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Design and Synthesis of Opioidmimetics Containing 2',6'-Dimethyl-L-tyrosine and a Pyrazinone-Ring Platform

Kimitaka Shiotani^a, Tingyou Li^a, Anna Miyazaki^b, Yuko Tsuda^{a,b}, Toshio Yokoi^{a,b}, Akihiro Ambo^c, Yusuke Sasaki^c, Sharon D. Bryant^d, Lawrence H. Lazarus^d, and Yoshio Okada^{a,b,*}

^aThe Graduate School of Food and Medicinal Sciences, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan

^bFaculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan

^cDepartment of Biochemistry, Tohoku Pharmaceutical University, Aoba-ku, Sendai 981-8558, Japan

^dMedicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, U.S.A.

Abstract

Twelve 2',6'-dimethyl-L-tyrosine (Dmt) analogues linked to a pyrazinone platform were synthesized as 3- or 6-[H-Dmt-NH(CH₂)_n]-3- or 6-R-2(1*H*)-pyrazinone (n = 1 - 4). 3-[H-Dmt-NH-(CH₂)₄]-6-β-phenethyl-5-methyl-2(1*H*)-pyrazinone **11** bound to μ-opioid receptors with high affinity ($K_{i\mu}$ = 0.13 nM; $K_i\delta/K_{i\mu}$ = 447) with μ-agonism (GPI IC₅₀ = 15.9 nM) and weak δ-antagonism (MVD pA₂ = 6.35). Key factors affecting opioid affinity and functional bioactivity are the length of the aminoalkyl chain linked to Dmt and the nature of the R residue. These data present a simplified method for the formation of pyrazinone opioidmimetics and new lead compounds.

Keywords

2',6'-Dimethyl-L-tyrosine; Pyrazinone; μ-Selective opioid ligand; μ-Agonist/δ-Antagonist; μ-Antagonist/δ-Antagonist

The N-terminal Tyr residue is essential for the opioid receptor interactions in opioid peptides, 1-6 except nociceptin,⁷ which contains Phe instead of Tyr. It is well known that replacement of Tyr in opioid peptides and opioidmimetics with 2',6'-dimethyl-L-tyrosine (Dmt) dramatically enhances receptor affinity and functional bioactivity.⁸ While H-Dmt-NHCH₃ specifically interacts with μ-opioid receptor ($K_{i\mu}$ = 7.45 nM), indicating that Dmt itself could act as a part of message domain, it was insufficient to trigger a biological reaction.⁹ Recently, the dimerization of the Dmt pharmacophore through diaminoalkane¹⁰ or diaminoalkyl-pyrazinone,¹¹ exhibited high μ-affinity and potent in vitro and in vivo functional bioactivity. It could be assumed that one Dmt residue interacts within message-binding domain, while another might lie in the address domain of the receptor.

The antinociceptive activity of one of Dmt-pyrazinone dimers, 3-[4'-(H-Dmt)-aminobutyl]-6-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone was 65-71 times greater than with morphine after icv administration in mice based on the tail-flick and hot-plate tests.¹² On the other hand, after sc administration it was equivalent to morphine in tail-flick test and 89% as effective by the hot-plate test; however, after oral administration it exhibited only 65% and 16% the activity of morphine on these tests, respectively.¹² The results indicated a degree of

*Corresponding author. Tel.: +81 78 974 1551; fax: +81 78 974 5689; e-mail: okada@pharm.kobegakuin.ac.jp

enzymatic stability and the ability to transit epithelial membranes through the gastrointestinal tract and blood-brain barrier.¹²⁻¹⁴ Thus, the pyrazinone-ring may be an ideal platform on which to develop stable opioids with sufficient lipophilicity suitable for clinical and therapeutic applications.

To further examine the role of the pyrazinone-ring in opioidmimetics, we prepared compounds (**1-12**) as shown in Figure 1 and examined their receptor affinities and in vitro functional bioactivities.

