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4-Substituted-kynurenic Acid Derivatives: A Novel Class of NMDA Receptor Glycine Site Antagonists

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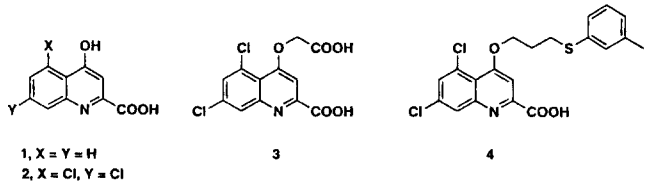
A series of 4-substituted-kynurenic acid derivatives possessing several different substituents at C4-position which are consisted of both a flexible propyloxy chain and an adjunct several type of carbonyl groups has been synthesized and evaluated for their *in vitro* antagonist activity at the glycine site on the NMDA receptor. Of them, N-benzoylthiourea **15c** and N-phenylthiourea **15a** were found to have the best *in vitro* binding affinity with IC_{50} of 3.95 and 6.04 μM, respectively. On the other hand, in compounds **12a~c** and **13** the displacement of a thiourea group to an amide or a carbamate caused a significant decrease of the *in vitro* binding affinity. In the SAR study of the 4-substituted kynurenic acid derivatives, it was realized that the terminal substitution pattern on a flexible C4-propyloxy chain of kynurenic acid nucleus significantly influences on the binding affinity for glycine site; the binding affinity to the NMDA receptor might be increased by the introduction of a suitable electron rich substituent at C4 of kynurenic acid nucleus.

Key words : NMDA receptor antagonists, Glycine modulatory site, [^{3}H]-Glycine displacement test, 4-Substituted-kynurenic acids

INTRODUCTION

Kynurenic acid **1**, an endogenous product of the tryptophan metabolism pathway, and its related compounds have attracted a considerable attention in neurochemistry. It has been known that kynurenic acid derivatives have selective NMDA antagonist activity, as a result of blockade of glycine modulatory site on the NMDA receptor (Kessler *et al.*, 1989; Kemp *et al.*, 1988; Baron *et al.*, 1990). Thus, they are considered as a class of potential therapeutic agents for the treatment of neurological disorders including ischemia, epilepsy, Alzheimer's disease, Huntington's disease and Parkinsonism (Bliss *et al.*, 1993; Klockgether and Turski, 1993; Meldrum *et al.*, 1990; Greenamyre and Young, 1989). The SAR study of the majority of the known NMDA antagonists implies that a hydroxyl group at C4 of kynurenic acids is capable to interact with a H-bonding domain on the receptor and its spatial orientation is very important for the binding activity (Carling *et al.*, 1992; Leeson *et al.*, 1992; Grimwood *et al.*, 1992). As a part of a program aimed at the discovery of new NMDA antagonists, we became interested in preparing a new class of kynurenic acids possessing various subsituents as H-bonding acceptor, at C4. Recently, it was disclosed 4-substituted kynurenic acids **3** and **4** also have micromolar range of NMDA receptor antagonist activity *in vitro* (Harrison *et al.*, 1990; Kim *et al.*, 1996). These findings, together with the precedent antagonists, prompted us to investigate alternative C4-substituents with enhanced biological properties. In this paper we describe synthesis and biological evaluation of a new series of kynurenic acid derivatives, in which a hydroxyl goup at C4 is replaced by a flexible propyloxy moiety fuctionalized with various carbonyl groups.



1, X = Y = H
2, X = Cl, Y = Cl

3

4

Fig. 1.

MATERIALS AND METHODS

All melting points were taken on a Thomas-Hoover melting point apparatus and were uncorrected. ^{1}H-NMR spectra were obtained on Varian Gemini 200

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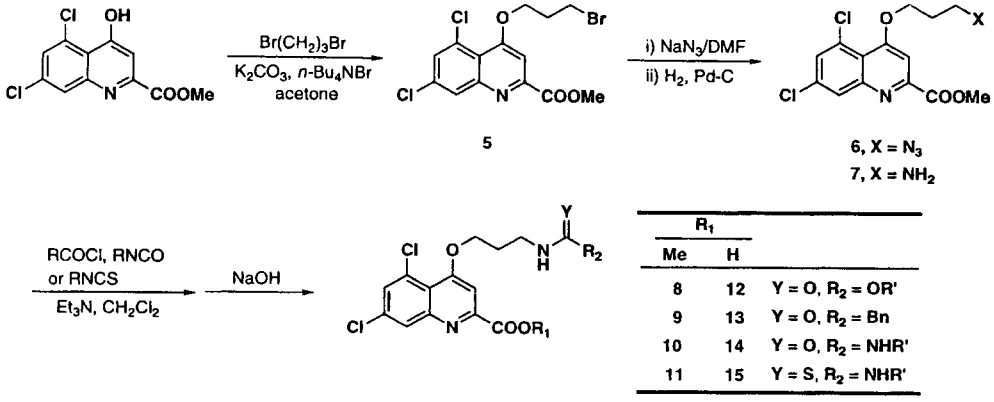


