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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ENANTIO-SEPARATION BASED ON DIASTEREOMER FORMATION WITH NEW FLUORESCENT CHIRAL QUINOXALINES

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Abstract – Two kinds of chiral 2,3-dimorpholinoquinoxalines bearing L-proline and *N*-bromoacetylproline residues at C-6 position were newly synthesized. The derivatization of (±)-naproxen, (±)-ibuprofen, and (±)-2-phenylpropionic acid with chiral quinoxalines afforded the diastereomers, which emitted an intense fluorescence. All the diastereomers were clearly separated within 16 min on reversed-phase (Finepak SIL C_{18}S) HPLC equipped with a fluorescence detector. The detection limit of the derivatized product was estimated to be 5 pmol/10 μL injection volume at S/N=5. Further, the quantum yield of the diastereomer was estimated to be 0.052 by using the relative quantum yield measurement with anthracene as a standard.

Numerous drugs have been developed and used for treatment of various diseases.[1] Enantiomers that exist in two mirror-image forms show identical chemical and physical properties, except for the direction of rotation of polarized light. When enantiomers are administered as drugs, they sometimes show different activities, toxicities, and pharmacokinetic properties. One of representative examples is thalidomide; the sedative hypnotic effect is associated with the *(R)*-isomer and the teratogenic effect is associated with the *(S)*-isomer.[2] Taking into account this medicinal disaster, chiral separation is quite important for the purpose of the investigation of difference in biological effect and the establishment of the specification for drug marketing.

High-performance liquid chromatography (HPLC) has been most widely used as a powerful tool for chiral analysis.[3] Many fluorescent chiral derivatization reagents have been developed; 1-(4-dansylaminophenyl)ethylamine (DAPEA),[4] 2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole (AP-MB),[5] 1-(4-dimethylamino-1-naphthyl)ethylamine (DANE),[6,7] 2-*tert*-butyl-2-methyl-(1,3-benzodioxole-

4-carboxylic acid (TBMB carboxylic acid),[8,9] □-methylbenzyl isothiocyanate (AMBI),[10] 1-methyl-2-(2,3-naphthalimido)ethyl trifluoromethanesulfonate (MNE-OTf),[11] 1-(9-fluorenyl)ethyl chloroformate (FLEC),[12] chiral amines (BOPA, FLOPA, and NAPA),[13,14] *o*-phthalaldehyde-*N*-acetyl-L-cysteine (OPTA-AcCys),[15,16] 1-(naphthyl)ethyl isocyanate (NEI),[17] 4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-amino-pyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-APy),[18] 4-nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxa-diazole (NBD-APy),[18] and 4-(aminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole (ABD-APy),[18] 4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole (NBD-PyNCS),[19] and 2-(anthracene-2,3-dicarboximido)-1-propyl trifluoromethanesulfonate (AP-OTf).[20,21] However, among them NBD-APy, ABD-APy, DBD-APy, and AP-OTf are only commercially available, to the best of our knowledge. In addition, these reagents are expensive and need many steps for synthesis. Therefore, the development of new fluorescent chiral derivatization reagents, which are inexpensive and easily availabe, is still desired. Previously we demonstrated that 2,3-disubstituted 6-aminoquinoxalines[22] and 6-(bromoacetyl)amino-2,3-dimorpholinoquinoxaline[23] showed excellent fluorescent characteristics and became possible to utilize as high sensitive fluorescence derivatization reagents for long-chain carboxylic acids.

