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4-Hydroxy-6-Oxo-6,7-Dihydro-Thieno[2,3-b] Pyrimidine Derivatives : Synthesis and Their Biological Evaluation for the Glycine Site Acting on the *N*-Methyl-D-Aspartate (NMDA) Receptor

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Bioisostere approach has been shown to be useful to augment potency or to modify certain physiological properties of a lead compound. Based upon well documented bioisosterism, an isosteric replacement of benzene ring of 4-hydroxy-2-quinolone compound (L-695902) with a thiophene moiety was carried out to prepare the title compounds, 4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b] pyrimidines **15**. The resulting bioisosteric compounds **15** were evaluated for their antagonistic activity (binding assay) for NMDA receptor glycine site.

Key words: NMDA Receptor, Glycine site, Bioisosteres, Thieno[2,3-b] pyrimidine, 4-Hydroxy quinolone

INTRODUCTION

L-Glutamate is a major excitatory amino acid neurotransmitter in the mammalian central nervous system (Monagham *et al.*, 1989 and Johnson *et al.*, 1988). Although the glutamate receptors are classified into two main groups, ionotropic and metabotropic, the *N*-Methyl-D-Aspartate (NMDA) type of ionotropic glutamate receptor plays a major role in the neurotoxic cascade following cerebral ischaemia and hypoxic events (Meldrum, 1990; McCullough,1992 and Rothman *et al.*, 1987). Additionally, the discovery that the glycine is a necessary coagonist for NMDA receptor activation (Johnson *et al.*, 1987) has stimulated research into the development of antagonists for the NMDA receptor glycine site. In comparison with MNDA receptor antagonists acting competitively at the glutamate site or uncompetitively as channel blockers, glycine site antagonists have significantly improved side-effect profiles (Kemp *et al.*, 1993). Therefore, NMDA receptor antagonists acting at the glycine site have been actively sought for their therapeutic potential in the treatment of CNS disease such as Alzheimer disease, stroke, head injury, epilepsy and schizophrenia (Leeson *et al.*, 1994; Iverson *et al.*, 1994; Kulagowski *et al.*, 1996 and Cai *et al.*, 1997).

Many classes of glycine antagonists with high affinity and selectivity have now been synthesized, and they can be categorized mainly as kynurenic acid **1** (Leeson *et al.*, 1991), quinoxalinedione **2** (Honore *et al.*, 1988; Epperson *et al.*, 1993 and Cai *et al.*, 1997), 2-carboxyindole **3** (Salituro *et al.*, 1992 and Fabio *et al.*, 1997), 2-carboxytetrahydroquinoline **4** (Leeson *et al.*, 1992) and 2-quinolone **5** (McQuaid *et al.*, 1992). Their representative chemical structures are depicted in Fig. 1.

In spite of many classes of compounds mentioned above, most of these lack activity in the central nervous system following systemic dosing. Evidently, significant improvements in blood-brain barrier permeability and bioavailability are important factors to be considered as a good drug candidate.Among those several kinds of glycine site antagonists the 4-hydroxy-2-quinolone derivatives **5** (Rowley *et al.*, 1993 and McQuaid *et al.*, 1992), where *in vitro* affinity has been shown to be strongly dependent on the nature of the 3-substituent, are known as only class of glycine antagonists with consistent *in vivo* activity. The 3-acyl series were optimized to provide ester **5a** (L-695902).

