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Synthesis of 3,6-bis[H-Tyr/H-Dmt-NH$(CH_2)_{m,n}$]-2(1*H*)pyrazinone derivatives: Function of alkyl chain length on opioid activity

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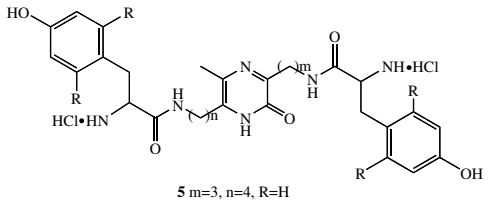
Abstract—Dimeric opioid analogues linked to a pyrazinone platform, 3-[Tyr/Dmt-NH$(CH_2)_m$]-6-[Tyr/Dmt-NH$(CH_2)_n$]-2(1*H*)-pyrazinone (*m*, *n* = 3 or 4), were synthesized. The Tyr-containing compound (*m* = 4, *n* = 3) exhibited μ-receptor affinity (K_iμ; 7.58 nM) comparable to that of morphine, while the Dmt derivatives exhibited considerably higher affinity (K_iμ; 0.021–0.051 nM) with corresponding agonism (IC_{50} = 1.79–4.93 nM). Interestingly one compound (*m* = 4, *n* = 3) revealed modest δ-opioid agonism; the converse analogue (*m* = 3, *n* = 4), however, was inactive in MVD assay.

The N-terminal Tyr residue is essential for the opioid receptor interactions in peptides,[1–6] except nociceptin,[7] which contains Phe in lieu of Tyr, and is an integral component of the 'message domain'.[8] While several amino acids were found to replace Tyr in opioids,[9] 2′,6′-dimethyl-L-tyrosine (Dmt) dramatically enhanced receptor affinity and functional bioactivity, and consistently altered receptor selectivity. In fact, H-Dmt-NH-CH_3[10] was found to specifically interact with the μ-opioid receptor (K_iμ = 7.45 nM), although H-Tyr-NH-CH_3 interacted with neither μ- nor δ-opioid receptors (K_iμ = 23,000 nM); however, H-Dmt-NH-CH_3 could not trigger a biological reaction. Incorporation of Dmt into opioid ligands greatly increased biological activity as seen with Dmt-Tic pharmacophore,[11] endomorphins,[12,13] [Dmt[1],Leu[5]]-enkephalin, [14] bis[Dmt-NH]alkane,[15] and the symmetrically substituted 3,6-bis[Dmt-aminoalkyl]-pyazinone derivatives.[16] The 3,6-bis[Dmt-aminoalkyl]-pyazinone analogues exhibited not only high μ- and δ-opioid affinities, but also unique in vitro functional biological activity profiles (GPI and MVD bioassays), and potent in vivo antinociceptive activity after intracerebroventricular (icv), subctaneous (sc), and oral (po) administration.[16] Thus, we directed our studies to the further development of 3,6-bis[Tyr- or Dmt-aminoalkyl]-pyrazinone derivatives for their application as possible therapeutics. Previously,[16] we synthesized eight kind of 3,6-bis[Tyr- or Dmt-aminoalkyl]-pyrazinone derivatives (Fig. 1, *m* = *n*: 1–4) and revealed that 3,6-bis[Tyr or Dmt-aminoalkyl]-pyrazinone derivatives (Fig. 1, *m* = *n*: 3) exhibited the highest



5 m=3, n=4, R=H
6 m=3, n=4, R=CH_3
7 m=4, n=3, R=H
8 m=4, n=3, R=CH_3

Figure 1. Structure of compounds **5**–**8**.

Keywords: 2′,6′-Dimethyl-L-tyrosine; Pyrazinone; μ-Selective opioid ligand.
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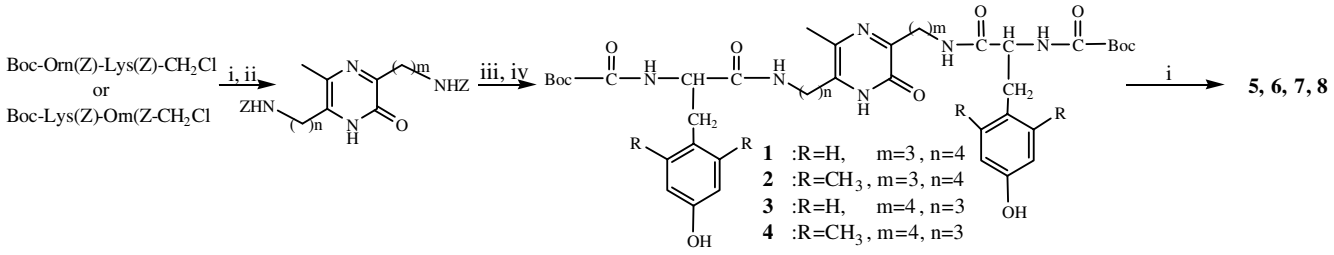
μ-opioid affinity ($K_i\mu$ = 25.7 and 0.042 nM for Tyr and Dmt derivative, respectively); and the compound ($m = n$: 4), followed the above compounds ($K_i\mu$ = 70.2 and 0.114 nM for Tyr and Dmt derivative, respectively). To determine the effect of different chain lengths of the aminoalkyl linkers of pyrazinone derivatives on opioid properties and to develop more potent opioidmimetic analgesics, the unsymmetrical aminoalkyl linkers (*m*, n = 3 or 4) were covalently bound at the positions 3 and 6 of pyrazinone ring platform (Fig. 1, **5**–**8**), which could provide a further basis for uncovering the discrete and subtle differences in opioidmimetic substances to produce biological effect.