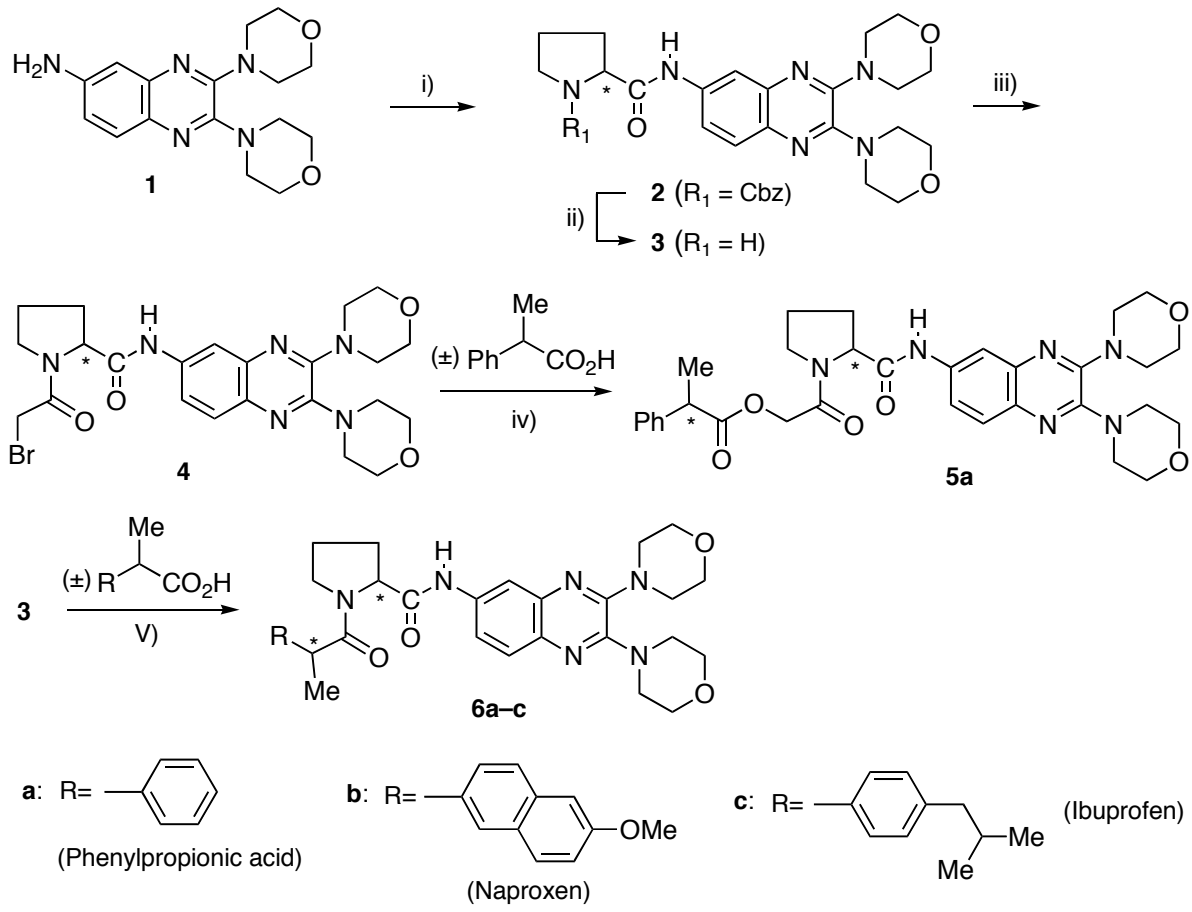
We would like to describe here synthesis of two kinds of new chiral 2,3-dimorpholinoquinoxalines bearing L-proline and *N*-(bromoacetyl)proline residues at C-6 position and their application to fluorescent chiral derivatization reagents for liquid chromatographic enantioseparation based on diastereomer formation.

RESULTS AND DISCUSSION

Synthesis of chiral fluorescent quinoxalines: 2,3-Dimorpholino-6-aminoquinoxaline (**1**) was prepared according to the literature method.[22] The coupling of 6-aminoquinoxaline (**1**) with Cbz-L-proline was carried out using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (water soluble carbodiimide: WSC) and 1-hydroxybenzotriazole (HOBt) to give *N*-benzyloxycarbonyl-L-prolylamino-2,3-dimorpholinoquinoxaline (**2**). The removal of the Cbz group with the catalytic hydrogenation in MeOH at room temperature gave 6-L-prolylamino-2,3-dimorpholinoquinoxaline (**3**). 6-Amino-quinoxaline (**3**) was subjected to the condensation with bromoacetic acid in the presence of DCC to afford 6-*N*-bromoacetyl-L-prolylamino-2,3-dimorpholinoquinoxaline (**4**). (Scheme 1)