R1		
Me	H	
8	12	Y = O, R2 = OR'
9	13	Y = O, R2 = Bn
10	14	Y = O, R2 = NHR'
11	15	Y = S, R2 = NHR'

Scheme 1.

or Varian AM-300 spectrometers. Chemical shifts were reported in parts per million (δ) relative to a tetramethylsilane as an internal standard. Mass spectra were obtained with VG 12-250 mass spectrometer. Analytical thin-layer chromatography was performed using 0.25 mm silica gel glass-backed plates. E. Merck silica gel (230-400 mesh) was used for flash chromatography. Solvents and reagents were dried and purified prior to use when deemed necessary. Reactions were carried out under nitrogen or argon atmosphere unless otherwise stated.

4-(3-Bromopropoxy)-5,7-dichloroquinoline-2-carboxylic acid methyl ester (5)

A suspension of 5,7-dichlorokynurenic acid methyl ester (0.54 g, 0.2 mmol), 1,3-dibromopropane (1.20 g, 6.0 mmol), K_2CO_3 (0.55 g, 4.0 mmol) and *n*-Bu_4NBr (108 mg, 20 w/w %) in 50mL of acetone was refluxed overnight. The resulting mixture was cooled down to room temperature and filtered. The filtrate was evaporated and purified by recrystallization from methanol to afford **5** (0.42 g, 54%) as a white powder: mp 144~145°C; ^{1}H NMR ($CDCl_3$) δ 2.50 (m, 2H, CH_2), 3.74 (t, 2H, J=6.2 Hz, CH_2Br), 4.07 (s, 3H, CO_2CH_3), 4.41 (t, 2H, J=6.4 Hz, OCH_2), 7.59 (m, 2H, ArH), 8.15 (d, 1H, J=2.2 Hz, ArH); MS m/e 393 [M^+], 334 [M^+-CO_2CH_3].

4-(3-Azidopropoxy)-5,7-dichloroquinoline-2-carboxylic acid methyl ester (6)

A suspension of the bromide **5** (0.39 g, 1.0 mmol) and sodium azide (2.65 g, 4.0 mmol) in 5 mL of DMF was heated at 50°C under a nitrogen atmosphere for 1 h. The mixture was cooled down to room temperature and the solvent was removed in vacuo. The residue was washed with water (10 mL×2) and ether (10 mL×2) and dried to give the azido compound **6** (0.33 g, 92%): mp 132~133°C; ^{1}H NMR ($CDCl_3$) δ 2.21 (m, 2H, CH_2), 3.06 (t,,2H, J=6.6 Hz, OCH_2), 4.06 (s, 3H, CO_2CH_3), 4.33 (t, 2H, J=5.8 Hz, OCH_2), 7.57 (s, 1H, ArH), 7.60 (d, 1H, J=2.2 Hz, ArH), 8.14 (d, 1H, J=2.2 Hz, ArH).

General procedure for preparation of 4-substituted quinoline-2-carboxylic acid methyl esters 8~11

The azido compound **6** (1.0 mmol) was dissolved in 100 mL of EtOAc and hydrogenated with a catalytic amount of 10 % Pd/C. After completion of the reaction, the used Pd/C catalyst was removed by a suction filtration on a celite pad with the aid of a 1:1 mixture of methanol/CH_2Cl_2 as an eluent. The filtrate was evaporated under reduced pressure to afford the corresponding amine **7** as a colorless solid in a quantitative yield. Without the further purification, the crude amine was dissolved in 15 mL of dry CH_2Cl_2 under a nitrogen atmosphere. To the above solution was added the appropriate isocyanates, thioisocyanates, acid chloride or chloroformates (1.2 mmol), and triethylamine (1.2 mmol) in 5 mL of dry CH_2Cl_2 by cannula. The mixture was stirred at room temperature until the starting amine disappeared. And then the mixture was diluted with CH_2Cl_2 (100 mL), washed with H_2O (100 mL×2) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the crude product. The crude was purified by a flash column chromatography (EtOAc:Hexane) to give the corresponding 4-substituted quinoline-2-carboxylic acid methyl esters **8~11**.

