Arch Pharm Res Vol 23, No 1, 31-34, 2000

Synthesis of 7,8-Dichloro-6-Nitro-1*H*-1,5-Benzodiazepine-2,4-(3*H*, 5*H*)-dione as a potential NMDA Receptor Glycine Site Antagonist

Ki-Jun Hwang

Department of Chemistry, College of Natural Science, Chonbuk National University, Dukjindong 664-14 Chonju, Korea 561-756

(Received September 19, 1999)

An efficient procedure for the preparation of 7,8-dichloro-6-nitro-1*H*-1,5-benzodiazepine-2,4-(3*H*, 5*H*)-dione(**7**) as a potential lead compound for the NMDA receptor glycine binding site antagonist, starting from readily available 4,5-dichloro-2-nitroaniline(**8**), is described. The key step in the synthesis involves the cyclization of malonic ester amide **10** to compound **11**.

Key words: NMDA Receptor, Glycine site, Quinoxalinedione, Benzodiazepine.

INTRODUCTION

The ionotropic *N*-methyl-*D*-aspartate(NMDA) receptor antagonists acting at the glycine site have broad therapeutic potential and offer a highly attractive target for CNS drug discovery such as Alzheimer disease, stroke, head injury, epilepsy and schizophrenia (Cotman *et al.*, 1981, Meldrum *et al.*, 1990, Carling *et al.*, 1993, Iverson *et al.*, 1994 and Leeson *et al.*, 1994). In comparison with NMDA receptor antagonists acting competitively at the glutamate site or noncompetitively as channel blockers, glycine antagonists may have significantly improved side-effect profiles (Kemp *et al.*, 1993). 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), 2-quinolone **2** (McQuaid *et al.*, 1992), 2-carboxytetrahydroquinoline **3** (Leeson et al., 1992), quinoxalinedione **4** (Honore *et al.*, 1988, Epperson *et al.*, 1993 and Cai *et al.*, 1997), 2-carboxyindole **5** (Salituro *et al.*, 1992 and Fabio *et al.*, 1997) and benzazepine-2,5-dione **6** (Swartz *et al.*, 1992). Their representative chemical structures are depicted in Fig. 1.

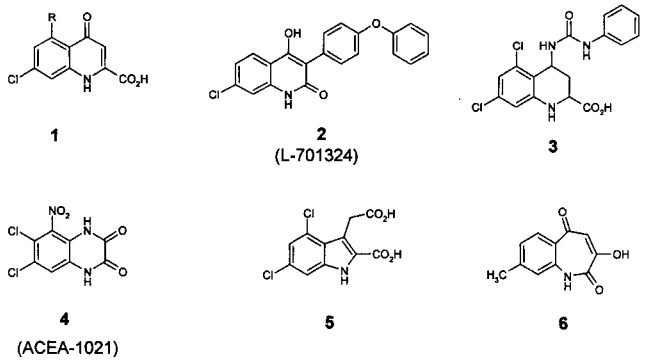


Fig. 1. Structure of Various Glycine Site Antagonist

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. The recently described compound ACEA 1021 (**4**), (Lessler *et al.*, 1989, Kessler *et al.*, 1989, Woodward *et al.*, 1993 and Cai *et al.*, 1997) and L-701324 (**3**), (Kulagowski *et al.*, 1994) appear to have the best systemic activity to date and are expected to provide further impetus for the evaluation of the glycine site *in vivo* (Leeson *et al.*, 1994).

In connection with designing new lead molecules for NMDA glycine site antagonist, we were interested in benzodiazepine type compound **7**; ring expansion product of ACEA 1021 and yet having similar structure to benzazepine **6** (Fig. 2).

Correspondence to: Ki-Jun Hwang, Department of Chemistry, College of Natural Science, Chonbuk National University, Dukjindong 664-14, Chonju, Korea 561-756.
E-mail: Kijun@moak.chonbuk.ac.kr

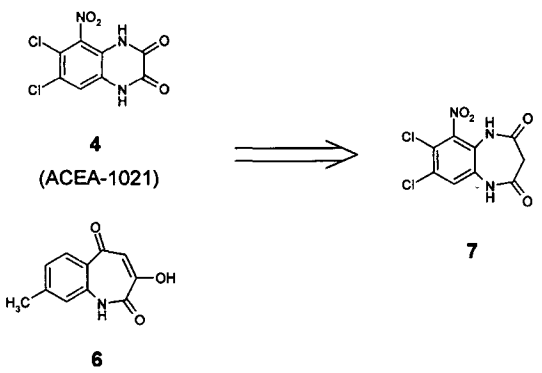
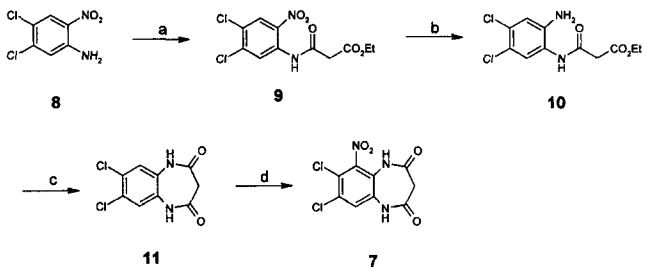


