1.06 Å). This resulted in somewhat different interactions with corresponding receptor residues which in some cases also have different side chain orientations between the two receptor forms. These distinct receptor interactions may explain the δ antagonist versus δ agonist behavior of compounds **4a** and **4**.

Compared to guanidinylated peptide **4**, the pseudopeptide Guan-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2 (**5**) showed a similar opioid

Table 1
Receptor binding affinities of TIPP- and TIPP-NH_2 analogues[a]

Compound		K_i^{δ} (nM)	K_i^{μ} (nM)	K_i^{κ} (nM)	Selectivity ratio (δ/μ/κ)
1	Guan-Tyr-Tic-Phe-Phe-OH	2.29 ± 0.51	875 ± 21	>5000	1/382/>2180
1a	H-Tyr-Tic-Phe-Phe-OH[b]	1.22 ± 0.07	1720 ± 50	>1000	1/1410/>820
2	Guan-Dmt-Tic-Phe-Phe-OH	0.146 ± 0.007	126 ± 10	2260 ± 10	1/863/15500
2a	H-Dmt-Tic-Phe-Phe-OH[b]	0.248 ± 0.025	141 ± 25	>1000	1/569/>4030
3	Guan-Tyr-TicΨ[CH_2NH]Phe-Phe-OH	0.968 ± 0.011	704 ± 82	>10,000	1/727/>10300
3a	H-Tyr-TicΨ[CH_2NH]Phe-Phe-OH[b]	0.308 ± 0.060	3230 ± 440	>1000	1/10500/>3250
4	Guan-Dmt-Tic-Phe-Phe-NH_2	0.146 ± 0.041	0.518 ± 0.047	35.8 ± 3.0	1/4/245
4a	H-Dmt-Tic-Phe-Phe-NH_2[b]	0.118 ± 0.016	1.19 ± 0.11	>1000	1/10/>8470
5	Guan-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2	0.789 ± 0.141	1.02 ± 0.19	40.1 ± 10.1	1/1/51
5a	H-Dmt-TicΨ[CH_2NH]Phe-Phe-NH_2[b]	0.447 ± 0.007	0.943 ± 0.052	>1000	1/2/>2240
6	Guan-Dmt-Tic-Lys-Phe-NH_2	2.20 ± 0.38	0.354 ± 0.005	1.69 ± 0.09	1/0.2/0.8
6a	H-Dmt-Tic-Lys-Phe-NH_2	0.306 ± 0.061	19.4 ± 2.2	2.23 ± 0.17	1/63/7
7	Guan-Dmt-TicΨ[CH_2NH]Lys-Phe-NH_2	1.83 ± 0.41	18.1 ± 3.1	24.7 ± 1.1	1/10/13
7a	H-Dmt-TicΨ[CH_2NH]Lys-Phe-NH_2	2.73 ± 0.80	9.11 ± 0.97	2.06 ± 0.59	1/3/0.8
8	Guan-Dmt-Tic-OH	2.66 ± 0.10	15.0 ± 1.3	>10,000	1/6/>3760
8a	H-Dmt-Tic-OH[b]	1.64 ± 0.07	1360 ± 160	>1000	1/829/>714

[a] Mean of 3 determinations ± SEM.
[b] Data taken from Ref. 3.