Structure–Activity Relationships of Dermorphin Analogues Containing Chiral Piperazin-2-one and Piperazine Derivatives

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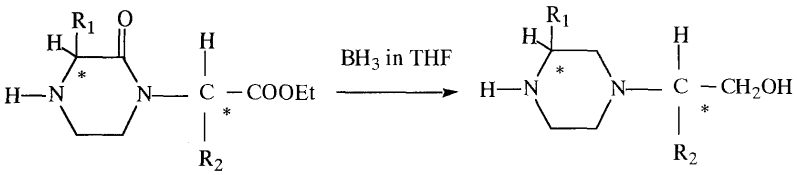
The amide and ester carbonyl groups of four piperazin-2-one derivatives (*N,N'*-ethylene-bridged dipeptide ethyl esters) constructed from (*R*)- or (*S*)-phenylalanine and glycine were reduced with borane-tetrahydrofuran complex to produce the corresponding piperazine derivatives in 70—80% yields. These piperazin-2-one or piperazine derivatives were used as the carboxyl-terminal residues of eight dermorphin analogues (H-tyrosyl-D-alanyl-piperazin-2-one or piperazine derivatives) whose opiate activities were examined *in vitro* by use of the guinea pig ileum and the mouse *vas deferens* assays. It was found in the guinea pig ileum assay that the configuration of phenylalanine and the replacement of the piperazin-2-one ring with a piperazine ring are important for enhancing or reducing the opiate activities of these analogues.

Key words dermorphin analogue; opiate activity; structure–activity relationship; *N,N'*-ethylene-bridged dipeptide; piperazine derivative

Dermorphin[1] is an opiate heptapeptide (H–Tyr–D-Ala–Phe–Gly–Tyr–Pro–Ser–NH_2) extracted from the skin of *Phyllomedusa sauvagei* (frog). Several workers[2–4] have investigated the biological activities of dermorphin and its analogues, and reported that shorter homologues (tetrapeptides = H–Tyr–D-Ala–Phe–Gly–NH_2 and H–Tyr–D-Ala–Phe–Phe–NH_2) of dermorphin display opiate activities. In our previous paper,[5] chiral *N,N'*-ethylene-bridged dipeptide ethyl esters [eXX'-OEt; X = F(Phe), X' = L(Leu) or M(Met)], constructed from (*R*)- or (*S*)-phenylalanine, -leucine and -methionine, were used as the carboxyl-terminal residues of Leu- and Met-enkephalin analogues (H–Tyr–D-Ala–Gly–eXX'-OEt), which are relatively lipophilic and conformationally restricted in comparison with the parent enkephalins. These enkephalin analogues were examined by mouse *vas deferens* assay, and showed opiate activities. However, it was found that differences of the configurations of α-amino acid residues in the eXX' had no clear influence on the biological activities of these pseudo-peptides. In this paper, piperazin-2-one derivatives[5–11] containing (*R*)- or (*S*)-phenylalanine and glycine (G) [*N,N'*-ethylene-bridged dipeptide ethyl esters = eXX'-OEt; **1a** = eF(*S*)G-,[7] **2a** = eF(*R*)G-, **3a** = eF(*S*)F(*S*)-[5,6,8] and **4a** = eF(*R*)F(*R*)-OEt] and their corresponding piperazine derivatives [eXX'-Red; **1b** = eF(*S*)G-, **2b** = eF(*R*)G-, **3b** = eF(*S*)F(*S*)- and **4b** = eF(*R*)F(*R*)-Red] were inserted into the third and fourth positions of shorter dermorphin analogues (H–Tyr–D-Ala–eXX'-OEt or -Red = DM-eXX'-OEt or -Red), respectively. The biological activities of the eight dermorphin analogues obtained here were assayed, in order to examine their structure–activity relationships, by means of the guinea pig ileum (GPI) and the mouse *vas deferens* (MVD) assays. It was found in GPI assay that the change [from (*S*) to (*R*)] of the configuration of phenylalanine residues in the eXX' and the substitution of the piperazin-2-one ring with the piperazine ring are useful for enhancing the opiate activities of these dermorphin analogues.