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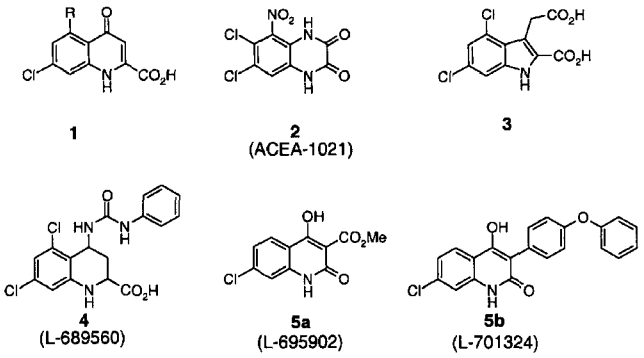


Fig. 1. Structure of various glycine site antagonist

Affinity for the receptor can be optimized in esters which take advantage of the bulk tolerance site exposed in earlier compound. Very recently, further optimization, based on combining the structural features contained in **5a** and other bulky compounds, has led to a breakthrough in systemic activity in a specific class of 3'-(aryloxy)-3-phenyl derivatives exemplified by **5b** (Kulagoski *et al.*, 1994). Compounds **5b** are the most potent glycine antagonists both *in vitro* and *in vivo*, yet described.

So far, all of the efforts to improve the biological activities in these 4-hydroxy-2-quinolone series have been made to modify only the 3-position of the quinolone moiety. So we were interested in modifying other part of the quinolone structure *via* bioisosteric approach. Bioisoteres are substituents or groups that have chemical or physical similarities, and which produce broadly similar biological properties (Silverman, 1992 and Blair *et al.*, 1999) Bioisosterism is a lead modification approach to attenuate toxicity and/or to improve the biological properties of the lead compounds. It is quite interesting to see that the undesirable side effect of anti-inflammatory analgesic pyroxicam (**7**) was greatly diminished by being converted to thiophene containing tenoxicam (**8**), and the herbicidal activity of Metsulfuron (**9**) was highly enhanced by switching to Thifensulfuron (**10**) (Fig. 2).

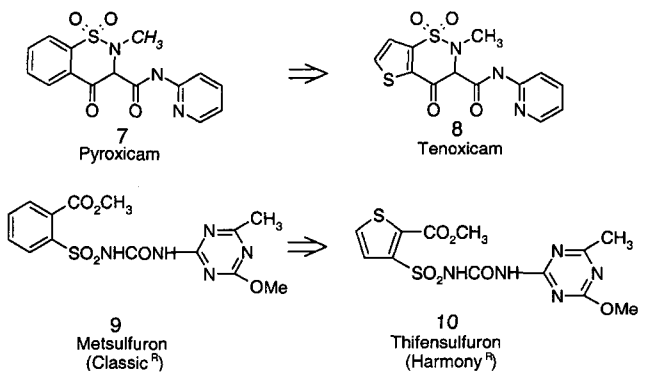
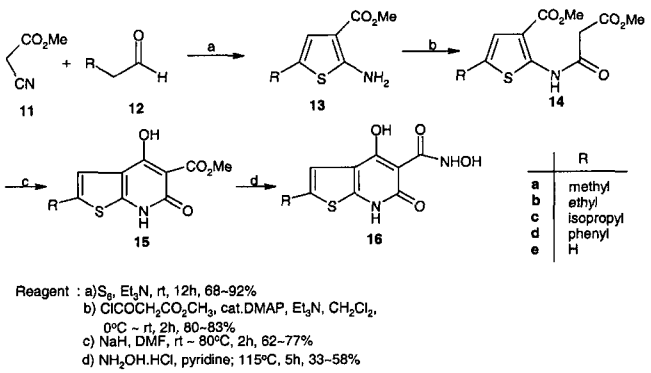


Fig. 2. Representative examples of commercial products derived by bioisosteric approaches



Scheme 1. Synthesis of 4-hydroxy-2-substituted thieno[2,3-b]pyridine derivatives

In connection with designing new lead molecules for NMDA glycine site antagonist exhibiting systemic activity along with good blood-brain barrier permeability, we were interested in thieno[2,3-b]pyrimidine type compounds **15**; the bioisoterically modified product from quinolone type compound **5** by substituting thiophene for the benzene moiety.

The present paper describes synthesis and biological evaluation of 4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b] pyrimidines **15** as a potential lead compound for the NMDA receptor glycine binding site antagonist (Scheme 1).

