Published in final edited form as:

Bioorg Med Chem Lett. 2006 November 15; 16(22): 5793–5796. doi:10.1016/j.bmcl.2006.08.079.

Synthesis of 3,6-bis[H-Tyr/H-Dmt-NH(CH₂)_{m,n}]-2(1*H*)pyrazinone derivatives: function of alkyl chain length on opioid activity

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

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

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

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

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

Abstract

Dimeric opioid analogues linked to a pyrazinone platform, 3-[Tyr-NH(CH₂)_m]-6-[Tyr-NH(CH₂)_n]-2(1*H*)-pyrazinone (n, m = 3 or 4) and their corresponding Dmt derivatives were synthesized. Whereas the Tyr-containing compounds were essentially inactive, the Dmt derivatives exhibited high affinity for the μ -opioid receptor ($K_i\mu$; 0.021–0.051 nM) with corresponding agonism (IC₅₀ = 1.79–4.93 nM). Interestingly, while one compound (m = 4, n = 3) revealed modest δ -agonism, the converse analogue (m = 3, n = 4) was inactive. The data suggest that the spatial conformation, linker length and covalent bonding position on the pyrazinone ring are important for opioid activity.

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-CH₃¹⁰ was found to specifically interact with the μ -opioid receptor even though Dmt when incorporated into opioid ligands, it greatly increased δ -opioid affinity as seen with Dmt-Tic pharmacophore,¹¹ endomorphins,^{12,13} [Dmt¹,Leu⁵]-enkephalin,¹⁴ bis-[DmtNH]-alkane,¹⁵ and the symmetrically substituted 3,6-bis-[Dmt-aminoalkyl]-pyrazinone derivatives.¹⁶ Thus, the Dmt residue, which is an essential part of the message domain anchors the opioid within the ligand binding domain of the receptor to affect *in vitro* and *in vivo* biological activity. To determine the effect of different lengths of aminoalkyl linkers on the opioid properties of pyrazinone derivatives, this communication also demonstrates the influence of Tyr or Dmt residues on receptor interaction. The unsymmetrical aminoalkyl linkers were covalently bound at the 3 and 6 positions of the pyrazinone ring platform (Figure 1) which could provide a further basis for uncovering the discrete and subtle differences in opioidmimetic substances that produce a biological effect.

Compounds were synthesized as shown in Scheme 1 starting from dipeptidyl chloromethyl ketones.¹⁷ 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-OH¹⁸ using PyBop reagent

*Corresponding author: Y. Okada: Tel: 81-78-974-1551, Fax: 81-78-974-5689 E-mail: okada@pharm.kobegakuin.ac.jp.

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, ¹H- and ¹³C-NMR, analytical HPLC and elemental analysis.¹⁸ The compounds exhibited greater than 98% purity.

The competitive displacement assay¹² was performed using [³H]DAMGO (H-Tyr-D-Ala-Gly-N^α-MePhe-Gly-ol) and [³H]deltorphin II for μ- and δ-opioid receptors, respectively. The binding affinities are summarized in Table 1 with the relative activity (RA, being the difference between Tyr and Dmt). Of the Tyr dimers, **7** (m = 4, n = 3) exhibited the lowest K_{iμ} (7.38 nM,) and substitution by Dmt (**8**) enhanced μ-opioid receptor affinity 361-fold (K_{iμ} = 0.021 nM). Similarly, the affinity of **5** increased 537-fold with the replace of Tyr by Dmt, comparable to published data;¹⁶ however, in the case of 1,4-bis-[Dmt-NH]-butane, the relative activity rose to 7,500,¹⁴ but only a 4.6-fold difference was observed with endomorphin-2.¹² 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. The δ-opioid receptor affinity of **5** and **7** are essentially non-existent (K_{iδ} = 2,770 and 924 nM, respectively), the Dmt derivatives (**6** and **8**) exhibited K_{iδ} = 18.8 and 31.9 nM, respectively with relative activity of 147- and 29-fold, respectively. Interestingly, although Dmt derivatives increased affinities compared with the corresponding Tyr derivatives for both μ- and δ-opioid receptors, they are still μ-selective opioid ligands (K_{iδ}/K_{iμ} for **5** and **7** were 65 and 220, respectively and **6** and **8**: 369 and 1,519, respectively).