Derivatization of racemic carboxylic acids with chiral fluorescent quinoxalines: The fluorescence derivatization was carried out using the following two methods. (±)-2-Phenylpropionic acid was treated with 6-*N*-bromoacetyl-L-prolylaminoquinoxaline (**4**) in the presence of 18-crown-6 and $KHCO_3$[23,24] at room temperature to give the derivatized product (**5a**). (±)-2-Phenylpropionic acid and anti-inflammatory drugs such as naproxen and ibuprofen was treated with 6-L-prolylaminoquinoxaline (**3**) in the presence of 2,2'-dipyridyl disulfide and Ph_3P[25] to give the derivatized products (**6a**-**c**). (Scheme 1)

On ^{1}H-NMR spectra of the derivatized products, two sets of signals for each proton were observed in the ratio of 1:1, indicating that the reaction rates were essentially the same for each enantiomer. Further, it is noteworthy that the derivatized products (**5a** and **6a**-**c**) emit an intense fluorescence.



Scheme 1 Reagents and conditions: i) Cbz-L-proline, WSC-HOBt, dry DMF-CH_2Cl_2; ii) H_2/10% Pd-C, MeOH; iii) $BrCH_2CO_2H$, DCC, dry DMF; iv) 18-crown-6, $KHCO_3$, MeCN; v) 2,2'-dipyridyl disulfide, Ph_3P, CH_2Cl_2.

Fluorescence characteristics of the derivatized products: Fluorescence spectra of the derivatized products (**5a** and **6a**-**c**) were measured in MeCN, and the data are summarized in Table 1. The fluorescence maxima appeared around 440 nm when the excitation wavelength of about 370 nm was applied. Large Stokes' shifts were also observed. The solvent effect upon the fluorescence intensity of the derivatized product (**6b**) was examined in various solvents, which are often used as components of a mobile phase on the reversed-phase column in HPLC, and the data are summarized in Tables 2. The relative fluorescence intensity in MeOH and aqueous solutions were remarkably decreased. This quenching may be attributable to the disruption of co-planarity between the amide bond and the quinoxaline ring. An intramolecular hydrogen bond between a lone pair of electrons of amide oxygen and an aromatic hydrogen may exsist in aprotic polar solvents such as MeCN. On the other hand, hydrogen bonds between the amide oxygen and the surrounding solvent predominantly exist in protic

polar solvents such as MeOH and aqueous solutions. (Figure 1) The co-planarity, therefore, is extinguished and the fluorescence intensity results in decrease.

Table 1 Data of fluorescence spectra of the derivatized products

	Fluorescence[a)]	
Compound	Ex (nm)	Fλ_{max} (nm)
5a	369	442
6a	367	442
6b	370	443
6c	369	442

a) 1.0×10^{-6} M in MeCN.

Table 2 The relative fluorescence intensity (RFI) of the derivatized product (**6b**) in various solvents

	Fluorescence[a)]		
Solvent	Ex (nm)	Fλ_{max} (nm)	RFI[b)]
MeCN	369	442	1.00
MeCN-H_2O (95:5)	370	447	0.35
MeCN-H_2O (75:25)	370	446	0.15
MeCN-H_2O (50:50)	370	448	0.067
MeOH	367	445	0.11

a) 1.0×10^{-6} M. b) Relative fluorescence intensity: the fluorescence intensity of **6b** in MeCN is arbitrarily taken as 1.00.

The fluorescence quantum yield is quite important when the fluorescence property, sensitivity, and the detection limit are considered. The relative quantum yield, therefore, was measured using anthracene as a standard, and that of the derivatized product (**6b**) was estimated to be 0.052.

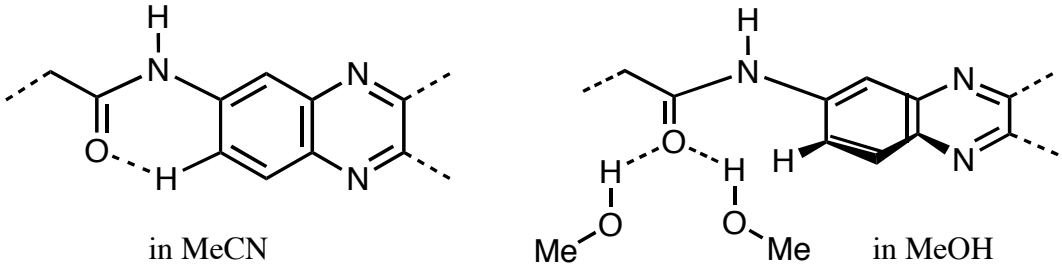


Figure 1 Possible hydrogen bonds

HPLC analysis: Separation of the derivatized diastereomeric product (**5a**) under various solvent systems as mobile phases was attempted by using the reversed-phase HPLC equipped with a fluorescence detector. Unfortunately, a satisfactory peak separation could not be obtained (not shown).