MATERIALS AND METHODS

Synthesis

Melting points were recorded on electrothermal melting point apparatus and are uncorrected. Mass spectra were recorded on a Shimadzu QP-1000 spectrometer (20 eV). ^{1}H-NMR and ^{13}C-NMR data were obtained from Jeol 400 MHz spectrometer and chemical shifts (δ) were reported in ppm in relation to tetramethylsilane (δ 0.00) and $CDCl_3$ (δ 77.0) for ^{1}H and ^{13}C NMR, respectively; J values are in hertz(Hz). Thin layer chromatography were performed on pre-coated silica gel 60 F-254 (layer thickness 0.2 mm, Merck). The Flash Column Chromatography was performed on Merck silica gel type 60 (230~400 mesh). The organic solvents and chemicals were obtained from Aldrich. Co. and purified by the appropriate methods before use.

General procedure for the preparation of compound 14

To a stirred solution of thiophene **13** (5 mmol) and DMAP (0.2 mmol) in CH_2Cl_2 (15 mL) was added a solution of methyl malonyl chloride (15 mmol) in CH_2Cl_2 (15 mL) at 0°C over 5 min. After stirring for 10 min Et_3N(15 mmol) was added at 0°C over 3 min. The whole reaction mixture was stirred at rt for 2 h, evaporated in vacuo. The

resulting residue was dissolved in EtOAc (100 mL), washed with sat.$NaHCO_3$ (30 mL) and brine (300 mL), dried over Na_2SO_4 and then concentrated to give a crude product, which was purified by column chromatography (EtOAc/Hexane:1/3) to provide pure product (1.2 g, 80% yield) generally as a yellow solid.

5-Methyl-2-(2-methoxycarbonyl-acetylamino)-thiophen-3-carboxylic acid methyl ester (14a)

Yield 80% ; mp 103~105°C ; ^{1}H-NMR ($CDCl_3$) δ 2.38 (s, 3H), 3.60 (s, 2H), 3.84 (s, 3H), 3.90 (s, 3H), 6.80 (s, 1H), 11.95 (s,1H); ^{13}C-NMR ($CDCl_3$) δ 168.42, 165.14, 161.76, 145.62, 129.92, 121.00, 113.12, 52.83, 51.72, 41.02, 14.58 ; MS: m/z 272(M^++1).

5-Ethyl-2-(2-methoxycarbonyl-acetylamino)-thiophen-3-carboxylic acid methyl ester (14b)

Yield 78% ; mp 65~67°C ; 1H-NMR ($CDCl_3$) δ 1.29 (t, 3H, J=7.8Hz), 2.73(q, 2H, J=7.8Hz), 3.60(s, 2H), 3.84 (s, 3H), 3.87 (s,3H), 6.89 (s , 1H); ^{13}C-NMR ($CDCl_3$) δ 168.40, 165.19, 161.78, 145.44, 137.31, 119.21, 112.94, 52.80, 51.69, 41.06, 22.75, 15.45; MS: m/z 286 (M^++1).

5-Isopropyl-2-(2-methoxycarbonyl-acetylamino)-thiophen-3-carboxylic acid methyl ester (14c)

Yield 82% ; yellow oil ; ^{1}H-NMR ($CDCl_3$) δ 1.31 (d, 6H, J=6.8Hz), 3.05 (sept, 1H, J=6.8Hz), 3.60 (s, 2H), 3.83 (s, 3H), 3.90 (s, 3H), 6.90 (s, 1H), 11.91 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 168.20, 164.95, 161.67, 145.09, 142.66, 117.60, 112.46, 52.53, 51.44, 40.89, 29.19, 24.04 ; MS : m/z 300 (M^++1).