In the functional bioactivity¹² (Table 1), **6** and **8** exhibited μ agonism (IC₅₀ = 4.93 and 1.79 nM, respectively) and they were either inactive (**6**) or had weak δ agonism (**8**); however, even at this level, the maximum inhibition of muscle contraction was only 60%. Other Dmt opioid derivatives^{12,15,16} exhibit full μ agonist activity, but only [Dmt¹]-endomorphin-2 was a full δ agonist.¹² While the differences in the opioid receptor affinity between **6** and **8** were not significant, the functional bioactivity revealed a major difference in 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.

These results provided further proof of the usefulness of our rapid and facile synthetic procedure of pyrazinone ring from dipeptidyl chloromethyl ketones.²⁰ The data also revealed that even in asymmetric Tyr dimers, Dmt can readily replace Tyr residue to enhance anchoring within opioid receptors to affect biological activity and differentially interact with the ligand-binding domains of μ- and δ-opioid receptors suggesting their possible application in computational analysis of the receptor pocket and docking of these ligands into opioid receptors.

Acknowledgement

This work was supported in part by a grant from Kobe Gakuin University to K.S. and the Intramural Research Program of the NIH, and NIEHS.

References and Notes

- (a) Cox BM, Goldstein A, Li CH. Proc. Natl. Acad. Sci. USA 1976;73:1821–1823. [PubMed: 1064855]
(b) Ling N, Burgus R, Guillemin R. Proc. Natl. Acad. Sci. USA 1976;73:3042.