On the other hand, the derivatized diastereomeric products (**6a**-**c**) were completely separated within 16 min. The capacity factors (*k'*), separation factors (□), and resolutions (R_s) are summarized in Table 3, and typical chromatograms of the derivatized diastereomeric products in the reversed-phase HPLC are shown in Figure 2. It is clearly indicative that the distance between two asymmetric carbons is one of the most important factors on separation of diastereomers, that is, the distance between two asymmetric carbons in compound (**6a**) is more proximal than that in compound (**5a**).[26]

Table 3 HPLC separations of the derivatized diastereomeric products (**6a**-**c**)[a)]

Compound	Enantiomer	*k'*	□	R_S
6a	*S*	1.18	1.24	2.02
	R	1.46		
6b	*S*	0.77	1.35	2.20
	R	1.04		
6c	*S*	1.22	1.34	2.82
	R	1.63		

a) Column: Finepak SIL $C_{18}S$; mobile phase: MeCN:H_2O (70:30) for **6a** and MeCN:H_2O (80:20) for **6b** and **6c**; Flow rate: 0.3 mL/min.

It is quite important to estimate the occurrence of racemizaton during the derivatization reaction. The derivatization of (*S*)-(+)-phenylpropionic acid with the chiral quinoxaline (**3**) was carried out. (*S*)-(+)-Phenylpropionic acid used in this work was ≧98.0% in purity, and the percentage of the peak was calculated to be ≧98.0%. It is concluded that a measurable racemization did not occur during the derivatization reaction.

The detection limit for the derivatized product (**6b**) was determined using the reversed-phase HPLC equipped with a fluorescence detector. A linear relationship between the peak area and the amount of the derivatized product (**6b**) was observed in the range from 5 pmol to 1 nmol/10 □L injection volume (a correlation coefficient = 0.999), and thus the detection limit was estimated to be 5 pmol/10 □L at S/N=5. The detection limits of commercially available fluorescence chiral derivatization reagents, NBD-APy,[18] ABD-APy,[18] DBD-APy,[18] and AP-OTf[20, 21] were 30, 15 and 10 fmol, (5 □L injection volume at S/N=2), and 4 fmol (10 □L injection volume at S/N=3), respectively. However, taking account of the synthetic simplicity, 6-L-prolylamino-2,3-dimorpholinoquinoxaline (**3**) is applicable to new fluorescent chiral derivatization reagents for liquid chromatographic enantioseparation based on diastereomer formation.

EXPERIMENTAL

Melting points were measured on a Mel-Temp apparatus in open capillaries and are uncorrected. IR and fluorescence spectra were recorded on JASCO JIR-3510 FT-IR and FP-6500 spectrophotometers, respectively. ^{1}H-NMR spectra were recorded on a JEOL GX-400 NMR spectrometer in $CDCl_3$, and are reported in ppm (□) downfield from internal Me_4Si. Matrix-assisted laser desorption/ionization time-of-

flight (MALDI-TOF) MS spectra were taken on a Shimadzu AXIMA-CFR plus with a linear mode. The nitrogen laser was set to deliver 337 nm wavelength pulses to the sample. Dithranol (for **2**, **3**, **5a**, and **6a-c**) and □-cyano-4-hydroxycinnamic acid (for **4**) were used as the matrixes. Thin layer chromatographic (TLC) analysis was performed on silica gel 60F-254 with a 0.2 mm layer thickness. High performance liquid chromatography (HPLC) was carried out with a JASCO 880-PU, a 875-UV and a 821-FP equipped with a JASCO 807-IT integrator by using a column packed with a Finepak SIL C_{18}S.