Compounds (**1-12**) were synthesized according to Scheme 1 starting from dipeptidyl chloromethyl ketones (**1a-l**).¹⁵ After removal of the Boc group from the dipeptidyl chloromethyl ketones (**1a-l**), the resulting amine hydrochlorides were treated in methanol at 60 °C to give Z-protected pyrazinone derivatives (**2a-l**), in which the different and desired moieties are covalently bound to positions 3 and 6. Z-Protection was removed by HBr/CH₃COOH to release the amine group, which was then coupled with Boc-Dmt-OH^{8b,16} using PyBop to produce Boc-protected Dmt analogues containing a pyrazinone-ring (**3a-l**). The Boc group was removed by 7 M HCl/dioxane to give the crude final compounds as hydrochloride salt (**1-12**), which were purified by semi-preparative HPLC [column: Cosmosil ODS (20 × 250 mm) in an initial 0.05% TFA acetonitrile/0.05% TFA water gradient (10:90) to (90:10) for 80 min, flow rate: 10 mL/min]. The identification and purity of the final compounds were assessed using MS, ¹H and ¹³C NMR, analytical HPLC¹⁷, and elemental analysis.¹⁸ The compounds exhibited greater than 98% purity.

The radioreceptor assay¹⁹ used [³H]DAMGO (H-Tyr-D-Ala-Gly-N-MePhe-Gly-ol) and [³H]deltorphin II for μ- and δ-opioid receptors, respectively. The affinities of compounds **1-12** are summarized in Table 1. Compounds **1**, **9**, **10**, **11** and **12** exhibited subnanomolar affinities ($K_{i\mu}$). All the compounds exhibited μ-selectivity ($K_{i\delta}/K_{i\mu} = 1.2 - 447$). Compounds **1** and **3** exhibited 92-fold and 3.6-fold higher μ-affinity relative to **2** and **4**, respectively, indicating that the Dmt residue at position 3 is preferable relative to position 6 of the pyrazinone-ring. Compound **3** exhibited the highest μ-affinity, indicating that the H-Dmt-NH-(CH₂)₄- moiety is the most suitable derivative among compounds **3**, **5-7**. Compound **1** ($R^1 = H$) exhibited subnanomolar μ-affinity, indicating that the pyrazinone-ring played a role to enhance affinity. Although the $K_{i\mu}$ of **9** ($R^1 = \text{benzyl}$) is only 1.5-fold higher than that of **12** ($R^1 = 2',6'$ -dimethylbenzyl), the increase in hydrophobicity introduced by dimethylation on phenyl-ring of benzyl moiety at position 6 did not substantially or significantly affect μ-affinity. Compound **11** (β-phenethyl) exhibited the highest μ-affinity ($K_{i\mu} = 0.125 \text{ nM}$) of the substances in Table 1. The phenyl moiety at R^1 contributed to increased μ-affinity.

The GPI (guinea-pig ileum) and MVD (mouse vas deferens) functional bioactivity assays were performed as described previously.¹⁹ The results are summarized in Table 1. All the compounds exhibited a relatively weak δ-antagonism ($pA_2 = 5.5$ to 6.61) with very low agonist potency (less than 34 % inhibition at a dose of 10 μM).

Compound **11** ($n = 4$, $R^1 = \beta\text{-phenethyl}$) exhibited mixed μ-agonism/δ-antagonism. The phenyl moiety at R^1 of **11** might be able to bind to the μ-opioid receptor as part of the address domain of the ligand. While **10** ($n = 3$, $R^1 = \beta\text{-phenethyl}$) and **12** ($n = 4$, $R^1 = 2',6'$ -dimethylbenzyl) had very weak μ-agonism ($IC_{50} = 5547$ and 6273 nM , respectively), **1-9** exhibited essentially no μ-agonism with inhibition of less than 47 % at a dose of 10 μM. Interestingly, only compounds **2-4**, **10** and **12** behaved as weak antagonists toward the μ-opioid receptor as well, which were not previously observed with other Dmt-pyrazinone analogues.¹⁰⁻¹²