2. Goldstein A, Tachibana S, Lowney LH, Hunkapiller M, Hood L. Proc. Natl. Acad. Sci. USA 1979;77:6666. [PubMed: 230519]
3. Hughes J, Smith TW, Kosterlitz HW, Forthergill LA, Morgan BA, Morris HR. Nature 1975;258:577. [PubMed: 1207728]
4. Zadina JE, Hackler L, Ge LJ, Kastin A. J. Nature 1997;386:499.
5. (a) Erspamer V, Melchiorri P. Trends Pharmacol. Sci 1980;1:391. (b) Broccardo M, Erspamer V, Falconieri, Erspamer G, Improta G, Linari G, Melchiorri P, Montecucchi PC. Br. J. Pharmac 1981;73:625. (c) Montecucchi PC, de Castiglione R, Piani S, Gozzini L, Erspamer V. Int. J. Peptide Prot. Res 1981;17:275. (d) Montecucchi PC, de Castiglione R, Erspamer V. Int. J. Peptide Prot. Res 1981;17:316. (e) Melchiorri P, Negri L. Gen. Pharmacol 1996;27:1099. [PubMed: 8981054]
6. (a) Kreil G, Barra D, Simmaco M, Erspamer-Falconieri G, Melchiorri P, Negri L, Severini C, Corsi R. Eur. J. Pharmacol 1989;162:123. [PubMed: 2542051] (b) Lazarus LH, de Castiglione R, Guglietta A, Wilson WE. J. Biol. Chem 1989;264:3047. [PubMed: 2563375] (c) Erspamer V, Melchiorri P, Erspamer-Falconieri G, Negri L, Corsi R, Severini C, Barra D, Simmaco M, Kreil G. Proc. Natl. Acad. Sci. USA 1989;86:5188. [PubMed: 2544892] (d) Lazarus LH, Bryant SD, Cooper PS, Salvadori S. Prog. Neurobiol 1999;57:377. [PubMed: 10080383]
7. Meunier J-C, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour J-L, Guillemot J-C, Ferrara P, Monsarrat B, Mazarguil H, Vassart G, Parmentier M, Costentin J. Nature 1995;377:532. [PubMed: 7566152]
8. (a) Schwyzler R. Biochemistry 1986;25:6335. [PubMed: 3024707] (b) Portoghese PS. Trends. Pharmacol. Sci 1989;10:230. [PubMed: 2549665]
9. Li T, Fujita Y, Tsuda Y, Mitazaki A, Ambo A, Sasaki Y, Jinsmaa Y, Bryant SD, Lazarus LH, Okada Y. J. Med. Chem 2005;48:586. [PubMed: 15658871]
10. Fujita Y, Tsuda Y, Motoyama T, Li T, Miyazaki A, Yokoi T, Sasaki Y, Ambo A, Niizuma H, Jinsmaa Y, Bryant SD, Lazarus LH, Okada Y. Bioorg. Med. Chem., Lett 2005;15:599. [PubMed: 15664820]
11. Salvadori S, Attila M, Balboni G, Bianchi C, Bryant SD, Crescenzi O, Guerrini R, Picone D, Tancredi T, Temussi PA, Lazarus LH. Mol. Med 1995;1:678. [PubMed: 8529134]
12. Okada Y, Fujita Y, Motoyama T, Tsuda Y, Yokoi T, Li T, Sasaki Y, Ambo A, Jinsmaa Y, Bryant SD, Lazarus LH. Bioorg. Med. Chem 2003;11:1983. [PubMed: 12670649]
13. Li T, Jinsmaa Y, Nedachi M, Tsuda Y, Ambo A, Sasaki Y, Bryant SD, Marchak E, Li Q, Swartzwelder HS, Lazarus LH, Okada Y. J. Med. Chem. submitted
14. Sasaki Y, Suto T, Ambo A, Ouchi H, Yamamoto Y. Chem. Pharm. Bull 1999;47:1506. [PubMed: 10553648]
15. Okada Y, Tsuda Y, Fujita Y, Yokoi T, Sasaki Y, Ambo A, Konishi R, Nagata M, Salvadori S, Jinsmaa Y, Bryant SD, Lazarus LH. J. Med. Chem 2003;46:3201. [PubMed: 12852751]
16. Jinsmaa Y, Miyazaki A, Fujita Y, Li T, Fujisawa Y, Shiotani K, Tsuda Y, Yokoi T, Ambo A, Sasaki Y, Bryant SD, Lazarus LH, Okada Y. J. Med. Chem 2004;47:2599–2610. [PubMed: 15115401]
17. Okada Y, Fukumizu A, Takahashi M, Yamazaki J, Yokoi T, Tsuda Y, Bryant SD, Lazarus LH. Tetrahedron 1999;55:14391.
18. Dygos JH, Yonan EE, Scaros MD, Goodmanson OJ, Getman DP, Periana RA, Beck JR. Synthesis 1992:741.
19. Physicochemical data of compounds **5–8** **6-[4'-(H-Tyr)-aminobutyl]-3-[3'-(H-Tyr)-aminoprop-yl]-5-methyl-2(1H)pyrazinone Hydrochloride (5)**: Yield 5.0 mg (65.0%), $[\alpha]_D^{20.2} +39.9^\circ$ (c=0.1, MeOH), R_f (nBuOH:AcOH:pyridine:H₂O=4:1:1:2) 0.32, t_R 20.7 (min), tof-MS m/z 565.50 (M +1)⁺. Calcd. 564.68. HPLC conditions: column, YMC pack R&D ODS (4.6×250mm); detection : 220nm, purity (99.7%). ¹H-NMR (pyridine-*d*₅) δ : 9.75 (1H, s, br, 3-CH₂-CH₂-CH₂-NH-), 9.63 (1H, br, 6-CH₂CH₂-CH₂-CH₂-NH-), 7.54 (d, 2H, *J*=1.80, 6-2', 6'-phenyl of Tyr), 7.52 (d, 2H, *J*=1.84, 3-2', 6'-phenyl of Tyr), 7.05 (s, 2H, 6-3', 5'-phenyl of Tyr), 7.03 (s, 2H, 3-3', 5'-phenyl of Tyr), 5.16 (t, 1H, *J*=6.88, 6 α -CH of Tyr), 5.09 (t, 1H, *J*=6.78, 3 α -CH of Tyr), 3.73 (br, 4H, 3 β -CH₂ of Tyr, 6 β -CH₂ of Tyr), 3.59 (m, 2H, 6-CH₂-CH₂-CH₂-CH₂-NH-), 3.50 (br, 2H, 3-CH₂-CH₂-CH₂-NH-), 3.00 (t, 2H, *J*=7.08, 6-CH₂-CH₂-CH₂-CH₂-NH-), 2.59 (br, 2H, 6-CH₂-CH₂-CH₂-CH₂-NH-), 2.23 (s, 3H, 5-CH₃), 2.17 (t, 2H, *J*=6.90, 6-CH₂-CH₂-CH₂-CH₂-NH-), 1.74 (br, 2H, 3-CH₂-CH₂-CH₂-NH-), 1.64 (br, 2H, 3-CH₂-CH₂-CH₂-NH-), ¹³C-NMR (pyridine-*d*₅) δ : 169.7 (q), 169.6 (q), 158.2 (q), 158.1 (q), 156.9 (q), 131.6 (t, phenyl Tyr), 126.2 (q), 116.5–116.4 (t, phenyl of Tyr), 56.2–56.1