Results and Discussion

The piperazine derivatives (eXX'-Red = **1b**, **2b**, **3b** and **4b**) were prepared by BH_3-reduction of the corresponding piperazin-2-one derivatives (eXX'-OEt = **1a**, **2a**, **3a** and **4a**) in tetrahydrofuran (THF), as shown in Chart 1. Dermorphin analogues [H–Tyr–D-Ala–eXX'-OEt = DM-eXX'-OEt = DM-eF(*S*)G(DM-**1a**)-, DM-eF(*R*)G(DM-**2a**)-, DM-eF(*S*)F(*S*)(DM-**3a**)- and DM-eF(*R*)F(*R*)(DM-**4a**)-OEt] were prepared from eXX'-OEt as starting



1a; R_1 = $PhCH_2$, *(*S*), R_2 = H — **1b**; R_1 = $PhCH_2$, *(*S*), R_2 = H

2a; R_1 = $PhCH_2$, *(*R*), R_2 = H — **2b**; R_1 = $PhCH_2$, *(*R*), R_2 = H

3a; R_1 = $PhCH_2$, *(*S*), R_2 = $PhCH_2$, *(*S*) — **3b**; R_1 = $PhCH_2$, *(*S*), R_2 = $PhCH_2$, *(*S*)

4a; R_1 = $PhCH_2$, *(*R*), R_2 = $PhCH_2$, *(*R*) — **4b**; R_1 = $PhCH_2$, *(*R*), R_2 = $PhCH_2$, *(*R*)

Chart 1

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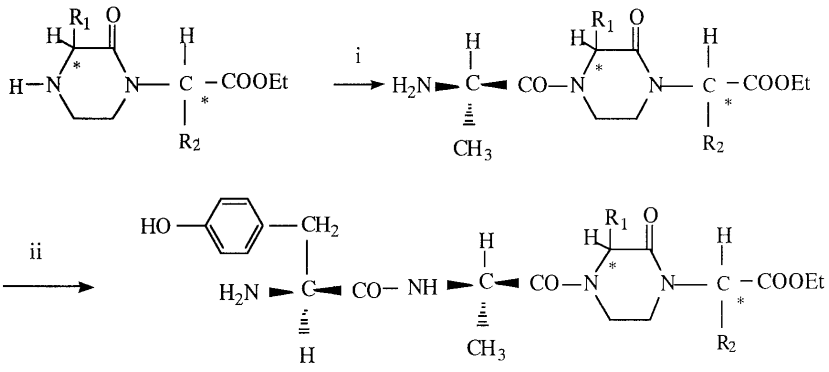

i: a) Boc-D-Ala, EEDQ, $CHCl_3$ b) 4 *N* HCl-DOX; ii: a) Boc-Tyr-OSu, $CHCl_3$ b) 4 *N* HCl-DOX, anisole

DM-1a; $R_1 = PhCH_2$, *(*S*), $R_2 = H$ **DM-2a**; $R_1 = PhCH_2$, *(*R*), $R_2 = H$

DM-3a; $R_1 = PhCH_2$, *(*S*), $R_2 = PhCH_2$, *(*S*) **DM-4a**; $R_1 = PhCH_2$, *(*R*), $R_2 = PhCH_2$, *(*R*)

Chart 2

Table 1. Analytical and Physical Data of Mono- and Di-hydrochlorides of Dermorphin Analogues