In the series of opioidmimetics which contains a single Dmt residue, μ-affinity is consistently lower than that of the opioidmimetic analogues with two Dmt residues.¹⁰⁻¹² In the case of Dmt dimers, both Dmt residues might participate in binding to the μ-opioid receptor as

constituents of the message and address domains, while in the Dmt monomeric analogues, the Dmt residue could act by anchoring the compound only within μ -opioid receptors. The distance between the two Dmt pharmacophores in the dimerized ligands might be better accommodated in the μ -opioid receptor. On the other hand, the distance between Dmt and another aromatic residue or the residue itself might not be appropriate for binding to the μ -opioid receptor. While μ -antagonists are important pharmacological tools, not only to delineate critical biochemical, pharmacological, and physiological roles played by these receptors, but also to serve as clinically and therapeutically relevant agents,²⁰ compounds **10** and **12** may be lead compounds for the further development of new μ -opioid receptor antagonists.

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17. The analytical HPLC condition for Compounds **1-12**. HPLC conditions: column, Cosmosil ODS (4.6×250 mm); solvents program, A: 0.05% TFA (trifluoroacetic acid)/water, B: 0.05% TFA/ acetonitrile, A:B (90:10) to A:B (10:90) in 40 min; flow rate: 1 mL/min; detection: UV 220 nm
18. Elemental analysis data of Compounds **1-12**. 3-[4'-(H-Dmt)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**1**): Calcd for C₂₀H₂₈N₄O₃· 2.5H₂O·HCl: C, 47.2; H, 6.85; N, 10.0. Found: C, 46.9; H, 6.93; N, 10.9. 6-[4'-(H-Dmt)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**2**): Calcd for C₂₀H₂₈N₄O₃· H₂O·HCl: C, 46.9; H, 6.93; N, 11.0. Found: C, 46.5 H, 6.88, N, 10.6. 3-[4'-(H-Dmt)-aminobutyl]-5,6-dimethyl-2(1*H*)-pyrazinone hydrochloride (**3**): Calcd for C₂₁H₃₀N₄O₃· 2.5H₂O·HCl: C, 50.6; H, 6.65; N, 10.27. Found: C, 49.77 H, 7.13, N, 11.1. 6-[4'-(H-Dmt)-aminobutyl]-3,5-dimethyl-2(1*H*)-pyrazinone hydrochloride (**4**): Calcd for C₂₁H₃₀N₄O₃· 4H₂O·HCl: C, 48.25; H, 6.87; N, 9.79. Found: C, 48.8 H, 6.22, N, 10.4. 3-[3'-(H-Dmt)-aminopropyl]-5,6-dimethyl-2(1*H*)-pyrazinone hydrochloride (**5**): Calcd for C₂₀H₂₈N₄O₃· H₂O·HCl: C, 48.9; H, 6.53; N, 10.5. Found: C, 49.0; H, 6.38; N, 10.4. 3-[2'-(H-Dmt)-aminoethyl]-5,6-dimethyl-2(1*H*) pyrazinone hydrochloride (**6**): Calcd for C₁₉H₂₆N₄O₃· 3H₂O·HCl: C, 47.9; H, 6.32; N, 10.7. Found: C, 47.9; H, 6.42; N, 10.6. 3-[(H-Dmt)-aminomethyl]-5,6-dimethyl-2(1*H*)-pyrazinone hydrochloride (**7**): Calcd for C₁₈H₂₄N₄O₄· 2H₂O·HCl: C, 51.9; H, 7.01; N, 13.4. Found: C, 51.7 H, 6.93, N, 13.5. 6-Benzyl-3-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**8**): Calcd for C₂₆H₃₂N₄O₃· 3H₂O·HCl: C, 54.9; H, 6.37; N, 9.09. Found: C, 54.5; H, 6.31, N, 8.83. 6-Benzyl-3-[4'-(H-Dmt)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**9**): Calcd for C₂₇H₃₄N₄O₃· H₂O·HCl: C, 62.7; H, 7.21; N, 10.8. Found: C, 62.9; H, 7.23; N, 11.2. 6-β-phenethyl-3-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**10**): Calcd for C₂₇H₃₄N₄O₃· 2.5H₂O·HCl: C, 59.6; H, 7.41; N, 10.3. Found: C, 59.6; H, 6.99; N, 10.7. 6-β-phenethyl-3-[4'-(H-Dmt)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**11**): Calcd for C₂₈H₃₆N₄O₃· 2H₂O·HCl: C, 61.3; H, 7.53; N, 10.2. Found: C, 61.5; H, 7.66; N, 9.81. 3-[4'-(H-Dmt)-aminobutyl]-6-[2',6'-dimethyl benzyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**12**): Calcd for C₂₉H₃₈N₄O₃· HCl·0.13H₂O: C, 60.2; H, 6.88; N, 9.22. Found: C, 60.2; H, 6.95; N, 9.25
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Figure 1.
The structure of Dmt-pyrazinone derivatives (**1-12**)

