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5-AMINOMETHYLQUINOXALINE-2,3-DIONES, PART III: ARYLAMIDE DERIVATIVES AS HIGHLY POTENT AND SELECTIVE GLYCINE-SITE NMDA RECEPTOR ANTAGONISTS

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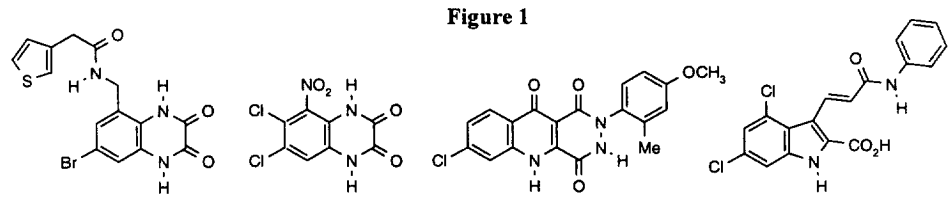
Abstract: A series of quinoxaline-2,3-diones with very high affinity to the glycine site of the NMDA receptor has been discovered. In contrast to the 7-nitro derivatives, the most potent 7-bromo substituted compounds were highly selective for the glycine site. Although none of the described compounds were active in the electroshock model in mice, **1a** displayed significant protection in the quinolinic acid-induced excitotoxicity model *in vivo*.

Glutamate is the most important known excitatory amino acid in the mammalian brain. Overactivation of its receptors is believed to play a major role in neurodegenerative disorders such as ischemia, stroke, head trauma, spinal cord injury, and epilepsy[1]. Glutamate receptors are divided into three classes: the metabotropic glutamate receptors (mGluRs), N-methyl-D-aspartate (NMDA) and (*S*)-2-amino-3-[5-methyl-3-hydroxyisoxazol-4-yl]propionic acid (AMPA)/kainate receptors. NMDA receptors mediate fast synaptic transmission via their associated calcium channels. Drugs which are capable of blocking the activation of these receptors are expected to lower the detrimental effects of ischemia by minimizing the extent of glutamate-induced brain damage[2]. Most of the known channel blockers as well as the antagonists of the glutamate binding site provoke strong adverse side effects. These side effects are expected to be less pronounced for modulatory glycine-site antagonists[3], making them a promising target[4]. Many different, highly potent glycine-site antagonists of NMDA receptors have been described in the literature[5], but only three of them, ACEA 1021 (**2**) (CoCensys)[6], ZD9379 (**3**) (Zeneca)[7], and GV150526A (**4**) (Glaxo Wellcome)[8] are reported to be currently under clinical investigation (Figure 1).

Figure 1



Amongst the different series of quinoxaline-2,3-diones which have been synthesized in our laboratories[9], several amidic derivatives of 5-aminomethylquinoxaline-2,3-dione showed interesting properties in the *in vitro* assays. In order to optimize for *in vitro* activity and selectivity, we synthesized and evaluated different amide derivatives of this series and tested many of them in the electroshock model for *in vivo* effects.

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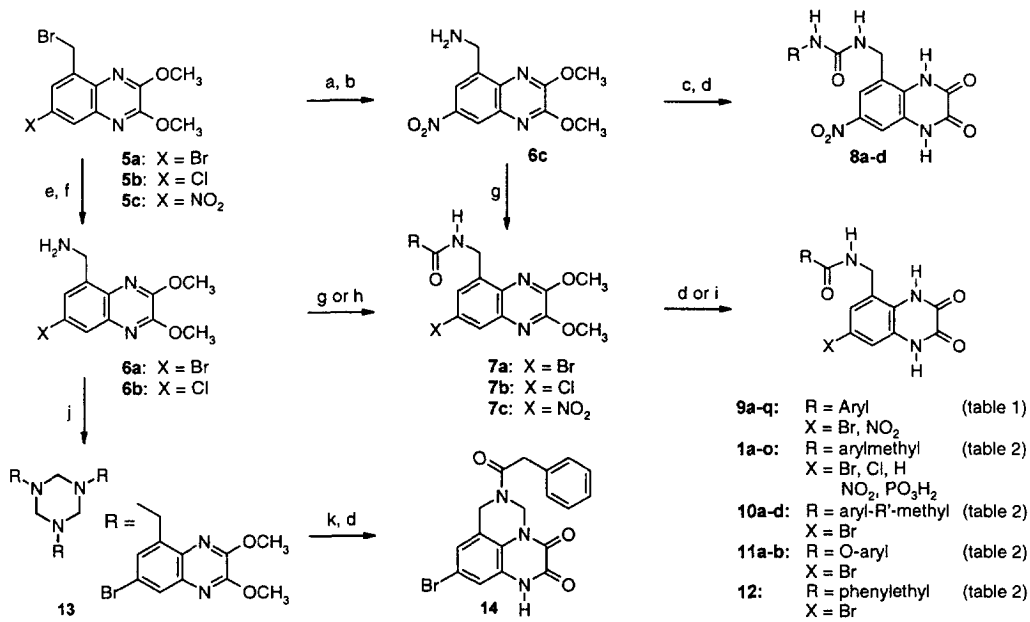
Chemistry

The synthesis of the amide derivatives is outlined in Scheme 1, starting from the 7-substituted 5-bromomethyl-2,3-dimethoxy-quinoxalines **5a-c**[10]. The 7-halo derivatives **5a** and **b** were treated with sodium azide in a water/toluene mixture in the presence of a phase-transfer catalyst. The resulting azide was catalytically reduced with Raney-Nickel to form the 7-bromo- and the 7-chloro-5-aminomethyl-2,3-dimethoxyquinoxalines **6a** and **6b**, respectively.