4-[3-(3-Benzyl-oxy-carbonylamino)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (8a)

0.32 g (70%); a white solid; ^{1}H NMR ($CDCl_3$) δ 2.18 (m, 2H, CH_2), 3.52 (m, 2H, $NHCH_2$), 4.03 (s, 3H, CO_2CH_3), 4.27 (m, 2H, OCH_2), 5.07 (s, 2H, $ArCH_2$), 5.14 (br s, 1H, NH), 7.25~7.40 (br s, 5H, ArH), 7.48 (s, 1H, ArH), 7.56 (d, 1H, J=2.2 Hz, ArH), 8.11(d, 1H, J=2.2 Hz, ArH).

4-[3-(3-n-Butyrylamido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (8b)

0.17 g (40%); a white solid; mp 120~123°C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J=7.2 Hz, CH_3), 1.32 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 2.18 (m, 2H, CH_2), 3.48 (m, 2H, $NHCH_2$), 3.99~4.05 (m, 2H, OCH_2), 4.05 (s, 3H, CO_2CH_3), 4.29 (t, 2H, J=5.4 Hz, OCH_2), 4.96 (br s, 1H, NH), 7.53 (s, 1H, ArH), 7.59 (d, 1H, J=2.2 Hz, ArH), 8.13 (d, 1H, J=2.2 Hz, ArH).

4-[3-(3-t-Butoxycarbonylamino)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (8c)

0.13 g (30%); a white solid; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, CH_3), 2.17 (m, 2H, CH_2), 3.45 (m, 2H, NCH_2), 4.06 (s, 3H, CO_2CH_3), 4.29 (t, 2H, J=6.0 Hz, OCH_2), 5.26 (br s, 1H, NH), 7.52 (s, 1H, ArH), 7.60 (d, 1H, J=2.0 Hz, ArH), 8.15 (d, 1H, J=2.0 Hz, ArH); MS m/e 429 [M^+], 372 [M^+-*t*-butyl], 356 [M^+-O-t-butyl].

4-[3-(3-phenylacetylamido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (9a)

0.15 g (26 %); a white solid; mp 171~173°C; ^{1}H NMR ($CDCl_3$) δ 2.13 (m, 2H, CH_2), 3.51 (m, 2H, CH_2), 3.56 (s, 2H, $PhCH_2$), 4.06 (s, 3H, CO_2CH_3), 4.19 (t, 2H, J=5.6 Hz, OCH_2), 5.63 (br s, 1H, NH), 7.16~7.25 (m, 5H, ArH), 7.47 (s, 1H, ArH), 7.57 (d, 1H, J=2.2 Hz, ArH), 8.13 (d, 1H, J=2.2 Hz, ArH); MS m/e 447 [M^+], 415 [M^+-OCH_3].

4-[3-(3-p-Tosylureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (10a)

0.27 g (40%); a white solid; mp 176~180°C; ^{1}H NMR ($CDCl_3$) δ 2.16 (m, 2H, CH_2), 2.38 (s, 3H, $PhCH_3$), 3.55 (m, 2H, $NHCH_2$), 4.06 (s, 3H, CO_2CH_3), 4.13 (t, 2H, J=5.4 Hz, OCH_2), 6.83 (br s, 1H, NH), 7.26 (d, 1H, J=7.8 Hz, ArH), 7.36 (s, 1H, NH), 7.52 (d, 1H, J=2.0 Hz, ArH), 7.76 (d, 1H, J=7.8 Hz, ArH), 8.04 (d, 1H, J=2.0 Hz, ArH).

5,7-Dichloro-4-[-(3-phenylureido)propoxy]-quinoline-2-carboxylic acid methyl ester (10b)

0.17 g (50%); a yellow solid; mp 208~209°C; ^{1}H NMR ($CDCl_3$) δ 2.18 (m, 2H, CH_2), 3.68 (m, 2H, NCH_2), 4.04 (s, 3H, CO_2CH_3), 4.19 (t, 2H, J=5.2 Hz, OCH_2), 5.98 (m, 1H, NH), 7.02 (m, 1H, ArH), 7.06 (br s, 1H, NH), 7.18~7.32 (m, 5H, ArH), 7.48 (d, 1H, J=2.0 Hz, ArH), 8.01 (d, 1H, J=2.0 Hz, ArH); MS m/e 357 [M^+-PhNH], 296 [M^+-PhNH, OCH_3].