5-Phenyl-2-(2'-methoxycarbonyl-acetylamino)-thiophen-3-carboxylic acid methyl ester (14d)

Yield 83% ; mp 113~115 °C ; ^{1}H-NMR ($CDCl_3$) δ 3.63 (s, 2H), 3.85 (s, 3H), 3.94 (s, 3H), 7.43 (s, 1H), 7.45 (m, 5H), 12.10(s, 1H); ^{13}C-NMR ($CDCl_3$) δ 168.37, 164.98, 162.02, 146.49, 133.92, 133.38, 128.73, 127.28, 125.24, 119.20, 114.28, 52.80, 51.83, 40.85; MS: m/z 334 (M^++1).

2-(2'-Methoxycarbonylacetyl amino)-thiolphene-3-carboxylic acid methyl ester(14e)

Yield 69%; mp 104~106°C; ^{1}H-NMR ($CDCl_3$) δ 3.62 (s, 2H), 3.85(s, 3H), 3.93(s, 3H), 6.76 (d, 1H), 7.23(d, 1H), 12.07 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 168.32, 165.14, 162.04, 143.74, 123.87, 116,31, 113.53, 52.81, 51.79, 40.98; MS: m/z 260(M^++1).

General procedure for the preparation of compound 15

NaH (20 mmol) was added to a solution of thiophene **14** (5 mmol) in anhydrous DMF (25 mL). The resulting reaction mixture was stirred at rt for 2 h and then heated at 80°C for 8 h. The mixture was then cooled, poured onto ice cold water (40 mL) and acidified with 2N HCl to provide precipitate as a crude product, which was collected by filtration and recrystallized from 20% aqueous DMF to give pure product as solid.

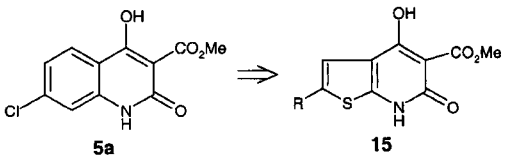


Fig. 3. A conceptual design for target compound **15**

2-Methyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid methyl ester(15a)

Yield 76.6%; mp 256~257°C; ^{1}H-NMR (DMSO-d_6) δ 6.42 (2, 3H), 3.83 (s, 3H), 6.93 (s, 1H), 12.25 (bs, 1H) 13.19 (bs, 1H); ^{13}C-NMR (DMSO-d_6) δ 171.00, 166.34, 159.21, 149.83, 130.80, 123.46, 116.56, 92.97, 52.01, 14.92; MS: m/z 240(M^++1).

2-Ethyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid methyl ester (15b)

Yield 36.0%; mp 193~194°C; ^{1}H-NMR(DMSO-d_6) δ 1.24 (t, 3H,J=7.3Hz), 2.77(q, 3H, J=7.3Hz), 3.83 (s, 3H), 6.95 (s, 1H), 12.24 (bs, 1H) 13.20 (bs, 1H); ^{13}C-NMR (DMSO-d_6) δ 171.15, 166.63, 159.39, 151.12, 138.08, 115.16, 113.37, 96.12, 52.13, 22.71, 15.19; MS: m/z 254 (M^++1).

2-Isopropyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid methyl ester (15c)

Yield 45.0%; mp 192~193°C ; ^{1}H-NMR(DMSO-d_6) δ 1.27 (d, 6H, J=6.8Hz), 3.11(sept, 1H, J=6.8Hz), 3.84 (s, 3H), 6.94 (s, 1H), 12.28 (bs, 1H), 13.21 (bs, 1H); ^{13}C-NMR (DMSO-d_6) δ 171.04, 166.66, 159.31, 150.71, 143.54, 113.69, 113.10, 96.02, 52.02, 29.14, 23.81; MS: m/z 302(M^++1).

2-Phenyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid methyl ester (15d)

Yield 62.0% ;light yellow solid; mp 231~232°C ; ^{1}H-NMR (DMSO-d_6) δ 3.85(s, 3H), 7.51 (m, 5H), 7.65 (s, 1H), 12.44 (bs, 1H), 13.22 (bs, 1H); ^{13}C-NMR(DMSO-d_6) δ 170.56, 167.00, 159.38, 149.70, 133.71, 132.40, 128.96, 127.78, 125.01, 115.28, 114.97, 96.82, 52.08; MS: m/z 302(M^++1).