Fig. 2. A conceptual design for target compound 7



Scheme 1. Synthesis of 7,8-dichloro-6-nitro-1*H*-1,5-benzodiazepine-2,4-(3*H*,3*H*)-dione

The present paper describes synthesis of 7,8-dichloro-6-nitro-1*H*-dihydro-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (7) as a potential lead compound for the NMDA receptor glycine binding site antagonist, starting from readily available 4,5-dichloro-2-nitroaniline (**8**) (Scheme 1).

MATERIALS AND METHODS

Mass spectra were recorded on a Shimadzu QP-1000 spectrometer (20eV). High Resolution Mass Spectra (HRMS) were obtained on a JEOL JMS-DX-305 high resolution mass spectrometer. ^{1}H-NMR spectra were recorded either on a JEOL-60 or on a Varian Gemini-200 MHz spectometer. Chemical shifts were expressed in ppm downfield from TMS used as an internal standard. Melting point was determined on an electrically heated Thomas-Hoover capillary melting point apparatus and uncorrected. All chromatographic separations were performed on Merck silica gel (Kieselgel 60, 230~400 mesh).

N-(4,5-Dichloro-2-nitrophenyl)malonic acid ethyl ester amide(9)

Ethyl malonyl chloride (7.26 ml, 51 mmol) was added to a well stirred solution of 4,5-dichloro-2-nitro aniline (10 g, 46.4 mmol) in anhydrous toluene (120 ml) at 15~20°C.

The reaction mixture was heated at 100~110°C for 4 h, cooled to room temperature and then washed with sat.$NaHCO_3$ solution (20 ml) and brine (30 ml). The organic layer was concentrated at reduced pressure to give product **9** (14.75 g, 99.1%). The crude product was practically pure and used for the next step without purification. An analytical sample was obtained by recrystallization from EtOAc/Hexane; mp 192-194°C; ^{1}H-NMR (200MHz, DMSO-d_6) δ 1.35(t, 3H), 3.64(s, 2H), 4.34(q, 2H), 8.35(s, 1H), 9.04(s, 1H), 11.40(bs, 1H); MS: m/z 320(M^+).

N-(2-Amino-4,5-dichlorophenyl)malonic acid ethyl ester amide(10)

A stirring mixture of compound **9** (14.6 g, 45.5 mmol) and Pd/C (1.5 g) in ethanol (250 ml) was hydrogenated under normal pressure at room temperature for 24 h. The resulting reaction mixture was filtered through celite and the organic solution was concentrated at reduced pressure to give the desired product **10** (12.5 g, 95.1%). The crude product was directly used for the next reaction and the analytical sample was obtained by recrystalization from ethanol; mp 186-188°C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.20(t, 3H), 3.45(s, 2H), 4.15(q, 2H), 5.40(bs, 2H), 6.89(s, 1H), 7.46(s,1H), 9.40(bs, 1H); MS: m/z 290(M^+).

7,8-Dichloro-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (11)

Sodium ethoxide (86 mg, 1.2 mmol) was added to a stirred solution of compound **10** (291 mg, 1 mmol) in absolute ethanol (10 ml). The reaction mixture was heated at 50°C for 8 h, cooled to room temperature, and then concentrated at reduced pressure. The resulting slurry was diluted with water (20 ml), then acidified with 6N-HCl to give white precipitate as crude product. The crude product was purified by flash column chromatography with 30 % hexane in ethyl acetate as an eluent to get pure product **11** (107 mg, 43.7%); mp 292-293°C; ^{1}H-NMR(200MHz, DMSO-d_6) δ 3.35(s, 2H), 7.32(s, 2H), 10.51(bs, 2H); MS: m/z 245(M^++1); HRMS : calc. for $C_9H_6N_2O_2Cl_2$ 243.9806, found 243.9797.

7,8-Dichloro-6-nitro-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione(7)

Compound **11** (122.5 mg , 0.5 mmol) was dissolved in c-H_2SO_4 (3 ml) at 0°C. To this solution was added KNO_3 (303 mg, 0.75 mmol) over 5 min and the resulting slurry was stirred at room temperature . After 30 min, the

reaction mixture was poured into ice-cold water (20 ml), and whole mixture was extracted with ethyl acetate (3 × 10 ml).