4-[3-(3-Benzylureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (10c)

0.20 g (45%); a yellow solid: mp 195~197°C; ^{1}H NMR ($CDCl_3$) δ 2.15 (dt, 2H, J_A=12 Hz, J_B=2.6 Hz, CH_2), 3.55 (m, 2H, $NHCH_2$), 4.02 (s, 3H, CO_2CH_3), 4.19 (t, 2H, J=5.8 Hz, OCH_2), 4.34 (d, 2H, J=5.6 Hz, $PhCH_2$), 4.96 (t, 1H, J=6.4 Hz, NH), 5.14 (t, 1H, J=5.6 Hz, NH), 7.15~7.27 (m, 5H, ArH), 7.34 (s, 1H, ArH), 7.50 (d, 1H, J=2.2 Hz, ArH), 8.05 (d, 1H, J=2.2 Hz, ArH); MS m/e 355 [M^+-BzNH].

4-[3-(3-Benzoylureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (10d)

0.14 g (38%); a white solid; mp 193~195°C; ^{1}H NMR ($CDCl_3$) δ 2.32 (m, 2H, CH_2), 3.74 (m, 2H, $NHCH_2$), 4.09 (s, 3H, CO_2CH_3), 4.48 (m, 2H, OCH_2), 7.42~7.87 (m, 7H, ArH), 8.17 (d, 1H, J=2.2 Hz, ArH), 8.49 (br s, 1H, NH), 8.83 (br s, 1H, NH); MS m/e 298 [M^+-PhCO, C_2OCH_3].

5,7-Dichloro-4-[3-(3-phenylthioureido)propoxy]-quinoline-2-carboxylic acid methyl ester (11a)

0.18 g (39%); a white solid; mp 176~178°C; ^{1}H NMR ($CDCl_3$) δ 2.27 (m, 2H, CH_2), 3.85 (br m, 2H, $NHCH_2$), 4.04 (s, 3H, CO_2CH_3), 4.44 (t, 2H, J=5.6 Hz, OCH_2), 7.13~7.22 (m, 1H, ArH), 7.32~7.65 (m, 4H, ArH), 7.64 (s, 1H, ArH), 7.93 (d, 1H, J=2.0 Hz, ArH), 8.00 (br s, 1H, NH), 8.20 (d, 1H, J=2.0 Hz, ArH), 9.58 (br s, 1H, NH); MS m/e 371 [M^+-PhNH], 328 [M^+-NH(CS)NHPh].

5,7-Dichloro-4-{3-[3-(4-methoxyphenyl)thioureido]propoxy}quinoline-2-carboxylic acid methyl ester (11b)

0.18 g (38%; a white solid; mp 177~179°C; ^{1}H NMR ($CDCl_3$) δ 2.29 (dt., J_A=12.8 Hz, J_B=6.4 Hz, CH_2), 3.69 (s, 3H, $ArOCH_3$), 3.93 (m, 2H, $NHCH_2$), 4.05 (s, 3H, CO_2CH_3), 4.26 (t, 2H, J=5.8 Hz, OCH_2), 6.10 (m, 1H, NH), 6.73 (d, 2H, J=6.6 Hz, ArH), 7.03 (d, 2H, J=6.6 Hz, ArH), 7.49 (s, 1H, ArH), 7.52 (d, 1H, J=2.2 Hz, ArH), 8.12 (d, 1H, J=2.2 Hz, ArH).

4-[3-(3-Benzoylthioureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid methyl ester (11c)

0.24 g (50%); a white solid; mp 204~205°C; ^{1}H NMR ($CDCl_3$) δ 2.43 (m, 2H, CH_2), 4.08 (m, 5H, CO_2CH_3), 4.39 (m, 2H, OCH_2), 7.45~7.64 (m, 5H, ArH), 7.78 (d, 1H, J=2.2 Hz, ArH), 7.82 (s, 1H, NH), 8.25 (d, 1H, J=2.2 Hz, ArH), 8.98 (s, 1H, NH); MS m/e 298 [M^+], 461[M^+-CO_2CH_3].