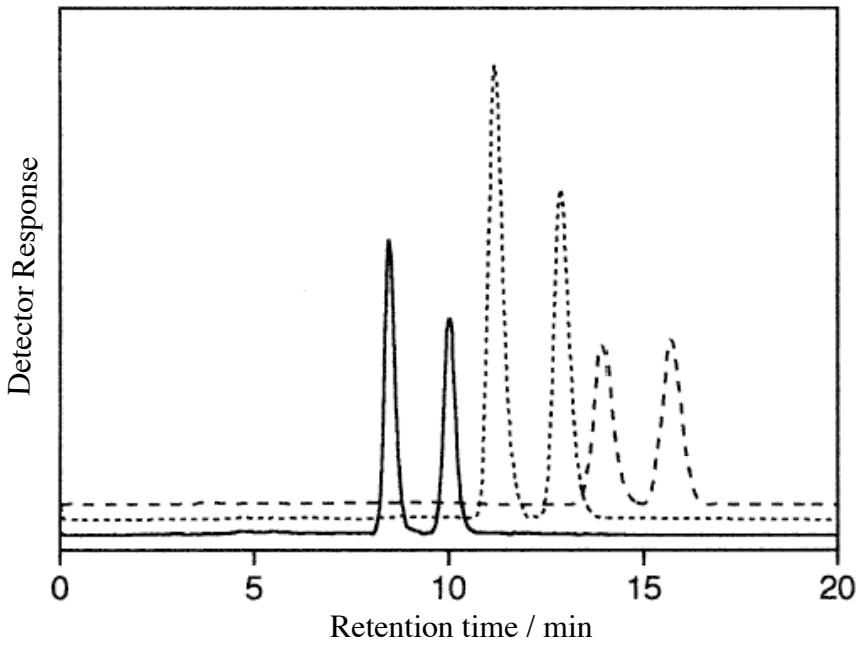


Figure 2 Chromatograms of the derivatized diastereomericproducts (**6a**-**c**) on reversed-phase HPLC:**6a** (_ _ _), **6b** (___), and **6c** (_____).

Benzyl 2-(2,3-dimorpholinoquinoxalin-6-ylcarbamoyl)pyrrolidine-1-carboxylate (2):

To a solution of compound (**1**)[22] (400 mg, 1.3 mmol), Cbz-L-proline (324 mg, 1.3 mmol), HOBt (190 mg, 1.4 mmol) in dry DMF (10 mL) was added WSC·HCl (270 mg, 1.4 mmol) in CH_2Cl_2 (10 mL) at -10 °C. The reaction mixture was stirred for 2 h at -10 °C and for another 19 h at rt. After removal of the solvent, the residue was dissolved in $CHCl_3$ (50 mL). The organic layer was successively washed with 5% HCl (30 mL x 2), 5% $NaHCO_3$ (30 mL x 2), H_2O (30 mL x 2), saturated NaCl (30 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel with $CHCl_3$-acetone-EtOH (100:10:2) mixture to give the pure product (**2**) (576 mg, 83%); mp: 115-118 °C; IR (KBr): 1697 cm^{-1} (□$_{C=O}$); ^{1}H-NMR (□, $CDCl_3$, 400 MHz): 1.82-2.47 (6H, m, -CH_2-CH_2-CH_2-N), 3.32-3.61 (8H, m, -CH_2-N-CH_2-), 3.71-3.93 (8H, m, -CH_2-O-CH_2-), 4.44-4.46 (1H, m, -C*H-), 5.15 (2H, s, Ph-CH_2-), 7.30-7.40 (5H, m, -C_6H_5), 7.51 (1H, dd, *J*=1.9 and 8.5 Hz, C7-H), 7.65 (1H, d, *J*=8.5 Hz, C8-H), 8.03 (1H, d, *J*=1.9 Hz, C5-H), and 9.40 ppm (1H, s, -C*CO-NH-); MALDI-TOF MS: m/z 547.49 ($[M+1]^+$); $[□]_D^{25}$= -112.4° (c=0.1 in MeOH). *Anal.* Calcd for $C_{29}H_{34}N_6O_5$: C, 63.72; H, 6.27; N, 15.37. Found: C, 63.60; H, 6.40; N, 15.14.