The combined extracts were washed with brine (20 ml), dried over Na_2SO_4, concentrated at reduced pressure to give crude product. The crude product waspurified by flash column chromatography with 30% hexane in ethyl acetate as an eluent to get pure product **7** (100 mg, 39.2%); mp 270-271°C; ^{1}H-NMR (200MHz, DMSO-d_6) δ 3.35(s, 2H), 7.59(s, 1H), 10.80(s, 2H); MS: m/z 290 (M^++1); HRMS: calcd. for $C_9H_5N_3O_4Cl_2$ 288.9657, found 288.9661.

RESULTS AND DISCUSSION

Quinoxaline-2,3-diones were introduced as the first antagonists of the AMPA-subtype of Non-NMDA excitatory amino acid receptor(Honore, *et al.*, 1988) and were subsequently shown to have comparable affinities for the glycine site. Efforts to improve the glycine site selectivity in this series have focused on both aromatic substitution and on modification of the heterocyclic ring. Several reports have appeared showing very significantly improved affinity and 10^3 fold selectivity (Woodward *et al.*, 1993) for the glycine site *vs* the AMPA receptor with the trisubstituted quinoxaline-2,3-dione ACEA 1021 (**4**). It is interesting to note that the benzazepine **6**, which also has b-dicarbonyl functionality, has been reported active in the gerbil model of global ischaemia (Swartz *et al.*, 1992)

With this background, it was envisioned that the 1,5-benzodiazepine **7**, which is ring expanded product of the ACEA 1021 and yet having silmilar structure to benzazepine **6**, might be potential lead compounds as NMDA glycine site antagonist.

At the beginning stage of the synthesis, it was thought that the target compound **7** could be easily prepared by condensing 4,5-dichloro-ortho-phenylendiamine, with either malonic acid or malonic acid ester. Several attamps, however, were proved to be unsuccessful. The synthesis had to be started with 2-nitro-4,5-dichloro aniline (**8**).

Treatment of compound **8** with ethyl malonyl chloride provided amide **9** in 99% yield. The nitrogroup of amide **9** was then reduced by hydrogen gas with Pd/C catalyst to give amino compound **10**. The amino compound **10** could be cyclized to compound **11** in both acidic and basic condition albeit in low yield (43.7%). The best condition for the cyclization was to utilize sodium ethoxide in ethanol solution. Final nitration to get target product **7** from compound **11** was accomplished by KNO_3/H_2SO_4, which was utilized by Cai *et al.* (Cai, *et al.*, 1997). The normal nitration conditions (HNO_3/H_2SO_4, HNO_3) were not succesful. With this synthetic strategy to prepare various 1,5-benzodiazepine-2,4-dione derivatives, the biological activities of the new synthetic compounds for the NMDA receptor site will be anticipated, hoping to develop new lead molecules with improved pharmacological properties.

REFERENCES

Cai, S. X., Kher, S. M., Zhou, Z.L., Ilyin, V., Espitia, S. A., Tran, M., Hawkinson, S. E., Woodward, R. M., Weber, E., and Keana, J. F., Structure-activity relationships of alkyl-and alkoxy-substituted 1,4-dihydroquinoxaline-2,3-diones: Potent and systemically active antagonists for the glycine site of the NMDA receptor, *J. Med. Chem.*, 40, 730-738 (1997).

Carling, R. W., Leeson, P. D., Moseley, A. M., Smith, J. D., Saywell, K., Triclebank, M. D., Kemp, J. A., Marshall, G. R., Foster, A. G., and Grimwood, S., Anticonvulsant activity of glycine site NMDA antagonists. 2. .trans-2 Carboxy-4-substituted tetrahydroquinolines. *Bioorg. Med. Chem. Lett.*, 3, 65-70 (1993).

Cotman, C. W., Foster, A. C., and Lanthorn, T. H., An overview of glutamate as a neurotransmitter. *Adv. Biochem. Biopharmacol.*, 27, 1-27 (1981).

Epperson, J. R., Hewawasam, P., Meanwell, N. A., Boissard, C. G., Gribkoff, V. K. and Postmunson, D., Synthesis and excitatory aminoacid pharmacology of some novel quinoxalinediones. *Biomed. Chem. Lett.*, 3, 2801-2804 (1993).

Fabio, R. D., Capelli, A. M., Conti, N., Cugola, A., Donati, D. Feriani, A., Gastaldi, P. and Gaviraghi, G., Substituted indole-2-carboxylates as in vivo potent antagonists acting as the strychnine-insensitive glycine binding site. *J. Med. Chem.*, 40, 841-850 (1997).