4-{3-[3-(4-Phenoxyphenyl)thioureido]propoxy}-5,7-dichloroquinoline-2-carboxylic acid methyl ester (11d)

0.35 g (64%); a pale yellow solid; mp 80~81°C; ^{1}H NMR ($CDCl_3$) δ 2.36 (m, 2H, CH_2), 3.98 (m, 2H, SCH_3), 4.09 (s, 3H, CO_2CH_3), 4.34 (t, 2H, J=5.8 Hz, OCH_2), 6.95 (s, 1H, NH), 6.96~7.04 (m, 4H, ArH), 7.15~7.22 (m, 3H, ArH), 7.35~7.43 (m, 2H, ArH), 7.56 (s, 1H, ArH),

7.60 (d, 1H, J=2.2 Hz, ArH), 8.18 (d, 1H, J=2.2 Hz, ArH); MS m/e 521 [M^+-OCH_3], 461[M^+-NHPh-O-Ph].

General Procedure for the hydrolyses of the 5,7-dichloro-4-substituted quinoline-2-carboxyic acid methyl esters

A solution of an appropriate quinoline-2-carboxylic acid methyl ester (1.0 mmol) and NaOH (2.0 mmol) in a 1:1 mixture of THF and H_2O was stirred at room temperature until the starting methyl ester disappeared. The reaction mixture was washed with ether and the aqueous phase was acidified with 1N HCl at pH 3. The precipitate was collected by filtration (or by extraction with dichloromethane) and dried in vacuo to give the corresponding carboxylic acid.

4-[3-(3-Benzyl-oxy-carbonylamino)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (12a)

0.27 g (60%); a white solid; ^{1}H NMR (DMSO-d_6) δ 2.10 (br s, 2H, CH_2), 4.32 (br s, 2H, OCH_2), 5.09 (s, 2H, $PhCH_2$), 7.27~7.48 (br s, 5H, ArH), 7.53 (br s, 1H, NH), 7.68 (br s, 2H, ArH), 7.68 (d, 1H, J=2.2 Hz, ArH), 8.39 (d, 1H, J=2.2 Hz, ArH).

4-[3-(3-n-Butyrylamido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (12b)

0.16 g (98%); a white solid; mp 140~142°C; ^{1}H NMR (DMSO-d_6) δ 0.95 (t, 3H, J=7.4 Hz, CH_3), 1.34~1.61 (m, 4H, CH_2), 2.09 (m, 2H, CH_2), 3.99 (t, 2H, J=7.0 Hz, OCH_2), 4.40 (t, 2H, J=5.6 Hz, OCH_2), 7.30 (m, 1H, NH), 7.64 (s, 1H, ArH), 7.94 (d, 1H, J=2.0 Hz, ArH), 8.19 (d, 1H, J=2.0 Hz, ArH).

4-[3-(3-t-Butoxycarbonylamino)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (12c)

0.15 g (60%); a white solid; mp 120~124°C; ^{1}H NMR (DMSO-d_6) δ 1.44 (s, 9H, CH_3), 2.06 (m, 2H, CH_2), 3.32 (m, 2H, NCH_2), 4.30 (br m, 2H, OCH_2), 7.05 (br m, 1H, NH), 7.64 (s, 1H, ArH), 7.94 (d, 1H, J=2.0 Hz, ArH), 8.19 (d, 1H, J=2.0 Hz, ArH).

4-[3-(3-phenylacetylamido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (13a)

0.056 g (39%); a white solid; mp 110~113°C; ^{1}H NMR (DMSO-d_6) δ 2.10 (m, 2H, CH_2), 3.42 (m, 2H, CH_2), 3.48 (s, 2H, Ph-CH_2), 7.28~7.35 (m, 5H, ArH), 8.06 (d, 1H, J=2.2 Hz, ArH), 8.19 (d, 1H, J=2.2 Hz, ArH), 8.26 (br m, 1H, NH); MS m/e 432 [M^+], 388 [M^+-CO_2], 339 [M^+-$PhCH_2$].

4-[3-(3-p-Tosylureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid(14a)

0.16 g (70%); a white solid; mp 155~160°C; ^{1}H NMR (DMSO-d_6) δ 1.93 (m, 2H, CH_2), 2.34 (s, 3H, $PhCH_3$), 3.25 (m, 2H, $NHCH_2$), 4.18 (t, 2H, J=5.4 Hz, OCH_2), 6.72 (t, 2H, J=4.6 Hz, NH), 7.35 (d, 2H, J=8.8 Hz, ArH), 7.48 (s, 1H, ArH), 7.75 (d, 2H, J=8.8 Hz, ArH), 7.72 (d, 1H, J=2.2 Hz, ArH), 8.08 (d, 1H, J=2.2Hz, ArH).