(t, 3 α -CH of Tyr 6 α -CH of Tyr), 39.8 (s, 6-CH₂-CH₂-CH₂-CH₂-NH-), 39.0 (s, 3-CH₂-CH₂-CH₂-NH-), 37.7 (s, 3 β -CH₂ of Tyr, 6 β -CH₂ of Tyr), 30.4 (s, CH₂-CH₂-CH₂-CH₂-NH-), 30.0 (s, 6-CH₂-CH₂-CH₂-NH-), 29.1 (s, 3-CH₂-CH₂-CH₂-NH-), 26.9 (s, 6-CH₂-CH₂-CH₂-CH₂-NH-), 26.2 (s, 3-CH₂-CH₂-CH₂-NH-), 18.7 (p, 5-CH₃). *Anal.* Calcd for C₃₀H₄₀N₆O₅•2HCl•5.6H₂O : C, 50.4; H, 6.96; N, 11.0. Found: C, 50.3; H, 6.64; N, 10.9. **6-[4'-(H-Dmt)-aminobutyl]-3-[3'-(H-Dmt)-aminopropyl]-5-methy-2(1H)-pyrazinone Hydrochloride (6):** Yield 9.0 mg (59.5%), [α]_D^{22.1} +32.4° (c=0.1, MeOH), *R*_f (nBuOH:AcOH:pyridine:H₂O=4:1:1:2) 0.64, *t*_R 31.9(min), tof-MS *m/z* : 622.05 (M+1)⁺. calcd. 621.78. HPLC conditions: column, YMC pack R&D ODS (4.6×250 mm); detection: 220 nm, purity (99.1%). ¹H-NMR (pyridine-*d*₅) δ : 8.56 (br, 1 H, 6-CH₂-CH₂-CH₂-NH), 8.10 (1H, s, br, 3-CH₂-CH₂-CH₂-NH-), 6.40 (s, 4H, 3, 6- β -CH₂ of Dmt), 4.60 (1H, br, 6- α -CH of Dmt), 4.17 (1H, br, 3- α -CH of Dmt), 3.69 (2H, br, 6-CH₂-CH₂-CH₂-CH₂-NH-), 3.01-2.89 (8H, m, 6-CH₂-CH₂-CH₂-CH₂-NH-, 3-CH₂-CH₂-CH₂-NH-, 2×phenyl of Dmt), 2.52 (2H, s, 6-CH₂-CH₂-CH₂-CH₂-NH-), 2.38 (2H, t, *J*=7.12, 3-CH₂-CH₂-CH₂-NH-), 2.19-2.17 (3H, s, 5-CH₃, 12H, m, 4×CH₃ of Dmt), 1.61-1.56 (2H, m, 6 β -CH₂), 1.32 (2H, t, *J*=7.30, 3 β -CH₂), ¹³C-NMR (pyridine-*d*₅) δ : (q), 191.0 (q), 168.0 (q), 167.8 (q), 155.5 (q), 138.2 (q), 122.6 (q), 114.9 (t, phenyl of Dmt), 114.8 (t, phenyl of Dmt), 51.7 (t, 6-CH₂-CH₂-CH₂-CH₂-NH-), 38.5 (s, 6-CH₂-CH₂-CH₂-CH₂-NH-), 38.2 (s, 3-CH₂-CH₂-CH₂-NH-), 30.5 (s, 3-CH₂-CH₂-CH₂-NH-), 30.4 (s, 6-CH₂-CH₂-CH₂-CH₂-NH-), 30.4 (s, 3-CH₂-CH₂-CH₂-NH-, 6-CH₂-CH₂-CH₂-NH-), 27.7 (s, 6-CH₂-CH₂-CH₂-CH₂-NH-), 19.8 (p, 5-CH₃), 17.6 (p, -CH₃ of Dmt). *Anal.* Calcd for C₃₄H₄₈N₆O₅•2HCl•5.5H₂O: C, 47.2; H, 6.42; N, 9.71. Found: C, 47.0; H, 6.23; N, 9.99. **3-[4'-(H-Tyr)-aminobutyl]-6-[3'-(H-Tyr)-aminopropyl]-5-methy-2(1H)-pyrazinone Hydrochloride (7):** Yield 5.0 mg (6.021%), [α]_D^{21.2} +3.58° (c=0.1, MeOH), *R*_f (nBuOH:AcOH:pyridine:H₂O=4:1:1:2) 0.42, *t*_R 19.1 (min), tof-MS *m/z*: 565.50 (M+1)⁺. Calcd. 564.68. HPLC conditions: column, YMC pack R&D ODS (4.6×250 mm); detection: 220nm, purity (99.7%). ¹H-NMR (pyridine-*d*₅) δ : 9.96 (br, 1H, 6-CH₂-CH₂-CH₂-NH-), 9.48 (br, 1H, 3-CH₂-CH₂-CH₂-NH-), 7.48 (s, 2H, phenyl of Tyr), 7.46 (s, 2H, phenyl of Tyr), 7.03 (d, 2H, *J*=2.60, phenyl of Tyr), 7.00 (d, 2H, *J*=2.60, phenyl of Tyr), 5.19 (t, *J*=7.00, 6- α -CH of Tyr), 5.02 (t, 1H, *J*=6.78, 3- α -CH of Tyr), 3.75 (m, 2H, 6-CH₂-CH₂-CH₂-NH-), 3.70 (m, 2H, 3-CH₂-CH₂-CH₂-CH₂-NH-), 