Compound[a]	Formula (Molecular weight)	Analysis (%)						mp (°C)	$[\alpha]_D$ (Ethanol)	MS *m/z* (M^+)
		Calcd			Found					
		C	H	N	C	H	N			
DM-eF(*S*)G-OEt (DM-**1a**)	$C_{27}H_{35}N_4O_6Cl \cdot 2H_2O$ (583.1)	55.61	6.74	9.61	55.65	6.53	9.57	153—158	+126°	510
DM-eF(*S*)G-Red (DM-**1b**)	$C_{25}H_{36}N_4O_4Cl_2 \cdot 7/4H_2O$ (559.0)	53.72	7.11	10.02	53.61	7.35	9.96	186—191	+47°	454
DM-eF(*S*)F(*S*)-OEt (DM-**3a**)	$C_{34}H_{41}N_4O_6Cl \cdot 5/4H_2O$ (659.7)	61.90	6.65	8.49	61.65	6.53	8.57	154—159	+53°	600
DM-eF(*S*)F(*S*)-Red (DM-**3b**)	$C_{32}H_{42}N_4O_4Cl_2 \cdot 5/2H_2O$ (662.6)	58.00	7.15	8.45	58.07	6.96	8.35	171—175	+36°	544
DM-eF(*R*)G-OEt (DM-**2a**)	$C_{27}H_{35}N_4O_6Cl \cdot 3H_2O$ (601.1)	53.94	6.87	9.32	53.66	6.68	9.16	155—168	−17°	510
DM-eF(*R*)G-Red (DM-**2b**)	$C_{25}H_{36}N_4O_4Cl_2 \cdot 6H_2O$ (635.6)	47.24	7.61	8.81	47.33	7.50	8.53	202—209	+30°	454
DM-eF(*R*)F(*R*)-OEt (DM-**4a**)	$C_{34}H_{41}N_4O_6Cl \cdot 5/4H_2O$ (659.7)	61.90	6.65	8.49	61.79	6.37	8.48	190—200	+23°	600
DM-eF(*R*)F(*R*)-Red (DM-**4b**)	$C_{32}H_{42}N_4O_4Cl_2 \cdot 4H_2O$ (689.7)	55.72	7.31	8.12	55.84	7.17	8.04	190—200	+61°	544

a) All samples were purified by washing them thoroughly with dry diethyl ether.

materials by the solution method, as outlined in Chart 2. Their reduced analogues [H–Tyr–D-Ala–eXX′-Red = DM-eXX′-Red = DM-eF(*S*)G(DM-**1b**)-, DM-eF(*R*)G(DM-**2b**)-, DM-eF(*S*)F(*S*)(DM-**3b**)- and DM-eF(*R*)F(*R*)(DM-**4b**)-Red] were similarly prepared using eXX′-Red(piperazine derivatives) as starting materials. A typical method for preparing dermorphin analogues is as follows. The deprotection of the *tert*-butyloxycarbonyl (Boc) group of Boc–D-Ala-eF(*S*)G-OEt, obtained from eF(*S*)G-OEt (**1a**) and Boc–D-alanine by using 2-ethoxy-1-ethyloxycarbonyl-1, 2-dihydroquinoline (EEDQ) as a condensing agent in chloroform ($CHCl_3$), was carried out in 4 N hydrochloric acid(HCl)–dioxane (DOX) solution. The tripeptide [H-D-Ala-eF(*S*)G-OEt] thus obtained was coupled with Boc-tyrosine *N*-hydroxysuccinimide ester (Boc–Tyr–OSu) in $CHCl_3$. After removal of the Boc-group with 4 N HCl–DOX using anisole as a scavenger, the desired tetrapeptide [H–Tyr–D-Ala–eF(*S*)G-OEt = DM-**1a**] was produced in 70% yield. The other dermorphin analogues were similarly prepared. Table 1 shows the analytical and physical data for the mono-hydrochlorides of DM-eXX′-OEt and di-hydrochlorides of DM-eXX′-Red.