5,7-Dichloro-4-[-(3-phenylureido)propoxy]-quinoline-2-carboxylic acid (14b)

0.10 g (63%); a white solid; mp 162~164°C; ^{1}H NMR (DMSO-d_6) δ 2.14 (m, 2H, CH_2), 4.45 (t, 2H, J=5.0 Hz, OCH_2), 6.49 (br m, 2H, NH), 6.92~6.98 (m, 1H, ArH), 7.24~7.32 (m, 2H, ArH), 7.43~7.47 (m, 2H, ArH), 7.67 (s, 1H, ArH), 7.94 (d, 1H, J=2.0 Hz, ArH), 8.19 (d, 1H, J=2.0 Hz, ArH), 8.59 (br s, 1H, NH).

4-[3-(3-Benzylureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (14c)

0.12 g, (63%); a white solid; mp 157~160°C; ^{1}H NMR (DMSO-d_6) δ 2.18 (m, 2H, CH_2), 4.28 (d, 2H, J=6.2 Hz, $PhCH_2$), 4.39 (m, 2H, OCH_2), 6.24 (m, 1H, NH), 6.41 (m, 1H, NH), 7.27~7.41 (m, 5H, ArH), 7.65 (s, 1H, ArH), 7.90 (d, 1H, J=2.2 Hz, ArH), 8.19 (d, 1H, J =2.2 Hz, ArH); MS m/e 355 [M^+-BzNH].

4-[3-(3-Benzoylureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (14d)

0.14 g (100%); a white solid; mp 218~220°C; ^{1}H NMR (DMSO-d_6) δ 2.21 (m, 2H, CH_2), 3.58 (m, 2H, $NHCH_2$), 4.43 (m, 2H, OCH_2), 7.52~7.65 (m, 4H, ArH), 7.85~7.99 (m, 3H, ArH), 8.14 (m, 1H, ArH), 8.83 (br s, 1H, NH).

5,7-Dichloro-4-[3-(3-phenylthioureido)propoxy]-quinoline-2-carboxylic acid (15a)

0.16 g, (94%); a white solid; mp 154~155°C; ^{1}H NMR (DMSO-d_6) δ 2.16 (m, 2H, CH_2), 3.80 (m, 2H, $NHCH_2$), 4.31 (m, 2H, OCH_2), 7.03~7.09 (m, 1H, ArH), 7.24~7.32 (m, 2H, ArH), 7.42~7.49 (m, 2H, ArH), 7.51 (s, 1H, ArH), 7.75 (br s, 1H, ArH), 8.03 (br s, 1H, ArH), 8.27 (br s, 1H, NH), 9.78 (br s, 1H, NH).

5,7-Dichloro-4-{3-[3-(4-methoxyphenyl)thioureido] propoxy}quinoline-2-carboxylic acid (15b)

0.10 g (59%); a white solid; mp 125~130°C; ^{1}H NMR (DMSO-d_6) δ 2.17 (m, 2H, CH_2), 3.73 (m, 5H, $ArOCH_3$, $NHCH_2$), 4.33 (m, 2H, OCH_2), 6.88 (d, 2H, J=8.8 Hz, ArH), 7.20 (d, 2H, J=8.8 Hz, ArH), 7.57 (s, 1H, ArH), 7.84 (d, 1H, J=2.2 Hz, ArH), 8.10 (d, 1H, J =2.2 Hz, ArH).

4-[3-(3-Benzoylthioureido)propoxy]-5,7-dichloroquinoline-2-carboxylic acid (15c)

0.21 g (91%); a white solid; mp > 160°C (dec.); ^{1}H

NMR (DMSO-d_6) δ 2.26 (m, 2H, CH_2), 3.96 (m, 2H, $NHCH_2$), 4.42 (m, 2H, OCH_2), 7.43~7.67 (m, 4H, ArH), 7.55~7.92 (m, 4H, ArH), 8.09 (d, 1H, J=2.2 Hz, ArH), 10.95 (m, 1H, NH), 11.24 (s, 1H, NH).

4-{3-[3-(4-Phenoxyphenyl)thioureido]propoxy}-5,7-dichloroquinoline-2-carboxylic acid (15d)

0.22 g (65%); a white solid; mp > 160°C (dec.); ^{1}H NMR (DMSO-d_6) δ 2.19 (br s, 2H, CH_2), 3.97 (br s, 2H, SCH_3), 4.31 (br s, 2H, OCH_2), 6.98~7.07 (m, 4H, ArH), 7.18~7.21 (m, 1H, ArH), 7.41~7.65 (m, 6H, ArH), 8.06 (d, 1H, J=2.2 Hz, ArH), 9.24 (br s, 1H, NH), 10.71 (br s, 1H, NH); MS m/e 284 [M^+-$(CH_2)_3$C (S)NH-ph-O-ph], 227.