***N*-(2,3-Dimorpholinoquinoxalin-6-yl)pyrrolidine-2-carboxamide (3):**

A suspension of 10% Pd-C (0.13 g) in distilled MeOH (100 mL) was prehydrogenated with H_2 for 1 h at rt. To this suspension was added a solution of compound (**2**) (0.79 g, 1.45 mmol) in distilled MeOH (100 mL). The reaction mixture was hydrogenated for 19 h under hydrogen atmosphere at rt. After filtration of the catalyst, the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with $CHCl_3$-MeOH (30:1) mixture to give the pure product (**3**) (536 mg, 90%); mp: 120-123 °C; IR (KBr): 1681 cm^{-1} ($\nu_{C=O}$); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 2.17-2.27 and 2.97-3.11 (6H, m, -CH_2-CH_2-CH_2-N), 2.32 (1H, br s, -NH-C*), 3.51-3.62 (8H, m, -CH_2-N-CH_2-), 3.81-3.91 (9H, m, -CH_2-O-CH_2- and -C*H-), 7.63 (1H, dd, *J*=1.9 and 8.5 Hz, C7-H), 7.66 (1H, d, *J*=8.5 Hz, C8-H), 8.08 (1H, d, *J*=1.9 Hz, C5-H), and 9.94 ppm (1H, s, -C*CO-NH-); MALDI-TOF MS: m/z 413.35 ($[M+1]^+$); $[\alpha]_D^{25}$= -32.5° (c=0.1 in MeOH). *Anal.* Calcd for $C_{21}H_{28}N_6O_3 \cdot 0.5H_2O$: C, 59.84; H, 6.93; N, 19.94. Found: C, 59.64; H, 6.90; N, 19.61.

1-(2-Bromoacetyl)-*N*-(2,3-dimorpholinoquinoxalin-6-yl)pyrrolidine-2-carboxamide (4):

To a solution of compound (**3**) (220 mg, 0.53 mmol) in dry DMF (10 mL) was added a mixture of bromoacetic acid (0.74 g, 5.3 mmol) and DCC (1.1 g, 5.3 mmol) in dry DMF (10 mL), and then the reaction mixture was stirred for 2 h at 0 °C and for another 1 h at rt. After removal of the solvent, the residue was dissolved in AcOEt (120 mL) and stood in a refrigerator. After filtration of 1,3-dicyclohexylurea, the filtrate was successively washed with 5% citric acid (30 mL), 5% $NaHCO_3$ (30 mL), H_2O (30 mL), and saturated NaCl (30 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel with $CHCl_3$-acetone-EtOH (100:10:2) mixture to give the pure product (**4**) (235 mg, 83%); mp: 118-120 °C; IR (KBr): 1660 cm^{-1} ($\nu_{C=O}$); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.88-2.68 (4H, m, -CH_2-CH_2-C*), 3.52-3.64 (8H, m, -CH_2-N-CH_2-), 3.64-3.75 (2H, m, -N-CH_2-C), 3.82-3.88 (8H, m, -CH_2-O-CH_2-), 3.92 and 4.15 (2H, ABq, *J*=13.0 Hz, Br-CH_2-), 4.78-4.85 (1H, m, -N-C*H-CO-), 7.50 and 7.51 (1H, dd, *J*=2.2 and 8.8 Hz, C7-H), 7.63 (1H, d, *J*=8.8 Hz, C8-H), 8.03 (1H, d, *J*=2.2 Hz, C5-H), and 9.41 ppm (1H, s, C*-CONH); MALDI-TOF MS: m/z 533.36 ($[M+1]^+$). *Anal.* Calcd for $C_{23}H_{29}N_6O_4Br \cdot 1.2H_2O$: C, 49.85; H, 5.70. Found: C, 49.85; H, 5.49.