Scheme 1

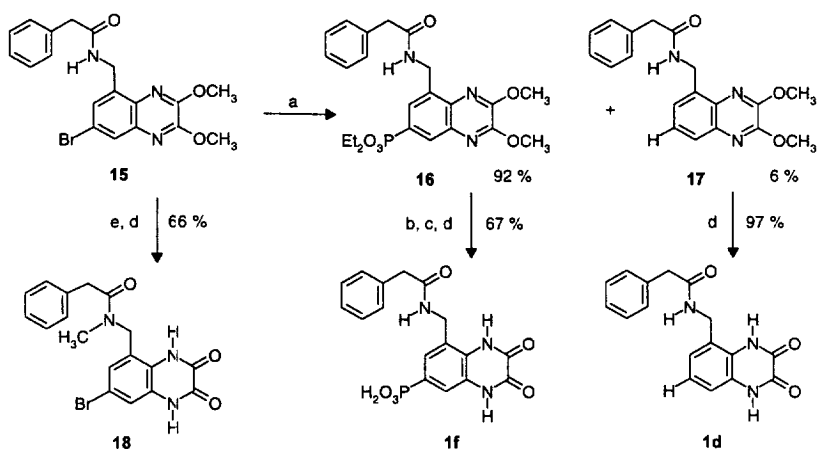


a) Di-t-butyl-iminodicarboxylate, Cs_2CO_3, DMF, 10h 50°C, 98%; b) TFA, 8h rt, 88%; c) RNCO, t-butyl-methylether, 3h rt, 83-90%; d) HBr (33%) in AcOH, 16h rt, 80-90%; e) NaN_3, Aliquat 336, toluene/water, 16h 55°C, 65%; f) Raney-Ni, THF, 15h rt, 63%; g) RCOOH, EDCI, DMAP, THF, 16h rt, 70-95%; h) RCOCl, Et_3N, THF, 72h 0°C-rt, 65-95%; i) 2N HCl, THF, 16h reflux, 35-85%; j) formalin (36%), ethanol, 1.5h rt, 90-95%; k) phenylacetyl chloride, CH_3CN, 72h 0°C - rt, 64%.

The corresponding 7-nitro compound **6c** was obtained by reaction of the benzylic bromide with di-t-butyl-iminodicarboxylate followed by cleavage of the BOC groups with trifluoroacetic acid (TFA). Treatment of **6a-c** with an acid chloride and triethylamine, or alternatively with the free carboxylic acid in the presence of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimid-hydrochloride (EDC) in THF led to the amides **7a-c**, respectively. Deprotection of **7a-c** either with hydrochloric acid, or in HBr-acetic acid gave the quinoxaline-2,3-diones **1a-d, 1f-o, 9a-q, 10a-d, 11a-b** and, **12**. The urea derivatives **8a-d** were obtained by treatment of the amine **6c** with isocyanates and subsequent deprotection. Treatment of the amine **6a** with formaldehyde in water and ethanol gave the triazinane **13** which yielded the tricylic quinoxaline-2,3-dione **14** after reaction with phenylacetyl chloride and subsequent deprotection.

The intermediate **15** was obtained from **6a** by treatment with phenylacetyl chloride. In a Heck-type reaction[11] with **15** and diethyl phosphite **16** and **17** were isolated in a 15 : 1 mixture (Scheme 2). The phosphonic acid ester **16** was deprotected with trimethylbromosilane in dichloromethane followed by ethanolysis of the silylated intermediate prior to the exposure to HBr in acetic acid to yield **1f**. The intermediate **17** gave **1d** upon deprotection with HBr in acetic acid. Methylation of the intermediate **15** with NaH/MeI and subsequent acidic deprotection gave the tertiary amide **18**.

Scheme 2



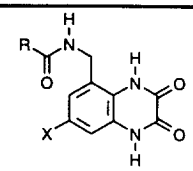
a) $(EtO)_2OPH$, $(Ph_3P)_4Pd$, Et_3N, toluene, 16h 90 - 100°C; b) TMS-Br, CH_2Cl_2, 16h rt; c) EtOH, 1h rt; d) HBr/AcOH 24h rt; e) NaH, MeI, THF, 1.5h reflux, CH_3I, 15h 0°C - rt.

