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Design and synthesis of opioidmimetics containing 2′,6′-dimethyl-L-tyrosine and a pyrazinone-ring platform

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Abstract—Twelve 2′,6′-dimethyl-L-tyrosine (Dmt) analogues linked to a pyrazinone platform were synthesized as 3- or 6-[H-Dmt-NH$(CH_2)_n$],3- or 6-*R*-2(1*H*)-pyrazinone (n = 1–4). 3-[H-Dmt-NH-$(CH_2)_4$]-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 IC_{50} = 15.9 nM) and weak δ-antagonism (MVD pA_2 = 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.

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-$NHCH_3$ 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 diaminoalkane[10] 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 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

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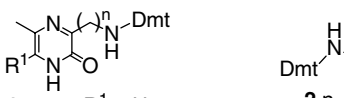


1 n = 4, R^1 = H
3 n = 4, R^1 = CH_3
5 n = 3, R^1 = CH_3
6 n = 2, R^1 = CH_3
7 n = 1, R^1 = CH_3
8 n = 3, R^1 = benzyl
9 n = 4, R^1 = benzyl
10 n = 3, R^1 = β-phenethyl
11 n = 4, R^1 = β-phenethyl
12 n = 4, R^1 = 2',6'-dimethylbenzyl

2 n = 4, R^2 = H
4 n = 4, R^2 = CH_3

Figure 1. The structure of Dmt-pyrazinone derivatives (**1**–**12**).

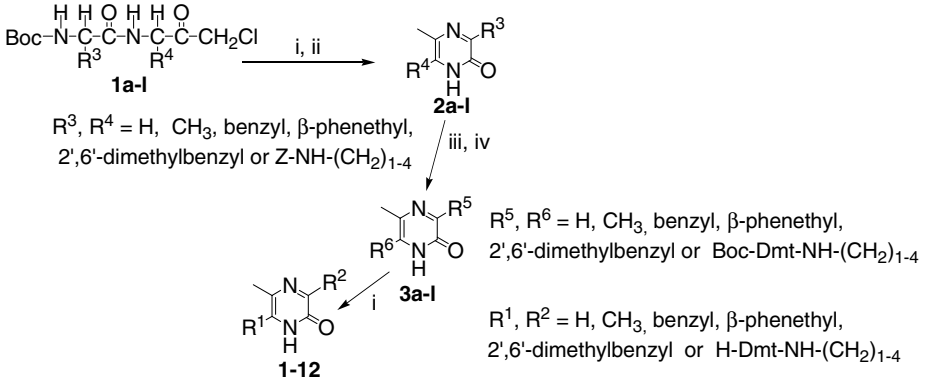
(**1a**–**l**).[15] After removal of the Boc group from the dipeptidyl chloromethyl ketones (**1a**–**l**), the resulting amine hydrochlorides were treated in methanol at 60 °C to give Z-protected pyrazinone derivatives (**2a**–**l**), in which the different and desired moieties are covalently bound to positions 3 and 6. Z-Protection was removed by HBr/CH_3COOH 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 ^{13}C NMR, analytical HPLC,[17] and elemental analysis.[18] The compounds exhibited greater than 98% purity.

The radioreceptor assay[19] 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_iμ). All the compounds exhibited μ-selectivity (K_iδ/K_iμ = 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-$(CH_2)_4$-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_iμ of **9** (R^1 = 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** (R^1 = β-phenethyl) exhibited the highest μ-affinity (K_iμ = 0.125 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 = β-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 = β-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



Scheme 1. Synthetic method for pyrazinone-ring containing opioidmimetics (**1**–**12**). Reagents and condition: (i) 4MHCl/dioxane; (ii) CH_3OH, at 60 °C; (iii) 25% HBr/CH_3COOH; (iv) Boc-Dmt-OH, PyBop, DIPEA.

Table 1. Opioid receptor binding affinity and functional bioactivity of compounds (**1**–**12**)