Derivatization of (±)-2-phenylpropionic acid with compound (4):

To a solution of compound (**4**) (30 mg, 0.056 mmol) in CH_3CN (8 mL) were added (±)-2-phenylpropionic acid (8.4 mg, 0.056 mmol), 18-crown-6 (15 mg, 0.056 mmol), and $KHCO_3$ (168 mg, 1.7 mmol), and then the reaction mixture was stirred for 50 min at rt. After removal of the solvent, the residue was dissolved in AcOEt (80 mL). The organic layer was washed with 5% $NaHCO_3$ (30 mL), and then dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel with $CHCl_3$-acetone-EtOH (100:5:1) mixture to give 2-phenylpropanoyloxyacetyl-*N*-(2,3-

dimorpholinoquinoxalin-6-yl)pyrrolidine-2-carboxamide (**5a**) as a mixture of diastereomers (27 mg, 80%); mp: 110-112 °C; IR (KBr): 1742, 1656, and 1683 cm^{-1} ($\nu_{C=O}$); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.58 and 1.60 (3H, d, *J*=5.0 Hz, Ph-C*-CH_3), 1.84-1.94, 2.00-2.08, and 2.50-2.58 (4H, m, -CH_2-CH_2-C*), 3.34-3.45 (2H, m, -N-CH_2-C), 3.52-3.63 (8H, m, -CH_2-N-CH_2-), 3.79-3.95 (9H, m, -CH_2-O-CH_2- and Ph-C*H(Me)-), 4.58 and 4.78 (1H, ABq, *J*=14.6 Hz, -O-C*H*H-), 4.66 and 4.70 (1H, ABq, *J*=14.6 Hz, -O-CH*H*-), 4.78-4.83 (1H, m, -N-C*H-CO-), 7.30-7.40 (5H, m, C_6H_5-C*-), 7.48 and 7.51 (1H, dd, *J*=2.4 and 9.0 Hz, C7-H), 7.59 and 7.60 (1H, d, *J*=9.0 Hz, C_8-H), 8.04 and 8.05 (1H, d, *J*=2.4 Hz, C5-H), and 9.35 and 9.42 ppm (1H, s, C*-CONH-); MALDI-TOF MS: m/z 603.51 ($[M+1]^+$); *Anal.* Calcd for $C_{32}H_{38}N_6O_6$: C, 63.77; H, 6.35; N, 13.94. Found: C, 63.47; H, 6.54; N, 13.89.

Derivatization of (±)-phenylpropionic acid with compound (3): A typical procedure:

To a solution of (±)-phenylpropionic acid (36 mg, 0.24 mmol) and 2,2'-dipyridyl disulfide (53 mg, 0.24 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise a solution of compound (**3**) (100 mg, 0.24 mmol) and triphenylphosphine (63 mg, 0.24 mmol) in dry CH_2Cl_2 (15 mL), and then the reaction mixture was stirred for 2 h at rt. After removal of the solvent, the residue was dissolved in $CHCl_3$ (50 mL). The organic layer was successively washed with 5% HCl (30 mL x 2), 5% $NaHCO_3$ (30 mL x 2), H_2O (30 mL x 2), saturated NaCl (30 mL x 2), and then dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by chromatography on silica gel with $CHCl_3$-acetone-EtOH (200:5:1) mixture and subsequent gel filtration on Toyopearl HW-40 with MeOH as an eluant to give 1-(2-phenylpropanoyl)-*N*-(2,3-dimorpholinoquinoxalin-6-yl)pyrrolidine-2-carboxamide (**6a**) as a mixture of diastereomers (118 mg, 89%); mp: 133-135 °C; IR(KBr): 1693 and 1625 cm^{-1} ($\nu_{C=O}$); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.51 (3H, d, *J*=6.8 Hz, -C*-CH_3), 1.63-1.80, 1.81-1.93, 1.97-2.03, 2.09-2.10, 2.52-2.61 and 3.23-3.36 (6H, m, -N-CH_2-CH_2-CH_2-C*-), 3.52-3.68 (8H, m, -CH_2-N-CH_2-), 3.81-3.92 (9H, m, -CH_2-O-CH_2- and -C*H(Me)-CO-), 4.82 and 4.93 (1H, d, *J*= 7.6 Hz, -N-C*H*CO-), 7.15-7.37 (5H, m, -C_6H_5), 7.44 and 7.56 (1H, dd, *J*=2.4 and 8.8 Hz, quino C7-H), 7.64 and 7.65 (1H, d, *J*=8.8 Hz, quino C8-H), 8.00 and 8.07 (1H, d, *J*=2.4 Hz, quino C5-H), and 9.63 and 9.93 ppm (1H, s, -CONH-); MALDI-TOF MS: m/z 545.17 ($[M+1]^+$). *Anal.* Calcd for $C_{30}H_{36}N_6O_4$: C, 66.16; H, 6.66; N, 15.43. Found: C, 66.23; H, 6.68; N, 15.57.