General procedure for the preparation of compound 16

NH_2OH HCl (30 mmol) was added to a solution of compound **15** (3 mmol) in pyridine (15 mL). The resulting reaction mixture was heated at 115°C for 5h, cooled

to rt and then acidified with 2N HCl to get precipitate which was collected by filtration and washed with 0.5N-HCl (10 mL × 2) and cold EtOAc (10 mL × 1) to give pure compound as solid.

2-Methyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid hydroxy amide (16a)

Yield 33.0%; mp 261~262°C; ^{1}H-NMR(DMSO-d_6) δ 2.44 (s, 3H), 6.92 (s, 1H), 9.67 (s, 1H), 11.86 (s, 1H), 12.63 (s,1H), 16.33 (s, 1H); ^{13}C-NMR (DMSO- d_6) δ 168.74, 168.12, 161.75, 148.24, 131.60, 116.73, 114.92, 93.39, 14.96; MS: m/z 241(M^++1).

2-Ethyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid hydroxy amide (16b)

Yield 57.2% ; mp 237~238°C ; ^{1}H-NMR(DMSO-d_6) δ 1.25 (t, 3H,J=7.3Hz), 2.79(q,2H, J=7.3Hz), 6.94 (s, 1H), 9.67 (s,1H), 11.87 (s, 1H), 12.68 (s, 1H), 16.34 (s, 1H); ^{13}C-NMR(DMSO- d_6) δ 168.89, 168.12, 161.81, 147.98, 138.76, 114.99, 114.75, 93.40, 22.68, 15.24; MS: m/z 255(M^++1).

2-Isopropyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b] pyridine-5-carboxylic acid hydroxy amide (16c)

Yield 47.0% ; mp 245~246°C; ^{1}H-NMR(DMSO-d_6) δ 1.28 (d, 6H, J=6.7Hz), 3.14 (sept, 1H, J=6.7Hz), 6.94(s, 1H), 9.67 (s,1H), 11.85 (s, 1H), 12.72 (s, 1H), 16.36(s, 1H); ^{13}C-NMR (DMSO-d_6) δ 169.00, 168.10, 161.81, 147.75, 144.37, 114.57, 113.64, 93.45, 29.25, 23.99; MS : m/z 269(M^++1)

2-Phenyl-4-hydroxy-6-oxo-6,7-dihydro-thieno[2,3-b]pyridine-5-carboxylic acid hydroxy amide(16d)

Yield 40.0%; mp 258~260°C ; ^{1}H-NMR(DMSO-d_6) 7.52 (m, 5H), 7.64 (s, 1H), 9.73 (s, 1H), 11.82 (s, 1H), 12.85 (s, 1H), 16.55 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 169.20, 167.85, 161.68, 148.46, 134.44, 132.27, 128.96, 127.89, 125.13, 116.00, 115.04, 93.66; MS: m/z 303 (M^++1).

Biological activity

Synaptic membrane preparation

Synaptic membranes for receptor binding studies were prepared as follows; Male Sprgue-Dawley rats (300-400g) were decapitated, the cerebral cortex and hippocampus were removed, chopped with scalpel and homogenized in 10 volumes of 0.32 M sucrose using a Teflon-glass homogenizer by 5 strokes. Following centrifugation at 1000 × g for 10 min in a Beckman J2/21 centrifuge (rotor: JA20), the supernatant was collected and centrifuged at 20,000 × g for 20min. The supernatant was discarded, and the pellet was resuspended in 20 volumes of ice-cold distilled-water using Brinkman Polytron homogenizer (setting No. 5, 30 sec). After incubation at 4°C for 30 min, the membrane suspension was then centrifuged at 8,000 × g for 20 min. The supernatant and buffy uppercoat were collected and centrifuged at 39,800 × g for 25 min in a Beckman L8-M Ultracentrifuge. The pellet was stored at -70°C overnight.

