Synthesis of Pyrazinone Ring-Containing Opioid Mimetics and Examination of Their Opioid Receptor-Binding Activity[1)]

Yoshio OKADA,*[,a] Atsuko FUKUMIZU,[a] Motohiro TAKAHASHI,[a] Toshio YOKOI,[a] Yuko TSUDA,[a] Sharon D. BRYANT,[b] and Lawrence H. LAZARUS[b]

Faculty of Pharmaceutical Sciences, Kobe Gakuin University,[a] Nishi-ku, Kobe 651–2180, Japan (E-mail: okada@pharm.kobegakuin.ac.jp) and Peptide Neurochemistry, LCBRA, National Institute of Environmental Health Sciences,[b] Research Triangle Park, NC 27709, U.S.A.

Received April 16, 1999; accepted June 21, 1999

Cyclization of dipeptidyl chloromethyl ketones gave 6-(4-aminobutyl)-3-carboxyethyl-5-methyl-2(1*H*)-pyrazinone, 3-(4-aminobutyl)-6-carboxyethyl-5-methyl-2(1*H*)-pyrazinone, and 3,6-bis(4-aminobutyl)-5-methyl-2(1*H*)-pyrazinone, which were inserted into the enkephalin sequence to give opioid mimetics. Thus, it was confirmed that a pyrazinone ring can be easily inserted into a peptide sequence in order to evaluate structural components required for biologically active peptides.

Key words peptide mimetic; opioid mimetic; pyrazinone ring; enkephalin sequence; receptor-binding activity; μ-selective

Previously, an easy and convenient synthetic procedure for the preparation of 2(1*H*)-pyrazinone derivatives from dipeptidyl chloromethyl ketones was developed.[2)] This novel method afforded 2(1*H*)-pyrazinone derivatives substituted with the desired functional groups at the 3 and 6 positions in high yield. Therefore, an amino and/or a carboxyl group can be easily introduced at position 3 or 6 of the pyrazinone ring by using appropriate amino acids.[3)] It was also reported that 5-methyl-6-β-phenethyl-3-tyrosylaminobutyl-2-(1*H*)-pyrazinone exhibited fairly potent binding activity to the μ-opioid receptor with a K_i value of 55.8 nM and to a δ-opioid receptor with a K_i value of 2165 nM and a $K_i\mu/K_i\delta$ value of 0.026, while 5-methyl-3-benzyl-6-tyrosylaminobutyl-2-(1*H*)-pyrazinone exhibited weak affinity for the δ-opioid receptor with a K_i value of 332.7 nM, and for the μ-opioid receptor with a K_i value of 3909 nM and $K_i\mu/K_i\delta$ value of 11.7.[4)] The above two compounds exhibited different binding activity profiles. One notable difference in structure between the above two compounds is the orientation of the carbonyl groups on the pyrazinione ring. The position of the carbonyl may be an important factor for μ-opioid receptor interaction.[4)] Therefore, in order to introduce conformational restrictions in the biologically active opioid-peptide enkephalin, three pyrazinone-ring containing compounds (**1**, **2**, **3**) were considered, as shown in Fig. 1. Using these templates, we designed opioid mimetics **I** and **II**, in which pyrazinone fragments were substituted for Gly^2 and Gly^3 in the enkephalin sequence[5)] and Leu^5 was deleted as shown in Fig. 2. We synthesized **I** and **II** and examined their opioid receptor binding activities. The synthesis and opioid receptor-binding activity of compound **III** (Fig. 2) will also be described.

The desired opioid mimetics (**I** and **II**) were prepared according to Chart 1. Two types of amino acids containing a pyrazinone ring (**1** and **2**) were prepared starting from Boc–Glu(OBzl)–Lys(Z)–CH_2Cl and Boc–Lys(Z)–Glu(OBzl)–CH_2Cl, respectively. The amino group was protected with a Fmoc group by using Fmoc-OSu to give compounds (**4** and **5**). Fmoc amino acids (**4** and **5**) were coupled with H–Phe–OBu^t by BOP reagent to give dipeptides (**6** and **7**, respectively). After removal of the Fmoc group, the resultant dipeptide amine was coupled with Boc–Tyr–OH by BOP reagent to give protected tripeptides (**8** and **9**, respectively). The Boc group and Bu^t groups were removed by TFA-anisole as usual. The resultant product was lyophilized from water containing 1 N HCl to give the desired peptide mimetics (**I** and **II**) as corresponding hydrochlorides.