Scheme 1.

Synthetic method for pyrazinone-ring containing opioidmimetics (**1-12**). i 4M HCl/dioxane; ii CH₃OH, at 60 °C; iii 25% HBr/CH₃COOH; iv Boc-Dmt-OH, PyBop, DIPEA.

Table 1
Opioid receptor binding affinity and functional bioactivity of compounds (1-12)

Compounds	K_{10} (nM) (n^a)	K_{10} (nM) (n^a)	K_{10}/K_{10}	GPI IC ₅₀ ^b (nM)	pA ₂ ^c	MVD IC ₅₀ ^b (nM)	pA ₂ ^c
DAMGO ^d	2.29	1.30	57	11.5	-	76	-
Deltorphin II ^e	272 ± 50 (11)	0.24 ± 0.06 (6)	1135 ^e	420 ± 95	-	0.14 ± 0.06	-
1	0.62 ± 0.068 (6)	73.80 ± 6.40 (5)	119	>10000	-	>10000	<5.5
2	56.9 ± 5.30 (3)	343.7 ± 26.0 (5)	6	>10000	<6.0	>10000	<5.5
3	4.23 ± 0.65 (3)	77.7 ± 6.1 (4)	18	>10000	6.70	>10000	5.60
4	15.4 ± 1.36 (3)	208.5 ± 23.0 (6)	14	>10000	<6.0	>10000	5.63
5	8.88 ± 0.77 (3)	269.4 ± 29.0 (3)	30	>10000	-	>10000	<5.5
6	76.5 ± 9.20 (7)	93.10 ± 16.0 (5)	1.2	>10000	-	>10000	5.87
7	129.1 ± 19.00 (3)	698.1 ± 30.0 (5)	5.4	>10000	-	>10000	5.83
8	1.00 ± 0.02 (3)	59.60 ± 5.3.0 (4)	60	>10000	-	>10000	5.91
9	0.42 ± 0.01 (3)	8.60 ± 0.70 (3)	20	>10000	-	>10000	6.23
10	0.55 ± 0.09 (3)	38.2 ± 2.60 (3)	69	5547 ± 741	<6.0	>10000	5.87
11	0.125 ± 0.002 (3)	55.9 ± 8.30 (3)	447	15.9 ± 2.8	-	>10000	6.35
12	0.66 ± 0.05 (5)	5.3 ± 0.8 (5)	8	6273 ± 895	<6.0	>10000	6.61

^aRepetitions (n) are five-seven times for each bioassay.

^bAgonists inhibited the electrically evoked twitch (IC₅₀). Values in parentheses indicate maximal inhibition of the tissue contraction at the concentration of 10,000 nM. Endomorphin 2 and deltorphin II were used as the μ - and δ -opioid receptor agonist standard peptides, respectively.

^cThe pA₂ (antagonism) is the negative logarithm (M = concentration) required to double the concentration of a δ -opioid receptor agonist (deltorphin II) in MVD assay or of a μ -opioid receptor agonist (endomorphin 2) in GPI assay to achieve the original response. - denotes no antagonism.

^dDAMGO is the standard μ -selective opioid agonist (data from ref. 21).

^eDeltorphin II is a δ -selective (K_{10}/K_{10}) amphibian opioid agonist (data from ref. 22).