3.58 (m, 1H, 6- β -CH₂ of Tyr), 3.44 (d, 2H, *J*=6.15, 3- β -CH₂ of Tyr), 2.85 (m, 2H, 3-CH₂-CH₂-CH₂-CH₂-NH-), 2.74 (m, 2H, 6-CH₂-CH₂-CH₂-NH-), 2.19 (s, 3H, 5-CH₃), 1.91 (m, 2H, 6-CH₂-CH₂-CH₂-NH-), 1.85 (m, 2H, 3-CH₂-CH₂-CH₂-CH₂-NH-), 1.66 (d, 2H, *J*=6.44, 3-CH₂-CH₂-CH₂-CH₂-NH-), ¹³C-NMR (pyridine-*d*₅) δ : 170.8 (q), 170.3 (q), 159.0 (q), 158.9 (q), 157.6 (q), 132.4 (q), 132.3 (q), 127.1 (q), 117.3 (t, phenyl Tyr), 117.2 (t, phenyl Tyr), 57.1 (t, 6 α -CH of Tyr), 57.0 (t, 3 α -CH of Tyr), 40.5 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 40.2 (s, 6-CH₂-CH₂-CH₂-NH-), 33.5 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 30.3 (s, 6-CH₂-CH₂-CH₂-NH-), 29.4 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 26.4 (s, 6-CH₂-CH₂-CH₂-NH-), 25.6 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 19.4 (p, 5-CH₃). *Anal.* Calcd for C₃₀H₄₀N₆O₅•2HCl•5.5H₂O: C, 50.4; H, 6.96; N, 11.0. Found: C, 50.4; H, 6.74; N, 11.4. **3-[4'-(H-Dmt)-aminobutyl]-6-[3'-(H-Dmt)-aminopropyl]-5-methy-2(1H)-pyrazinone Hydrochloride (8):** Yield 7.0 mg (48.2%), [α]_D²⁵ : +76.3° (c=0.1, MeOH), *R*_f (nBuOH:AcOH:pyridine:H₂O=4:1:1:2) 0.48, *t*_R 25.7(min), tof-MS *m/z* : 621.88 (M+1)⁺ calcd. 621.78. HPLC conditions: column, YMC pack R&D ODS (4.6×250 mm); detection: 220nm, purity (98.3%). ¹H-NMR (pyridine-*d*₅) δ : 9.62 (t, 1H, *J*=5.48, 6-CH₂-CH₂-CH₂-NH), 9.11 (t, 1H, *J*=5.48, 3-CH₂-CH₂-CH₂-CH₂-NH), 6.88 (s, 2H, 6- β -CH₂ of Dmt), 6.87 (s, 2H, 3 β -CH₂ of Dmt), 4.84-4.86 (q, 1H, *J*=5.23 6- α -CH of Dmt), 4.6-4.70 (1H, q, *J*=5.13, 3 α -CH of Dmt), 3.74-3.83 (2H, m, 6-CH₂-CH₂-CH₂-NH-), 3.60-3.68 (m, 2H, 3-CH₂-CH₂-CH₂-CH₂-NH-), 3.32-3.59 (m, 4H, Dmt), 2.82-2.85 (m, 2H, 3-CH₂-CH₂-CH₂-CH₂-NH-), 2.65-2.85 (m, 2H, 6-CH₂-CH₂-CH₂-NH-), 2.41 and 2.42 (2s, *J*=5.60, 12H, 4×CH₃ of Dmt), 2.20 (s, 3H, 5-CH₃), 1.75-1.77 (m, 2H, 6-CH₂-CH₂-CH₂-NH-), 1.51-1.57 (m, 2H, 3-CH₂-CH₂-CH₂-CH₂-NH-), ¹³C-NMR (pyridine-*d*₅) δ : 170.2 (q), 170.0, 157.5 (q), 157.4 (q), 139.5 (q), 139.4 (q), (q) 116.3 and 116.2 (2×t, phenyl of Dmt), 54.1 (t, 6 α -CH of Dmt), 54.0 (t, 3 α -CH of Dmt), 39.6 (s, 2× β -CH₂ of Dmt), 39.1 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 32.5 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 31.9 (s, 6-CH₂-CH₂-CH₂-NH-), 29.0 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 28.8 (s, 6-CH₂-CH₂-CH₂-NH-), 24.6 (s, 6-CH₂-CH₂-CH₂-NH-), 24.4 (s, 3-CH₂-CH₂-CH₂-CH₂-NH-), 20.7 (p, 4×CH₃ of Dmt), 18.5 (p, 5-CH₃). *Anal.* Calcd for C₃₄H₄₈N₆O₅•2HCl•6H₂O: C, 50.9; H, 7.49; N, 10.5. Found: C, 50.7; H, 7.23; N, 10.1.