Analytical HPLC profiles of **I** [FAB-MS: m/z=564 $(M+1)^+$] and **II** [FAB-MS: m/z=564 $(M+1)^+$] are shown in Fig. 3. Compounds **I** and **II** exhibited opioid receptor-binding activity with $K_i\delta$ of 1610 nM and 1040 nM, and $K_i\mu$ of 3120 nM and 4080 nM, respectively. These peptide mimetics contained functional groups known to be important for opioid receptor interaction; namely the hydroxyl group on tyrosine, the *N*-terminal amine and the presence of two aromatic groups.[6)] Yet both compounds were inactive in the radiolabeled ligand-binding assay. Since both compounds were inactive, it could be surmised that the distance between the two aromatic rings was too large to manifest opioid receptor

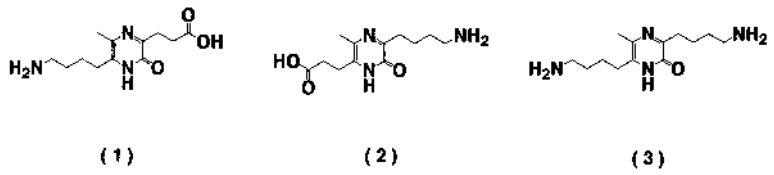

Fig. 1. Design of Three Templates

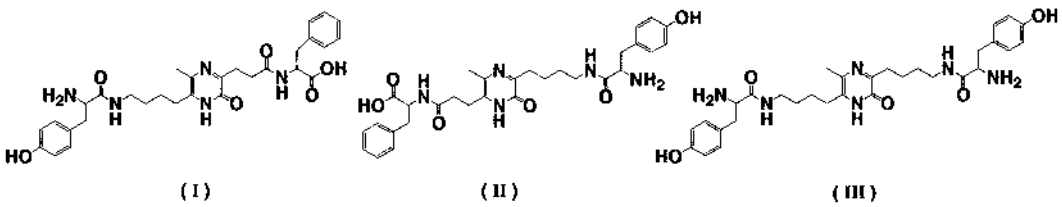

Fig. 2. Design of Opioid Mimetics Containing a Pyrazinone Ring (**I**, **II** and **III**)

* To whom correspondence should be addressed.

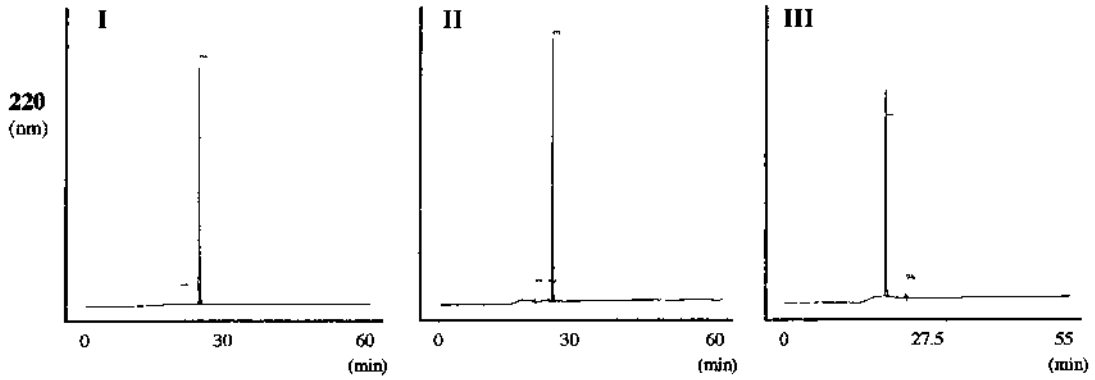

Fig. 3. HPLC Profiles of Opioid Mimetics **I**, **II**, and **III**

Column, COSMOSIL 5C18-AR (4.6×250 mm); eluent, 0.05% TFA in H_2O (A) and 0.05% TFA in MeCN (B); A : B 95 : 5 for 5 min, A : B from 95 : 5 to 60 : 40 in 15 min, 60 : 40 to 30 : 70 in 30 min, 30 : 70 to 10 : 90 in 10 min; flow rate, 1 ml/min; detection, 220 nm.