[^{3}H]-Glycine binding assay

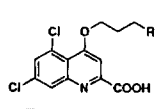
Synaptic membranes for receptor binding studies were prepared by the modified methods of Foster and Fagg (1987) and Murphy *et al.* (1988). [^{3}H]-Glycine binding assays were performed as described by Baron *et al.* (1991). For saturation binding analysis of [^{3}H]-Glycine, synaptic membranes (100 μg of membrane protein) were incubated at 4°C for 30 min in 13×100 mm borosilicated glass tube in a final volume of 0.5 mL reaction mixture containing 50 mM Tris-acetate buffer, pH 7.1, and 5~500 nM [^{3}H]-Glycine. For drug displacement analysis, various concentrations of testing compounds were incubated as described above, in a reaction mixture containing synaptic membranes (100 μg of membrane protein), 50 nM of [^{3}H]-Glycine and 50 mM Tris-acetate buffer, pH 7.1. After incubation, the reaction was terminated by the addition of 2.5 mL of ice-cold 50 mM Tris-acetate buffer, pH 7.1, and the bound radioactivity was separated using a Brandel cell harvester (Brandel M-12R) by rapid filtration through Whatman GF/B glass fiber filter which was presoaked in 0.3% polyethylenimine in the assay buffer. The filters were washed twice with 2.5 mL of cold buffer within 10 sec. The trapped radioactivity on the filter was measured by a liquid scintillation counter (Beckman LS 6000TA) using 3 mL Luma gel at a counting efficiency of 50~55%. Non-specific binding was determined in the presence of 1 mM glycine. All testing compounds were dissolved in dimethylsulfoxide (DMSO), and serially diluted to various concentrations for binding assays.

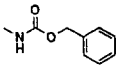
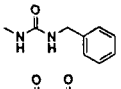
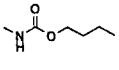
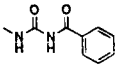
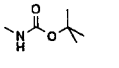
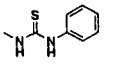
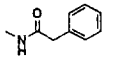
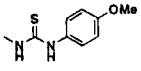
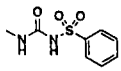
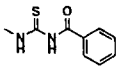
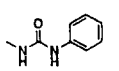
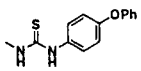
RESULTS AND DISCUSSION

Synthesis of target kynurenic acids outlined in Scheme 1 was started from methyl 5,7-dichlorokynurenate which was readily prepared according to literature procedures (Surrey and Hammer, 1946; Baker *et al.*, 1972). Treatment of methyl 5,7-dichlorokynurenate with 1,3-dibromopropane in presence of excess Na_2CO_3 and 10% (w/w)n-Bu_4NBr provided the bromide **5**. The azide **6** was obtained by treatment of the bromide **5** with NaN_3 in DMF at 50°C. Hydrogenation of the azide **6** using a catalytic amount of 10% Pd-C provided an amine **7** as a key intermediate. Simultaneously, reaction of the intermediate amine **7** with the appropriate carbonyl compounds including acid chloride, chloroformate, isocyanate, or thioisocyanate in presence of Et_3N was followed. Typical basic hydrolysis afforded a series of new kynurenic acid derivatives **12~15** as listed in Table I.

The compounds synthesized were evaluated for their ability to compete with [^{3}H]glycine for strychnine-insensitive binding site on rat cortical membranes (Baron *et al.*, 1991). First, the percent value (Inhibition %) representing the extent of [^{3}H]glycine displacement at 100 μM of testing compound was determined for comparison of its relative binding activity with others. Second, IC_{50} value was determined for compounds which show greater than 50% inhibition in the above preliminary [^{3}H]glycine displacement test. IC_{50} values reported, representing the concentration of testing compound required to reduce glycine binding by 50%, were mean values from two or more experiments. Since one of the aims of the present study was an examination of the effect of substituent variation at C4 of the kynurenic acids on NMDA glycine site affinity, a set of substituents was rationally selected to provide

Table I. Biological data of 4-(3-substitued-propyloxy)-kynurenic acid derivatives