Compounds were synthesized as shown in Scheme 1 starting from dipeptidyl chloromethyl ketones.[17] After removal of Boc group of dipeptidyl chloromethyl ketone, the resulting hydrochlorides were treated in acetonitrile at 60 °C to afford Z-protected pyrazinone derivative, in which the different and desired aminoalkyl moieties are covalently bound to positions 3 and 6. Z-Protection of compounds was removed by HBr/AcOH to release the amine groups, which were then coupled with Boc-Tyr-OH or Boc-Dmt-OH[18] using PyBop reagent to produce asymmetrical Boc-protected 3,6-bis(Tyr- or Dmt-aminoalkyl)-pyrazinone derivatives (**1**–**4**). The Boc group was removed by 7 N HCl/dioxane to give the 3,6-bis(H-Tyr- or H-Dmt-aminoalkyl)-pyrazinone·2HCl (**5**–**8**). The crude final products were purified by semi-preparative HPLC [column: YMC pack R&D ODS (4.6 × 250 mm) in an initial acetonitrile/water gradient (10:90) for 20 min to 50:50 for 40 min and finally 90:10 for 5 min]. The identification and purity of the final compounds were assessed using MS, ^{1}H and ^{13}C NMR, analytical HPLC, and elemental analysis.[19] The compounds exhibited greater than 98% purity.

The competitive displacement assay[12] was performed using [^{3}H]DAMGO (H-Tyr-D-Ala-Gly-N$^{\alpha}$-MePhe-Gly-ol) and [^{3}H]deltorphin II for μ- and δ-opioid receptors, respectively. The affinities are summarized in Table 1 with the relative activity (RA, the difference between Tyr and Dmt). Of the Tyr dimers, **7** (m = 4, n = 3) exhibited a higher μ-receptor affinity ($K_i\mu$ 7.38 nM), similar to that of morphine with a higher receptor selectivity ($K_i\delta/K_i\mu$ = 220) compared with a previous report ($K_i\mu$ = 25.7–460 nM and $K_i\delta/K_i\mu$ = 4.0–31).[16] Substitution by Dmt (**8**) enhanced μ-opioid receptor affinity 361-fold ($K_i\mu$ = 0.021 nM) with higher selectivity ($K_i\delta/K_i\mu$ = 1519). Similarly, the affinity of **5** increased 537-fold upon replacement of Tyr by Dmt (**6**), comparable to published data;[16] however, in the case of 1,4-bis-[Dmt-NH]butane, the relative activity rose to 7500,[15] while in the case of endomorphin-2, RA value was only 4.6.[12] These results further support the observations that Dmt in opioidmimetics plays an important role to anchoring the compounds within opioid receptors to elicit a higher degree of binding[12–16] and suggest that an unsymmetrical alkyl chain at position 3 or 6 of the pyrazinone ring produced a more favorable conformation for affinity and selectivity to the receptor. The δ-opioid receptor affinity of **5** and **7** is essentially non-existent, whereas the Dmt derivatives (**6** and **8**) exhibited $K_i\delta$ = 18.8 and 31.9 nM, respectively, with relative activity of 147- and 29-fold, respectively. Interestingly, although Dmt derivatives increased affinities compared with those of the corresponding Tyr derivatives for both μ- and δ-opioid receptors, they are still μ-selective opioid ligands.

In the functional bioactivity[12] (Table 1), **6** and **8** exhibited μ-agonism and they were either inactive (**6**) or exhibited weak agonism (**8**); however, even at this level, the maximum inhibition of muscle contraction was only 60% in the MVD assay. Other Dmt opioid derivatives[12,15,16] were reported to exhibit full μ-agonist activity, but [Dmt^1]-endomorphin-2 was a full μ and δ agonist.[12] While the differences in the opioid receptor affinity between **6** and **8** are not significant, the functional bioactivity revealed a major difference in the effect of the length of the aminoalkyl chain and its position on the pyrazinone ring; namely, relative to **6**, the agonism elicited by **8** for μ- and δ-opioid receptors more than doubled and increased by over 40-fold, respectively.

Thus, from the data on asymmetrical pyrazinone derivatives (**5**–**8**), differences in not only chain lengths, but also the position (3 or 6) on the pyrazinone ring to which aminoalkyl chains are bound, affected biological activity and allowed the opioidmimetics to differentially interact with the ligand-binding domains of μ- and δ-opioid receptors. These data also opened further possibility for rational design of potential opioid mimetic drugs that are more μ-selective. It is also emphasized that our rapid and facile synthetic procedure of pyrazinone ring from dipeptidyl chloromethyl ketones[20] could provide the asymmetric pyrazinone derivatives.



Scheme 1. Synthesis of bis-Tyr-pyrazinone (**5** and **7**) or bis-Dmt-pyrazinone opioidmimetics (**6** and **8**). Reagents and conditions: (i) 7 N HCl/dioxane; (ii) CH_3CN, 60 °C; (iii) 25% HBr/AcOH; (iv) Boc-Tyr-OH or Boc-2′,6′-dimethyl-L-tyrosine (Boc-Dmt-OH), PyBop, DIEA, DMF; Z = benzyloxycarbonyl.