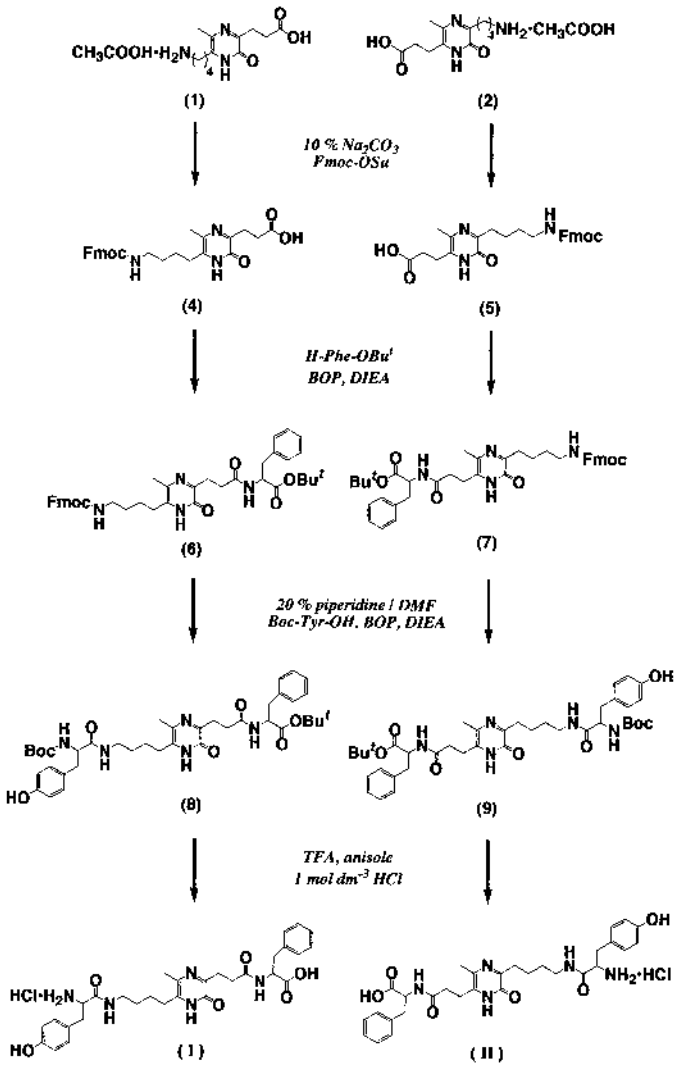

Chart 1. Synthetic Scheme for Opioid Mimetics **I** and **II**

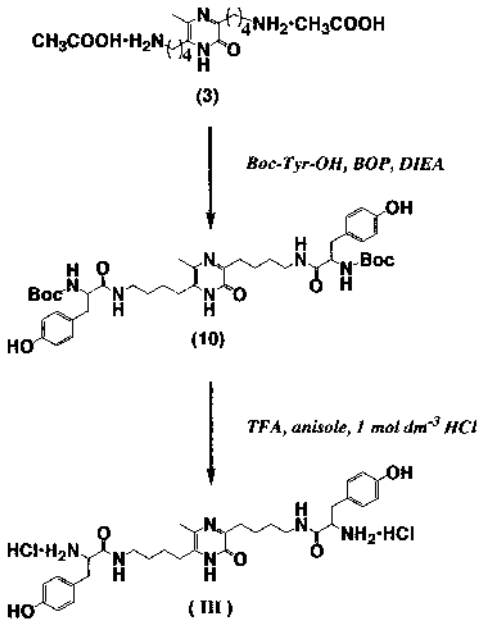

Chart 2. Synthetic Scheme for Opioid Mimetic **III**

binding activity.