On the next day, the pellet was thawed at room temperature for 10 min, resuspended in 20 volumes of 50 mM Tris-acetate (pH 7.1 at 4°C) containing 0.04% Triton X-100, incubated at 37°C for 20 min, and centrifuged at 39,800 × g for 20 min as above. The pellet was washed three times by centrifugation as above with 20 volumes of 50 mM Tris-acetate, pH 7.1, and protein concentration was determined using Bio-Rad reagent (Bradford, 1976). The resuspending buffer volume was adjusted to give a membrane protein concentration of 1 mg/ml, and aliquots were stored at 70°C.

[^{3}H]MDL 105,519 binding experiments

[^{3}H]MDL 105,519 binding assays were performed in 96-well plates to test the binding affinities of compounds on glycine site. The synaptic membranes (50 ug per well) were used in a final volume of 0.25 ml of reaction mixture and incubated at 25°C for 30 min with 50 mM Tria-acetate buffer. For drug screening, 1 and 10 uM of testing compounds were incubated as described above, in a reaction mixture containing 4 nM of [^{3}H]MDL 105,519. After incubation, the reaction was terminated by the rapid filtration and washed 9 times with 200 μl of ice-cold 50 mM Tris-acetate buffer using a Inotech harvester (Inotech, Switzland) through Wallac GF/A glass fiber filter(Wallac, Finland) which was presoaked in the assay buffer. The filter was covered with MeltiLex, sealed in a sample bag followed by drying in the oven, and counted by Micro Beta Plus at a counting efficiency of 30~40%. The assays were performed in a quadruplicate for the respective testing compounds.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the target compounds was started from the preparation of thiophene **13** by a previously described method(Hwang *et al.*, 1991). Thus, treatment of methyl cyanoacetate **11** with various aldehydes, elemental sulfur and triethylamine in DMF at room temperature provided thiophenes **13** in good yields. Although, this procedure provides convenient access to various amino thiophenes **13**, preparation of compound **13e** (R=H), however, should be modified due to the poor reactivity of acetaldehyde and methyl cyano acetate **11**. Thus compound **13e** was prepared by treating **11** with 1,4-dithiane-2,5-diol in pyridine and methanol in 82 % yield. The amino thiophenes **13** were then acylated with methyl malonyl chloride (methyl-3-chloro-3-oxopropionate) at 0°C in the presence of triethyl amine and catalytic amount of DMAP to give the

Table I. Effect of compound **15** and **16** (at 100 μM) on the specific binding of [^{3}H]-MDL-105519, a selective ligand for NMDA receptor glycine site

Compound **15**	% Inhibition	Compound **16**	% Inhibition
15a	42.0	16a	20.6
15b	43.8	16b	18.0
15c	37.1	16c	26.3
15d	34.3	16d	24.0
L-695902	81.7		

desired intermediates **14** in 80~83% yield. Since the initial target compounds **15** exhibited desired biological activities, the compounds **15** were further derivatized by treating them with hydroxylamine in pyridine solvent at 115°C to provide another type compounds, hydroxyamides **16** in 33~58% yield.

Binding assay for NMDA receptor glycine site

The target compounds (**15a~15d, 16a~16d**) were tested with [^{3}H]-MDL-105519 for their *in vitro* activity at the glycine site, and the percent values (inhibition %) representing the extent of [^{3}H]-MDL-105519 displacement at 100 μM of testing compounds were determined for comparison of their relative binding activities with others. L-695,502 (**5a**) was used as standard material and their results are reported in the Table I. Judging from the data shown in Table I, the bioisosterically modified products **15** exhibited inferior biological activities to the quinolone lead compound **5a** (L-695902).The activity of hydroxyamides **16** was even further lower than ester form **15**. Although the biological activities of the target compounds toward the NMDA/glycine site as antagonists were not exciting, this experiment clearly demonstrate that the bioisostere approaches still can be utilized as a general tool to develope new lead compounds from the existing molecules.

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