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New series of potent δ-opioid antagonists containing the H-Dmt-Tic-NH-hexyl-NH-R motif

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Abstract—Heterodimeric compounds H-Dmt-Tic-NH-hexyl-NH-R (R = Dmt, Tic, and Phe) exhibited high affinity to δ- ($K_i\delta$ = 0.13–0.89 nM) and μ-opioid receptors ($K_i\mu$ = 0.38–2.81 nM) with extraordinary potent δ antagonism (pA_2 = 10.2–10.4). These compounds represent the prototype for a new class of structural homologues lacking μ-opioid receptor-associated agonism (IC_{50} = 1.6–5.8 μM) based on the framework of bis-[H-Dmt-NH]-alkyl (Okada, Y.; Tsuda, Y.; Fujita, Y.; Yokoi, T.; Sasaki, Y.; Ambo, A.; Konishi, R.; Nagata, M.; Salvadori, S.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H. *J. Med. Chem.* **2003**, 46, 3201), which exhibited both high μ affinity and bioactivity.

The combination of two unnatural amino acids, 2′,6′-dimethyl-L-tyrosine (Dmt) and 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), produced a new family of δ-opioid antagonists.[1] This dipeptide H-Dmt-Tic-OH not only had δ-opioid receptor antagonism (pA_2 = 8.2) with high affinity [$K_i\delta$ = 0.022 nM] and extraordinarily selectivity for δ-opioid receptors [$K_i\mu/K_i\delta$ = 150,800],[1] but also exhibited in vivo antagonism following systemic administration against deltorphin II-induced analgesia.[2] Due to the unique biological properties of the Dmt-Tic motif, intensive structure–activity studies were conducted in order to develop bifunctional or heterofunctional opioid ligands.[3] The extensive modifications included *N*-alkylation,[4] change of the position of hydroxyl and methyl groups on the tyramine ring,[5] introduction of a β-methyl to the Dmt residue,[6] modification of the 5–7 positions of Tic aromatic ring with various groups, such hydroxyl, halogen, nitro, and phenyl,[7] as well as substitution of Tic with its D-isomer, D- or L-Phe,[8] heteroaromatic or heteroaliphatic nuclei,[9] the conversion of the C-terminal carboxyl group to amide,[1,4b] ester,[4b] and alcohol,[1,10] and conjugation of a third aromatic center at the C-terminal with or without interposing linkers.[4c,11] Many of the analogues exhibited the desired properties, including enhanced δ-opioid antagonism,[1–11] conversion from a δ antagonist to a δ agonist,[11a] the appearance of opioids with mixed μ agonist/δ antagonist properties,[4b,c,11a,b] and the development of an irreversible fluorescent δ antagonist.[11c]

Recently, a series of homodimeric Dmt-Tic compounds were prepared by covalent linkage through a diaminoalkane and a symmetric or asymmetric 3,6-diaminoalkyl-2(1*H*)-pyrazinone.[12] The extraordinarily high δ-opioid-mediated antagonism (pA_2 = 10.3–11.2) was independent of the chemical nature or length of the linker between the Dmt-Tic pharmacophores.[12] Previously, Balboni et al.[11a] proposed that Dmt-Tic-mediated antagonism in C-terminally extended analogues containing benzimidazole or phenyl as the third aromatic center primarily depended on the length of the interposing linker between Tic and to a lesser degree on the aromatic moiety. In order to investigate the effect of another type of aromatic pharmacophore

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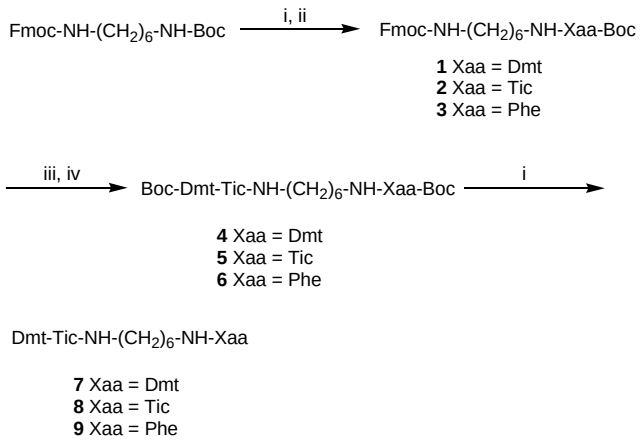

Scheme 1. Synthesis of Dmt-Tic-NH-$(CH_2)_6$-NH-Xaa. Reagents: (i) TFA/anisole; (ii) Boc-Xaa-OH, DIPEA, PyBop, DMF; (iii) 20% piperidine/DMF; (iv) Boc-Dmt-Tic-OH, DIPEA, PyBop, DMF.

and a longer alkyl spacer, heterodimers of H-Dmt-Tic-NH-hexyl-NH-R (R = Dmt, Tic, and Phe) were prepared (Scheme 1). These compounds had a biological activity profile similar to that of the Dmt-Tic homodimers, suggesting that the activity was affected predominantly by the Dmt-Tic pharmacophore. Furthermore, the third aromatic center (Dmt, Tic, or Phe) appeared to enhance interaction with μ-opioid receptors, but not biological activity as observed in other Dmt-Tic analogues containing C-terminal hydrophobic moieties[3,4] and various enkephalin analogues.[13]