Honore, T., Davies, S. N., Drejer, J., Fletcher, E. J., Jacobsen, P., Lodge, D. and Nielsen, F. E., Quinoxalinediones: Potent competitive non-NMDA glutamate receptor antagonists. *Science*, 241, 701-703 (1988).

Iversen, L. L., and Kemp, J. A., Noncompetitive NMDA antagonists as drugs. In the NMDA Receptor, 2nd ed., Collingridge, G. L., Watkins, J. C.(Eds.), IRL press, Oxford, England, pp 469-486, 1994.

Kemp, J. A. and Leeson, P. D., The glycine site of the NMDA receptor-Five years on. *Trends Pharmacol. Sci.*, 14, 20-25 (1993).

Kulagowski, J. J., Baker, R., Curtis, N. R., Leeson, P. D., Mawer, I. M., Moseley, A. M., Ridgill, M. P., Rowley, M., Stansfield, I., Foster, A. C., Grimwood, S., Hill, R. G., Kenp, J. A., Marshall, G. R., Saywell, K. L. and Tricklebank, M. D., 3-(Arylmethyl-and 3-(Aryloxy)-3 phenyl-4-hydroxyquinolin-2(1H)-ones: Orally Active Antagonists of the Glycine site on the NMDA Receptor. *J. Med. Chem.*, 37, 1402-1405 (1994).

Kessler, M., Baudry, M. and Lynch, G., Quinoxaline Derivatives are High affinity Antagonists of the NMDA Receptor-associated Glycine Sites. *Brain Res.*, 489, 377-

382 (1989).

Leeson, P. D., Baker, R., Carling, R. W., Curtis, N. R., Moore, K. W., William, B. J., Foster, A. C., Donald, A. E., Kemp, J. A. and Marshall, G. R., Kynurenic acid derivatives, Structure-activity relationships for excitatory aminoacid antagonism and identification of potent and selective antagonists at the glycine site on the NMDA receptor, *J. Med. Chem.*, 34, 1243-1252 (1991).

Leeson, P. D., Carling, R. W., Moore, K. W., Moseley, A. M., Smith, G. D., Stevenson, G., Chan, T., Baker, R., Foster, A. C., Grimwood, S., Kemp, G. A., Marshall, G. R. and Hoogsteen, K., 4-Amido-2-carboxytetrahydroquinolines. Structure-activity relationships for antagonism at the glycine site of the NMDA receptor, *J. Med. Chem.*, 35, 1954-1968 (1992).

Leeson, P. D. and Iversen, L. L., The glycine site on the NMDA receptor: Structure-activity relationship and therapeutic potential, *J. Med. Chem.*, 37, 4053-4067 (1994).

Lester, R. A. J., Quarum, M. L., Parker, J. D., Weber, E. and Jahr, C. E., Interaction of 6-Cyano-7-nitroquinoxaline-2,3-dione with the N-Methyl-D-Aspartate Receptor Associated Glycine Binding Site. *Mol. Pharmacol.* 35, 565-570(b) (1989).

McQuaid, L. A., Smith, E. C. R., Lodge, D., Pralong, E., Wikel, J. H., Calligaro, D. O. and OMalley, P. J., 3-Phenyl-4-hydroxyquinolin-2(1H)-ones: Potent and Selective Antagonists at the Strychnine-Insensitive Glycine Site on the N-Methyl-D-aspartate Receptor Complex. *J. Med. Chem.*, 35, 3423-3425 (1992).

Meldrum, B.. Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission. *Cerebrovasc. Brain Metab. Rev.*, 2, 27-57 (1990).

Salituro, F. G., Harrison, B. L., Baron, B., Nyce, P. L., Stewart, K. T., Kehne, J. H., White, H. S. and McDonald, I. A., 3-(2-Carboxyindol-3-yl)propionic acid-based antagonists of the N-methyl-D-aspartic acid receptor associated glycine binding site. *J. Med. Chem.*, 35, 1791-1799 (1992).

Swartz, K. J., Koroshetz, W. J., Ress, A. H. and Heuttner, J. E., Competitive antagonism of glutamate receptor channels by substituted benzazepines in cultured cortical neurons. *Mol. Pharmacol.*, 41, 1130-1141 (1992).

Woodward, R. M., Huettner, J. E., Guastella, J. and Weber, E., *In vitro* Characterisation of ACEA-1021: A Potent NMDA Receptor Glycine Site Antagonist. *Soc. Neurosci. Abstr.*, 19, 296.14 (1993).