Compound	$K_i\mu$ (nM) (*n*)[a]	$K_i\delta$ (nM) (*n*)[a]	$K_i\delta/K_i\mu$	GPI IC_{50}[b] (nM)		pA_2[c]	MVD IC_{50}[b] (nM)		pA_2[c]
DAMGO[d]	2.29	130	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	>10,000	(47.6%)	—	>10,000	(25.7%)	<5.5
2	56.9 ± 5.30 (3)	343.7 ± 26.0 (5)	6	>10,000	(37.1%)	<6.0	>10,000	(28.1%)	<5.5
3	4.23 ± 0.65 (3)	77.7 ± 6.1 (4)	18	>10,000	(35.1%)	6.70	>10,000	(26.2%)	5.60
4	15.4 ± 1.36 (3)	208.5 ± 23.0 (6)	14	>10,000	(30.1%)	<6.0	>10,000	(0.0%)	5.63
5	8.88 ± 0.77 (3)	269.4 ± 29.0 (3)	30	>10,000	(22.7%)	—	>10,000	(31.1%)	<5.5
6	76.5 ± 9.20 (7)	93.10 ± 16.0 (5)	1.2	>10,000	(45.0%)	—	>10,000	(25.2%)	5.87
7	129.1 ± 19.00 (3)	698.1 ± 30.0 (5)	5.4	>10,000	(20.6%)	—	>10,000	(28.1%)	5.83
8	1.00 ± 0.02 (3)	59.60 ± 5.30 (4)	60	>10,000	(36.7%)	—	>10,000	(34.6%)	5.91
9	0.42 ± 0.01 (3)	8.60 ± 0.70 (3)	20	>10,000	(39.5%)	—	>10,000	(19.9%)	6.23
10	0.55 ± 0.09 (3)	38.2 ± 2.60 (3)	69	5547 ± 741		<6.0	>10,000	(4.1%)	5.87
11	0.125 ± 0.002 (3)	55.9 ± 8.30 (3)	447	15.9 ± 2.8		—	>10,000	(12.8%)	6.35
12	0.66 ± 0.05 (5)	5.3 ± 0.8 (5)	8	6273 ± 895		<6.0	>10,000	(6.4%)	6.61

—, denotes no antagonism.

[a] Repetitions (*n*) are 5–7 times for each bioassay.

[b] Agonists inhibited the electrically evoked twitch (IC_{50}). 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.

[c] The pA_2 (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.

[d] DAMGO is the standard μ-selective opioid agonist (data from Ref. 21).

[e] Deltorphin II is a δ-selective ($K_i\mu/K_i\delta$ = 1135) amphibian opioid agonist (data from Ref. 22).

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 $C_{20}H_{28}N_4O_3 \cdot HCl \cdot 2.5\ H_2O$: C, 47.2; H, 6.85; N, 10.0. Found:C, 46.9; H, 6.93; N, 10.3. 6-[4′-(H-Dmt)-aminobutyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**2**): Calcd for $C_{20}H_{28}N_4O_3 \cdot HCl \cdot H_2O$: C, 46.9; H, 6.93; N, 11.0. Found:C, 46.5 H, 6.88, N, 10.7. 3-[4′-(H-Dmt)-aminobutyl]-5,6-dimethyl-2(1*H*)-pyrazinone hydrochloride (**3**): Calcd for $C_{21}H_{30}N_4O_3 \cdot HCl \cdot 2.5H_2O$: C, 50.6; H, 6.93; N, 10.3. Found:C, 50.3; H, 7.13, N, 10.7. 6-[4′-(H-Dmt)-aminobutyl]-3,5-dimethyl-2(1*H*)-pyrazinone hydrochloride (**4**): Calcd for $C_{21}H_{30}N_4O_3 \cdot HCl \cdot 4\text{-}H_2O$: C, 48.3; H, 6.87; N, 9.79. Found:C, 48.5 H, 6.82, N, 10.0. 3-[3′-(H-Dmt)-aminopropyl]-5,6-dimethy-2(1*H*)-pyrazinone hydrochloride (**5**): Calcd for $C_{20}H_{28}N_4O_3 \cdot HCl \cdot H_2O$: 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-dimethy-2(1*H*) pyrazinone hydrochloride (**6**): Calcd for $C_{19}H_{26}N_4O_3 \cdot HCl \cdot 3H_2O$: 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-dimethy-2(1*H*)-pyrazinone hydrochloride (**7**): Calcd for $C_{18}H_{24}N_4O_4 \cdot HCl \cdot 2H_2O$: 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 $C_{26}H_{32}N_4O_3 \cdot HCl \cdot 3H_2O$: 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 $C_{27}H_{34}N_4O_3 \cdot HCl \cdot H_2O$: 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 $C_{27}H_{34}N_4O_3 \cdot HCl \cdot 2.5H_2O$: 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 $C_{28}H_{36}N_4O_3 \cdot HCl \cdot 2H_2O$: 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′-dimethylbenzyl]-5-methyl-2(1*H*)-pyrazinone hydrochloride (**12**): Calcd for $C_{29}H_{38}N_4O_3 \cdot HCl \cdot 1.3H_2O$: C, 60.2; H, 6.88; N, 9.22. Found: C, 60.2; H, 6.95; N, 9.25.
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