Comp No.	R	IC_{50} (μM)[a]	Inh (%)[b]	Comp No.	R	IC_{50} (μM)[a]	Inh (%)[b]
12a		>100	24	**14c**		>100	41
12b		>100	11	**14d**		24.3	81
12c		50.6	52	**15a**		6.04	84
13		>100	26	**15b**		20.2	71
14a		>100	35	**15c**		3.95	90
14b		ND[c]	56	**15d**		23.5	ND[c]

[a]IC_{50}, 50% inhibition, was defined as the concentration of testing compound required to reduce [^{3}H]glycine binding by 50%. Values given are the means of two to four experiments.
[b]Percent value represents the extent of [^{3}H]glycine displacement at 100 μM of testing compound.
[c]ND, not determined.

a systematic variation in hydrophobic, electronic, steric and H-bonding properties. To determine the relationships between binding affinity and these properties, 3-substituted propyloxy groups were introduced at C4 position of a parent kynurenic acid. The 3-substituents we tested were comprised by two distinct characters, i. e., H-bonding moiety such as carbamate, amide, urea and thiourea, as well as an adjunct hydrophobic interaction moiety. It is apparent from the data presented in Table 1 that *in vitro* binding ability of the compounds prepared was found to be quite sensitive to the structural character of substituents at C4 of a parent kynurenic acid nucleus. The comparison of *in vitro* binding affinities of this series of compounds demonstrated that a thiourea group behaving as a H-bonding moiety seems to most favorably influence on binding ability to the NMDA receptor and also implied that the effectiveness of a H-bonding moiety is in the order of thiourea > urea > carbamate > amide. For example, N-phenylthiourea 15a and N-benzoylthiourea **15c** showed the highest *in vitro* binding affinity with IC_{50} of 6.04 and 3.95 μM, respectively. Indeed, compounds **15a** and **15c** were more potent in *in vitro* asssay than the precedent known homologs **3** (IC_{50}, 9.0 μM) and **4** (IC_{50}, 7.49 μM). On the other hand, the replacement of a thiourea group to an amide or a carbamate group results in significant decreases of binding affinity as demonstrated in compounds **12a~c** and **13**. A possible cause for this behavior could be explained because of an unfavorable electronic movement on a C=O bond of amide or carbamate group, compared to that of urea or thiourea. It is believed that H-bonding ability of a carbonyl group generally depends on the electron density on an oxygen atom. Since two nitrogen atoms of an urea or a thiourea group behave as an electron donor, the ability of H-bonding acceptor of the C=O (or C=S) bond in **14** (or **15**) might be significantly increased compared to that of the C=O bond in **12** or **13**. In addition, it was also found that the size and electronic characters of adjunct hydrophobic interaction moieties play a important role in the ligand-receptor binding (Fersht *et al.*, 1985). For example, compound **12c** containing *t*-butyl carbamate group has higher binding affinity than compound **12b** containing *n*-butyl carbamate group. In common with the carbamate derivatives, chain extention in the ureas also reduces binding as shown in compounds **14b** and **14c**. Although a benzoyl group is not significantly different from a benzyl in size, the urea **14d** show much higher affinity than **14c**. This might be due to the fact that a C=O bond of a N-benzoyl group favorably cooperates with a neighbor urea C=O bond when H-bonding interaction ocurrs in ligand-receptor binding. In order to obtain the rationallized details for this unique behavior of N-benzoyl urea group, we have currently further investigated the structure-activity relationships (SAR) of 4-(3-substituted-propyloxy)kynurenic acid derivatives possessing the N-acyl urea groups as well as other related groups. Further syntheses and the expanded SAR studies of these novel series will be the subject of future reports from our laboratory.

In conculsion, a series of 4-(3-substituted-propyloxy)-5,7-dichloro-kynurenic acid derivatives were synthesized and some of them were found to be reasonably potent antagonists for NMDA receptor acting at the glycine recognition site. In the SAR study of the 4-substituted kynurenic acid derivatives so far, it was realized that the 3-substitution pattern on a flexible C4-propyloxy chain of kynurenic acid nucleus significantly influences on binding affinity for glycine site by at least two structural factors, the type of H-bonding and an adjunct hydrophobic interaction moietiy. As a result, the modification at C4 of kynurenic acid nucleus with various substituents suggested that the the binding ability to the NMDA receptor might be increased by the introduction of suitable electron rich substituents at C4 of kynurenic acid nucleus. This hypothesis may offer some benefit in the design of new potent NMDA receptor antagonists acting at glycine binding site.

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