Dmt was prepared according to Dygos et al.[14] and the catalyst, (*R*,*R*)-(−)-1,2-bis[(*O*-methoxyphenyl)(phenyl)-phosphino]ethane-(1,5-cyclooctadiene)rhodium(I) tetrafluorobroate,[14] was a product of Strem Chemicals Inc. Boc-Dmt-Tic-OH was prepared as described.[4b] Fmoc-NH-$(CH_2)_6$-NH-Boc was prepared from Boc-NH-$(CH_2)_6$-NH_2[15] by the usual method. Asymmetric bipharmacophoric compounds (**7**–**9**) were synthesized as shown in Scheme 1. Removal of Boc-group of Fmoc-NH-$(CH_2)_6$-NH-Boc with TFA/anisole and precipitation of the Fmoc-protected diaminohexane with ether yielded an oily material; therefore, the solvent was removed in vacuo and the oily material was used directly for condensation with Boc-Xaa-OH (Xaa = Dmt, Tic, and Phe) using PyBop as coupling reagent to give compounds **1**–**3**. The Fmoc-group of **1**–**3** was removed with 20% piperidine in DMF. After removal of solvent in vacuo, the residue was coupled with Boc-Dmt-Tic-OH using PyBop as coupling reagent in DMF to give compounds **4**–**6**. The Boc-protecting group was removed with TFA/anisole, and the crude compounds (**7**–**9**) were precipitated with ether and purified by semi-preparative RP-HPLC.[16]

The δ- and μ-opioid receptor affinities of the heterodimers were determined as previously published with rat brain membranes[17] (Table 1). The heterodimers **7**–**9** had quite high binding affinities to both δ- and μ-opioid receptors, comparable to those of the Dmt-Tic homodimers[12] and other Dmt-Tic analogues.[3b] Relative to the parent compound H-Dmt-Tic-NH_2 (Table 1), the heterodimers increased affinity to δ-opioid receptors by several fold, while μ affinity rose by over two orders magnitude. The third aromatic center interacted with and enhanced the affinity toward μ-opioid receptors as seen consistently with other Dmt-Tic analogues containing hydrophobic or aromatic groups at the C-terminus.[3b,4b] Interestingly, the replacement of one H-Dmt-Tic pharmacophoric unit from bis-[H-Dmt-Tic-NH]-hexyl[12] with H-Dmt (**7**) increased μ-opioid receptor affinity nearly 5-fold ($K_i\mu = 0.38$ nM) (Table 1). This phenomenon supports the observations that Dmt primarily improves the affinity to μ-opioid receptors more than to δ-opioid receptors,[18] although as a single amino acid (H-Dmt-NH-CH_3) it binds only to μ-opioid receptors.[19] The combination of Dmt with Tic, on the other hand, greatly enhanced the interaction with δ-opioid receptors.[1] Furthermore, substitution of Dmt (**7**) by Tic (**8**) or Phe (**9**) revealed a weaker μ-opioid receptor affinity than **7** demonstrating the efficacy of Dmt in the interaction with opioid receptor binding sites. Although Phe was observed to enhance the interaction with μ-opioid receptors,[4c,8] Tic and Phe had similar K_i values to that of the homodimer,[12] substantiating earlier studies that Tic has a preference for δ-opioid receptors.[1–11]

The functional biological activities were evaluated using classic pharmacological methods: isolated guinea-pig ileum (GPI) for μ-opioid receptors and mouse vas deferens (MVD) for δ-opioid receptors[20] (Table 2). A third aromatic center in the heterodimers (**7**–**9**) induced a greater than 50-fold increase in δ-receptor antagonism comparable to that observed with homodimers,[12] and being 2–3 orders of magnitude greater than the parent compounds and 10-fold greater

Table 1. Rat brain membrane opioid receptor binding affinity of Dmt-Tic heterodimers

Compound	Peptide	$K_i\delta$ (nM)[a,b]	$K_i\mu$ (nM)[a,c]	$K_i\delta/K_i\mu$
7	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Dmt-H	0.23 ± 0.02 (3)	0.38 ± 0.03 (3)	2
8	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Tic-H	0.13 ± 0.03 (4)	2.81 ± 0.16 (3)	22
9	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Phe-H	0.89 ± 0.10 (4)	1.91 ± 0.26 (5)	2
	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Tic-Dmt-H[d]	0.13 ± 0.03	1.79 ± 0.08	14
	H-Dmt-Tic-OH[e]	0.022	3320	150,780
	H-Dmt-Tic-NH_2[e]	1.22	277	227

[a] K_i values reported as mean ± SE; the number of independent repetitions is noted in parentheses (*n*).
[b] Versus [^{3}H]deltorphin II.
[c] Versus [^{3}H]DAMGO.
[d] Data cited from Ref. 12.
[e] Data cited from Ref. 1.