Derivatization of (±)-naproxen with compound (4):

The derivatization was carried out in the same manner described above to give 1-(2-(2-methoxynaphthalen-6-yl)propanoyl)-*N*-(2,3-dimorpholinoquinoxalin-6-yl)pyrrolidine-2-carboxamide (**6b**) as a mixture of diastereomers (108 mg, 71%); mp: 145-148 °C; IR(KBr): 1691 and 1619 cm^{-1} (νC=O); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.56 and 1.58 (3H, d, *J*=6.8 Hz, C*-CH_3), 1.78-1.90, 1.91-2.02, 2.08-2.21, 2.50-2.61, and 3.24-3.38 (6H, m, -N-CH_2-CH_2-CH_2-C*-), 3.52-3.70 (8H, m, -CH_2-N-CH_2-), 3.83-3.91 (8H, m, -CH_2-O-CH_2-), 3.92 (3H, s, -OCH_3), 3.94-3.99 (1H, m, -N-C*H-), 4.86 and 4.96 (1H,

d, *J*=6.8 Hz, -C*H(Me)CO-), 6.98-7.04 (1H, m, naphthyl C7-H), 7.13-7.18 (1H, m, naphthyl C5-H), 7.36 and 7.58 (1H, dd, *J*=2.2 and 9.5 Hz, quino C7-H), 7.38-7.47 (1H, m, naphthyl), 7.58-7.63 (3H, m), 7.71 and 7.73 (1H, d, *J*=9.5 Hz, quino C8-H), 8.01 and 8.09 (1H, d, *J*=2.2 Hz, quino C5-H), and 9.66 and 9.96 ppm (1H, s, -CONH-); MALDI-TOF MS: m/z 625.10 ($[M+1]^+$). *Anal.* Calcd for $C_{35}H_{40}N_6O_5 \cdot 1.5H_2O$: C, 64.44; H, 6.60; N, 12.89. Found: C, 64.74; H, 6.64; N, 12.61.

Derivatization of (±)-ibuprofen with compound (4):

The derivatizationwas carried out in the same manner described above to give 1-(2-(4-isobutylphenyl)propanoyl)-*N*-(2,3-dimorpholinoquinoxalin-6-yl)pyrrolidine-2-carboxamide (**6c**) as a mixture of diastereomers (103 mg, 71%); IR(KBr): 1693 and 1625 cm^{-1} ($\nu_{C=O}$); ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 0.82 and 0.90 (6H, d, *J*=7.0 Hz, -C(CH_3)$_2$-), 1.50 (3H, d, *J*=6.6 Hz, -C*(CH_3)-CO-), 1.71-1.80, 1.81-1.91, 1.95-2.10, 2.52-2.61, and 3.25-3.37 (6H, m, -N-CH_2-CH_2-CH_2-C*-), 2.37 and 2.45 (2H, d, *J*=7.0 Hz, -Ph-CH_2-), 3.52-3.65 (8H, m, -CH_2-N-CH_2-), 3.77-3.90 (9H, m, -CH_2-O-CH_2- and -C*H(Me)-CO-), 4.82 and 4.92 (1H, d, *J*=7.6 Hz, -N-C*H-CO-), 7.02 and 7.17 (2H, d, *J*=8.0 Hz, phenyl C4-H and C6-H), 7.12 and 7.20 (2H, d, *J*=8.0 Hz, phenyl C1-H and C3-H), 7.42 and 7.55 (1H, dd, *J*=2.2 and 8.8 Hz, quino C7-H), 7.63 and 7.65 (1H, d, *J*=8.8 Hz, quino C8-H), 8.01 and 8.07 (1H, d, *J*=2.2 Hz, quino C5-H), and 9.58 and 9.96 ppm (1H, s, -CONH-); MALDI-TOF MS: m/z 601.04 ($[M+1]^+$); *Anal.* Calcd for $C_{34}H_{44}N_6O_4 \cdot H_2O$: C, 65.99; H, 7.49; N, 13.58. Found: C, 66.00; H, 7.69; N, 13.26.

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