The results of GPI and MVD assays of the mono- and di-hydrochlorides of dermorphin analogues prepared in this experiment are summarized in Table 2. As shown in Table 2, distinct differences in activity are observed in GPI assay rather than in MVD assay. The values of IC_{50} ($0.94—1.60 \times 10^4$ nM) for these dermorphin analogues obtained in MVD assay are comparable with those ($0.45—1.10 \times 10^4$ nM) for enkephalin analogues[5] reported earlier. Table 2 shows that the IC_{50} values for DM-

Table 2. Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD) Assays of Dermorphin Analogues

Compound	GPI (IC_{50}/nM)[a] [$\times 10^4(\pm \times 10^3)$]	MVD (IC_{50}/nM)[a] [$\times 10^4(\pm \times 10^3)$]	GPI/MVD (IC_{50}-ratio)
HCl·DM-eF(*S*)G-OEt (DM-**1a**)	920 (±400)	1.40 (±1.10)	657
2HCl·DM-eF(*S*)G-Red (DM-**1b**)	3.10 (±1.81)	1.10 (±0.75)	2.82
HCl·DM-eF(*S*)F(*S*)-OEt (DM-**3a**)	4.00 (±2.88)	0.94 (±0.80)	4.25
2HCl·DM-eF(*S*)F(*S*)-Red (DM-**3b**)	2.00 (±1.08)	0.96 (±0.52)	2.11
HCl·DM-eF(*R*)G-OEt (DM-**2a**)	0.14 (±0.09)	1.20 (±0.80)	0.12
2HCl·DM-eF(*R*)G-Red (DM-**2b**)	0.25 (±0.19)	1.60 (±0.95)	0.16
HCl·DM-eF(*R*)F(*R*)-OEt (DM-**4a**)	2.60 (±1.38)	1.40 (±1.45)	1.86
2HCl·DM-eF(*R*)F(*R*)-Red (DM-**4b**)	0.13 (±0.08)	1.10 (±0.98)	0.12
Dermorphin[3]	0.00033	0.0029	0.11
H–Tyr–D-Ala–Phe–Gly–NH_2[3]	0.0110	0.0892	0.12

a) Results are expressed as means ± S.E. of three experiments.

eF(*S*)G-Red (DM-**1b**) and DM-eF(*R*)F(*R*)-Red (DM-**4b**) obtained in GPI assay are 3.10 and 0.13×10^4 nM, and those for DM-eF(*S*)G-OEt (DM-**1a**) and DM-eF(*R*)F(*R*)-OEt (DM-**4a**) are 920 and 2.6×10^4 nM, respectively. These results suggest that the activity of DM-**1b** (DM-**4b**) with a piperazine ring, of which the conformation is more flexible than that of a piperazin-2-one ring, is about 300 (20) times higher than that of DM-**1a** (DM-**4a**) with a piperazin-2-one ring. Also, Table 2 reveals that the activities of dermorphin analogues with (*R*)-phenylalanine residues are superior to those of analogues with (*S*)-phenylalanine residues, except for DM-eF(*S*)F(*S*)-OEt (DM-**3a**) and DM-eF(*R*)F(*R*)-OEt (DM-**4a**), in GPI assay. In particular, DM-eF(*R*)G-OEt (DM-**2a**) ($IC_{50} = 0.14 \times 10^4$ nM) is about 6500 times more active than the corresponding eF(*S*)G-OEt (DM-**1a**) ($IC_{50} = 920 \times 10^4$ nM). Accordingly, it is apparent from the results of GPI assays that the activities of the dermorphin analogues used here are enhanced markedly by replacing (*S*)-phenylalanine residues with (*R*)-phenylalanine residues, and the piperazin-2-one ring with the piperazine ring, though their activities are lower than that [$IC_{50} = 3.3$ nM] of the parent dermorphin.[3] Moreover, the large value (657) of the IC_{50} ratio (GPI/MVD) observed for DM-**1a** implies that DM-**1a** binds the δ-receptor in preference to the μ-receptor. However, further investigations are needed to explain the observations fully.