20. Okada Y, Taguchi H, Nishiyama Y, Yokoi T. Tetrahedron Lett 1994;35:1231.

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

Scheme 1.

Synthesis of bis-Tyr-pyrazinone (**5** and **7**) or bis-Dmt-pyrazinone opioidmimetics (**6** and **8**).

(i) 7N HCl/dioxane; (ii) CH₃CN, 60°C; (iii) 25% HBr/AcOH, (iv) Boc-2',6'-dimethyl-L-tyrosine (Boc-Dmt-OH), pyBop, DIEA, DMF; Z = benzyloxycarbonyl.

The competitive opioid receptor affinity and functional bioactivity of compounds (**5–8**) Displacement of [³H]-DAMGO (μ -selective) and [³H]-deltorphin II (δ -selective) from rat membrane synaptosome (P₂ fraction)¹⁶. Values are means $\pm$ S.E. with (n) the number of independent repetitions. (RA: relative activity of Dmt derivative compared with the corresponding Tyr derivatives.)

Compounds		Receptor binding <i>K_i</i> (nM)			Receptor selectivity			Functional bioactivity IC ₅₀ $\pm$ S.E.(nM)	
		μ	(n)	RA	δ	(n)	RA	GPI assay ^a	MVD assay ^a
5 R = Tyr, m = 3, n = 4		27.4 $\pm$ 5.2	(5)	1	2770 $\pm$ 46	(5)	1		
6 R = Dmt, m = 3, n = 4		1 0.051 $\pm$ 0.009	(5)	537	18.8 $\pm$ 2.9	(3)	147	4.93 $\pm$ 1.73	>10,000 ^{**}
7 R = Tyr, m = 4, n = 3		7.58 $\pm$ 0.21	(5)	1	924.4 $\pm$ 125	(4)	1		
8 R = Dmt, m = 4, n = 3		0.021 $\pm$ 0.003	(5)	361	31.9 $\pm$ 2.9	(5)	29	1.79 $\pm$ 0.57	25.8 $\pm$ 4.3 [*]

^aThe data are the means of over five independent repetitions used different isolated tissue preparations.

^{*} Partial agonist (Max inhibition 60%)

^{**} Partial agonist (Max inhibition 44%).