Next, as shown in Chart 2, 3,6-bis(4-aminobutyl)-5-methyl-2(1*H*)-pyrazinone (**3**), which was prepared from Boc–Lys(Z)–Lys(Z)–CH_2Cl, was coupled with Boc-Tyr-OH by BOP reagent to give **10** and then treated with TFA-anisole and converted to its hydrochloride (**III**). The analytical HPLC profile of **III** [FAB-MS: m/z=580 $(M+1)^+$] is shown in Fig. 3. While compound **III** exhibited μ-receptor-binding activity ($K_i\mu$ of 61 nM), it did not display a significant effect on δ-receptor binding affinity ($K_i\delta$ of 1920 nM), resulting in a $K_i\mu/K_i\delta$ value of 0.032, indicating that compound **III** is μ-selective. Previously, it was reported that Tyr^4-enkephalin exhibited only 0.1% and 0.3% activity in GIP and MVD assay, respectively, compared with the parent molecule, enkephalin, and this compound was rather δ-selective, as enkephalin itself.[7] Kondo *et al.*[8] prepared a cystamine–enkephalin dimer and examined its opioid receptor binding activity. Their compound was almost five times more potent for δ-opioid receptors and four times more potent for μ-opioid receptors than the cystamine monomer and was rather δ-selective. This molecule contains two molecules of [D-Ala^2, Leu^5] enkephalin cross-linked at the *C*-terminal leucine with cystamine. In addition, other studies involving dimeric peptides have demonstrated enhanced biological properties.[9] Com-

pound **III** exhibited quite a different opioid receptor-binding activity profile compared with Tyr^4-enkeplalin and a cystamine–enkephalin dimer. Therefore, it can be deduced that this result exhibits the possibility that compound **III** acts at different μ-opioid receptors.

In conclusion, the pyrazinone platform serves as a template for developing diverse opioid peptides that may prove useful for clinical and therapeutic applications, and compound **III** is a candidate of a lead compound for developing μ-receptor agonists and antagonists.

Acknowledgement This work was supported in part by grants from the Science Research Promotion Fund of the Japan Private School Promotion Foundation, Grant-in-Aid for Scientific Research(C) 11694326, Japan Society for the Promotion of Science and Grant-in-Aid for Health Science Research of Kobe Gakuin University.

References and Note

1) The customary L-configuration for amino acid residues is omitted. Abbreviations used in this report for amino acids, peptides and the derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485—2489 (1966); **6**, 362—364 (1966); **11**, 1726—1732 (1972). The following additional abbreviations are used: TFA, trifluoroacetic acid; Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; OBu^t, *tert*-butyl ester; Fmoc, 9-fluorenylmethyloxycarbonyl; OSu, *N*-hydroxysuccinimide ester; BOP, Benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate; GPI, guinea pig ileum; MVD, mouse vas deferens.
2) Taguchi H., Yokoi T., Tsukatani M., Okada Y., *Tetrahedron*, **51**, 7361—7372 (1995).
3) Taguchi H., Yokoi T., Okada Y., *Chem.Pharm.Bull*., **44**, 2037—2041 (1996).
4) Okada Y., Tsukatani M., Taguchi H., Yokoi T., Bryant S. D., Lazarus L. H, *Chem.Pharm.Bull*., **46**, 1374—1382 (1998).
5) Hughes J., Smith T. W., Kosterlitz H. W., Forthergill L. A., Morgan B. A., Morris H. R., *Nature*, **258**, 577—579 (1975).
6) Balboni G., Guerrini R., Salvadori S., Tomatis R., Bryant S. D., Bianchi C., Attila M., Lazarus L. H., *Biol. Chem*., **378**, 19—29 (1997).
7) Morgan B. A., Smith C. F. C., Waterfield A. A., Hughes J., Kosterlitz H. W., *J. Pharm. Pharmac*., **28**, 660—661 (1976).
8) Kondo M., Kodama H., Costa T., Shimohigashi Y., *Int. J. Peptide Protein Res*., **27**, 153—159 (1986).
9) Lazarus L. H., Guglietta A., Wilson W. E., Irons B. J., de Castiglione R., *J. Biol. Chem*., **264**, 354—362 (1989).