Table 2. Functional bioactivities of Dmt-Tic heterodimers

Compound	Peptide	MVD[a]		GPI[a]	
		Agonism IC_{50}, nM	Antagonsim[b] pA_2	Agonism IC_{50}, nM	Antagonism pA_2
7	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Dmt-H	>10,000	10.2	5900 ± 780	ND
8	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Tic-H	>10,000	10.4	1600 ± 250	ND
9	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Phe-H	>10,000	10.3	5550 ± 900	ND
	H-Dmt-Tic-NH-$(CH_2)_6$-NH-Tic-Dmt-H[c]	>10,000	10.6	2720 ± 1360	ND
	H-Dmt-Tic-OH	—	8.5	—	—
	H-Dmt-Tic-NH_2[d]		7.2	>10,000	—
	Naltrindole[d]	—	9.2	—	7.3

A dash (—) = none recorded; ND = not determined.

[a] The data are means of at least five to six independent repetitions using different isolated tissue preparations.

[b] Versus deltorphin II as the δ agonist.

[c] Data cited from Ref. 12.

[d] Data cited from Ref. 1.

than naltrindole (Table 2). Thus, while a single Dmt-Tic pharmacophoric unit contributed to the δ antagonism, the presence of a third aromatic center (Dmt, Tic, or Phe) C-terminal to the hexyl linker in the heterodimers apparently played an auxiliary role in recognition by the μ-opioid receptor,[3,4] or possibly acted in the stabilization of the ligand within the receptor.[18d] In contrast to the high μ-opioid affinity, the heterodimers **7–9** exhibited very weak μ agonism. In fact, while the presence of a third aromatic center in H-Dmt-Tic-NH-linker-R compounds generally enhances μ-receptor affinity with or without μ-opioid receptor-associated agonism,[4c,11a,11b] the lack of a biological response by these analogues suggests a difference between receptor binding and the triggering of μ agonism as observed previously.[3,4,13] Although the argument has been advocated that the difference in tissues used between assays might account for the discrepancies observed, the literature is replete with complete correlations between receptor affinity and functional bioactivity and considered sine qua non;[6,7,10,19] however, in some notable cases, a discrepancy occurs among these assays that lacks a plausible answer.[3,4,13c]

In conclusion, we developed a new series of Dmt-Tic heterodimers (H-Dmt-Tic-NH-hexyl-NH-R) in which the R group is an aromatic or hydrophobic center that contributes to their significantly increased μ affinity, although the compounds remained potent δ antagonists. Moreover, they should exhibit greater stability than the original dipeptides, H-Dmt-Tic-OH and H-Dmt-Tic-NH_2, which spontaneously cyclize to diketopiperazines.[21] Although the presence of a third aromatic center permitted interaction with μ-opioid receptor, that binding was insufficient to engage the tissue-bound receptor (GPI) to initiate μ agonism, presumably due to the considerably more potent effect dictated by the Dmt-Tic pharmacophore that is known to predominantly elicit δ antagonism.[3–5,7]

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16. Physicochemical data of compounds **7**–**9**. Dmt-Tic-NH-$(CH_2)_6$-NH-Dmt·2HCl (**7**). Yield 104.3 mg (94%); R_{f1} = 0.19 (*n*-BnOH:H_2O:AcOH = 4:1:5); R_{f2} = 0.73 (*n*-BnOH:H_2O:AcOH:pyridine = 4:1:1:2); $[\alpha]_D^{25}$ +58.13 (*c* = 0.42, H_2O); *m/z* 659 (MH^+). Anal. Calcd for $C_{38}H_{51}N_5O_5$·2HCl·4H_2O: C, 56.9; H, 7.60; N, 8.72. Found: C, 56.6; H, 7.21; N, 8.75.
Dmt-Tic-H-$(CH_2)_6$-NH-Tic·2HCl (**8**). Yield 95.4 mg (86.8%); R_{f1} = 0.18 (*n*-BnOH:H_2O:AcOH = 4:1:5); R_{f2} = 0.75 (*n*-BnOH:H_2O:AcOH:pyridine = 4:1:1:2); $[\alpha]_D^{25}$ −14.53 (*c* = 0.52, H_2O); *m/z* 627 (MH^+). Anal. Calcd for $C_{37}H_{47}N_5O_4$·2HCl·3H_2O: C, 59.0; H, 7.31; N, 9.30. Found: C, 58.8; H, 6.95; N, 9.29.
Dmt-Tic-NH-$(CH_2)_6$-NH-Phe·2HCl (**9**). Yield 104.6 mg (95.1%); R_{f1} = 0.17 (*n*-BnOH:H_2O:AcOH = 4:1:5); R_{f2} = 0.72 (*n*-BnOH:H_2O:AcOH:pyridine = 4:1:1:2); $[\alpha]_D^{25}$ +34.2 (*c* = 0.58, H_2O); *m/z* 615 (MH^+). Anal. Calcd for $C_{36}H_{47}N_5O_4$·2HCl·3.5H_2O: C, 57.7; H, 7.47; N, 9.34. Found: C, 57.8; H, 7.19; N, 9.39.
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