Experimental

Chemistry All samples were measured at room temperature using JASCO IRA-1(IR spectra), JEOL GX-400 (^{1}H-NMR spectra; solutions in $CDCl_3$, tetramethylsilane as an internal standard) and JASCO DIP-370 (optical rotations) instruments. Mass spectra (FAB-MS) were obtained on JEOL Q-300 [m/z (M^+); in the case of the hydrochlorides or their hydrates, m/z = molecular weight -1 or 2 HCl and $-n H_2O$]. α-Amino acids, their derivatives and reagents for synthesis of peptides were purchased from Peptide Institute Inc., Osaka, and Kokusan Chemical Works Ltd., Tokyo.

Bioassay All dermorphin analogues prepared here are effective at inhibiting electrically induced contractions in the bioassays examined. Their values of IC_{50} were determined at 37 °C in Krebs–Ringer solution ($NaCl$–KCl–$CaCl_2$–KH_2PO_4–$NaHCO_3$–D-glucose = 118 : 4.75 : 2.45 : 1.19 : 25.0 : 11.0 mM) using the *vas deferens* of ddY mice (8—9 weeks of age) and the ileum of guinea pigs (Hartley strain) (4 weeks of age).

Preparation of *N,N'*-Ethylene-Bridged Dipeptide Ethyl Esters (eXX'-OEt = 1a, 2a 3a and 4a) Ethyl-(3'*R*)-2-(2'-oxo-3'-benzyl-1'-piperazinyl)-acetate (**2a**) was synthesized according to the method[7] used for preparing the corresponding (*S*)-isomer (**1a**), and was purified from ethyl acetate (EtOAc)/dichloromethane (CH_2Cl_2) as the hydrochloride. The physical and analytical data for the hydrochloride of **2a** are as follows. mp 171—173 °C [170—174 °C for (*S*)-isomer (**1a**)]; $[\alpha]_D$ +100° (c = 1.2, EtOH) (−104° for **1a**). *Anal.* Calcd for $C_{15}H_{21}N_2O_3Cl \cdot 1/3H_2O$: C, 56.51; H, 6.85; N, 8.79. Found: C, 56.78; H, 6.78; N, 8.78. Ethyl-(2*R*, 3'*R*)-3-phenyl-2-(2'-oxo-3'-benzyl-1'-piperazinyl)propionate (**4a**) prepared by our method[5,7,8] was purified from EtOH as the hydrochloride. The physical and analytical data for the hydrochloride of **4a** are as follows. mp 192—198 °C [192—197 °C for (*S*)-isomer (**3a**)]; $[\alpha]_D$ +171° (c = 0.5, EtOH) [−168° for **3a**]. *Anal.* Calcd for $C_{22}H_{27}N_2O_3Cl$: C, 65.58; H, 6.75; N, 6.95. Found: C, 65.56; H, 6.77; N, 6.94. The ^{1}H-NMR, IR and MS data for **2a** (**4a**) were identical with those[5,7] reported previously for **1a** (**3a**).

Preparation of Piperazine Derivatives The preparation of the dihydrochloride of (3'*S*)-2-(3'-benzyl-1'-piperazinyl)-ethanol [eF(*S*)G-Red = **1b**] is described as a typical example. Sodium borohydride (4.5 g, 0.120 mol) was suspended in THF (50 ml) at 0 °C, and methyl iodide (17.0 g, 0.120 mol) was added. After 1 h, **1a** (3.7 g, 0.012 mol) was added to the THF solution containing BH_3 with stirring, and then the reaction was continued at room temperature for 24 h. At the end of the reaction, water (10 ml) was added slowly to the solution in order to decompose excess BH_3. After removal of the solvent, the oily residue obtained was treated with aqueous K_2CO_3, and then extracted with CH_2Cl_2. After addition of 4 N HCl–DOX (5 ml) to the extract, a solid was precipitated. It was collected by filtration, and found to be almost pure by TLC. Its IR spectrum showed the disappearance of the amide (1643 cm^{-1}) and ester (1736 cm^{-1}) carbonyl group absorptions of **1a**. This solid was subjected to silica-gel column chromatography [$CHCl_3$: MeOH = 4 : 1], and purified from isopropanol/ether as the di-hydrochloride of **1b** (2.7 g, 0.009 mol) in 75% yield. mp 185—189 °C [185—190 °C for (*R*)-isomer (**2b**)]. MS m/z = 220. $[\alpha]_D$ −15° (c = 1.0, EtOH) (+13° for **2b**). *Anal.* Calcd for $C_{13}H_{22}N_2OCl_2 \cdot 1/10H_2O$: C, 52.92; H, 7.58; N, 9.49. Found: C, 52.89; H, 7.70; N, 9.31. (2*S*,3'*S*)-3-Phenyl-2-(3'-benzyl-1'-piperazinyl)-propanol (**3b**) or **4b** obtained similarly was purified as the di-hydrochloride from MeOH in 70—80% yield. **3b**: mp 254—262 °C [256—262 °C for (*R*)-isomer (**4b**)]. MS m/z = 310. $[\alpha]_D$ −24° (c = 1.1, EtOH) (+23° for **4b**). *Anal.* Calcd for $C_{20}H_{28}N_2OCl_2 \cdot 1/10H_2O$: C, 62.37; H, 7.38; N, 7.27. Found: C, 62.35; H, 7.44; N, 7.22.

Preparation of Dermorphin Analogues The preparation of the hydrochloride of DM-eF(*S*)G-OEt (DM-**1a**) [HCl·H–Tyr–D-Ala–eF(*S*)G-OEt] is shown as a typical example.

(1) A solution of Boc–D-alanine (0.46 g, 2.40 mmol) and **1a** (0.66 g, 2.40 mmol) in $CHCl_3$ (50 ml) was treated with EEDQ (0.59 g, 2.40 mmol) at room temperature with stirring. After 2 d, the solution was washed with aqueous $KHSO_4$, aqueous $NaHCO_3$ and water, and then dried over anhydrous Na_2SO_4. After removal of the solvent, the oily residue obtained was purified by silica-gel column chromatography (benzene : EtOAc = 3 : 2) to afford Boc-D-Ala-**1a** (0.63 g, 1.40 mmol) in 58% yield.

(2) Boc-D-Ala-**1a** (0.63 g, 1.40 mmol) obtained in **1** was dissolved for the removal of the Boc-group in 4 N HCl–DOX (7 ml, 28.0 mmol) at room temperature. The solution was evaporated to dryness, followed by the addition of dry ether. The solid obtained was collected by filtration, giving the hydrochloride of H-D-Ala-**1a** (0.40 g, 1.04 mmol) in 74% yield. This product was used in (**3**) without further purification.

(3) A solution of HCl·H-D-Ala-**1a** (0.40 g, 1.04 mmol), Boc–Tyr–OSu

(0.41 g, 1.08 mmol) and triethylamine (0.12 g, 1.19 mmol) in $CHCl_3$ (50 ml) was stirred at room temperature for 24 h. The solution was washed with aqueous $KHSO_4$, aqueous $NaHCO_3$ and water, and then dried over anhydrous Na_2SO_4. After removal of the solvent, Boc–Tyr–D-Ala-**1a** (0.67 g, 0.98 mmol) was obtained in 94% yield as a powder. The Boc group was removed with 4 N HCl–DOX (10 ml, 40.00 mmol) in the presence of anisole (0.11 g, 1.02 mmol) as a scavenger. The powder was purified by silica-gel column chromatography ($CHCl_3$: MeOH = 4 : 1), and washed thoroughly with dry ether, affording the desired dermorphin analogue (HCl·Tyr–D-Ala-**1a** = DM-**1a**, 0.37 g, 0.68 mmol) in 70% yield. The other dermorphin analogues (DM-eXX′-OEt = DM-**2a**, -**3a** and -**4a**. DM-eXX′-Red = DM-**1b**, **2b**, **3b** and **4b**) were similarly synthesized according to the procedure described above for the preparation of DM-**1a**.

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References and Notes

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