Results and Discussion

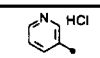
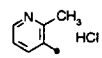
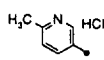
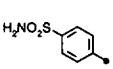
All compounds were evaluated for their ability to bind to AMPA/kainate receptors and to the glycine-site of NMDA receptors. The results of the *in vitro* binding assays are described as their IC_{50} values for the displacement of [^{3}H]-AMPA[12] and [^{3}H]-(Z)-2-carboxy-4,6-dichloroindole-3-(2'-phenyl-2'-carboxy)-ene [^{3}H]-MDL-105,519[13], respectively. The SAR of the 7-substituted 5-arylamidomethylquinoxaline-2,3-diones **9a-q** with the aromatic moiety attached directly to the carbonyl carbon is summarized in Table 1. In this series, the 2- and 3-thienyl derivative **9d** and **9g** showed the highest affinity, with IC_{50} values of 10 nM in the MDL-assay. Even small structural changes, like the replacement of the thienyl substituent by a phenyl (**9a**) or a 2-furyl ring (**9c**), resulted in a significant loss of affinity. Addition of a methyl group to **9d** at positions 3 (**9e**) or 5 (**9f**) of the thienyl ring lowered the binding affinity approximately to the same extent as a N-methyl-2-pyrrolylamide substituent (**9h**). These data suggest that the conformational space of the glycine binding pocket is highly restricted. The loss of the binding affinity by the introduction of the sterically demanding trifluoromethyl groups (**9b** vs. **9a**) corroborates this pharmacophore hypothesis.

The pyridine derivatives **9i-l** are soluble to about 1% in water, making them better suitable for i.v. application. However, these compounds also exhibited lower binding affinities to the glycine-site, compared to the hardly soluble compound **9a**.

Table 1: IC_{50} values [μM] for AMPA and NMDA receptors of derivatives **9a-q**. The IC_{50} values are means of 2 to 4 separate experiments, obtained from 6 or 12 concentrations o feach compound, run in triplicate. Variation between the experiments were less than 50%.



	subst. R	X	MDL	AMPA
9a		Br	0.06	1.7
9b		Br	> 10	>> 10
9c		Br	0.04	6.8
9d		Br	0.01	1.6
9e		Br	0.02	~ 10
9f		Br	0.03	1.7
9g		Br	0.01	2.0
9h		Br	0.02	> 10

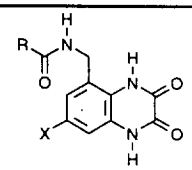
	subst. R	X	MDL	AMPA
9i		Br	0.28	4.3
9j		Br	0.25	~ 10
9k		Br	1.8	~ 10
9l		Br	0.11	~ 10
9m		NO_2	0.04	0.15
9n		NO_2	0.15	0.13
9o		NO_2	0.20	0.064
9p		NO_2	0.17	0.3
9q		NO_2	0.63	0.2

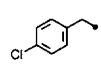
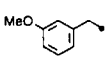
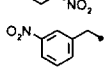
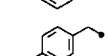
The results of the *in vitro* binding tests of α-unsubstituted 5-arylmethylamide derivatives **1a-o**, α-substituted derivatives **10a-d**, carbamates **11a-b** and urea-type derivatives **8a-d** of 5-aminomethylquinoxaline-2,3-diones are summarized in Table 2. The binding affinity at the glycine site remains unchanged if the 7-bromo substituent in the original lead compound **1b** is replaced by a chlorine atom (**1c**). It is slightly decreased when the bromine atom is replaced by a hydrogen atom (**1d**) or a nitro group (**1e**). A phosphonate group at the 7-position (**1f**) leads to a complete loss of activity. Whereas substituents in the meta position of the benzyl ring of **1b** are tolerated (**1h, j, l**), substitutions in the para position lower the binding affinity dramatically (**1i, m**). However, substituents in the ortho position seem to be favored (**1g** and **1k**). It is intriguing to note that the 3,4-dimethoxy derivative **1n** is only slightly less active than the 3-methoxy derivative **1j**, whereas the 3,4-methylenedioxy derivative **1o** is inactive.

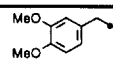
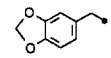
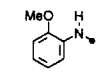
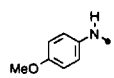
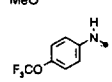
A substitutent at the sp^3 α-carbon does not or only weakly influence the binding affinity (**10a-c**), but a change to sp^2 geometry results in a loss of activity (**10d**). The binding affinity to the glycine site is lost if the α-carbon in **1b** is replaced by a hetero atom (**8a, 11a**). However, it can be partially restored by appropriate substitution (**8b-d**) or by chain elongation as in **11b**, which is even more active than its carbon analogue **12**.

The tertiary amide **18** (Scheme 2) has a 10 times lower binding affinity to the glycine site than its nor-derivative **1b**. Interestingly, the tricyclic derivative **14** (Scheme 1) proved to be inactive in the[^{3}H]-MDL-105,519 binding assay, indicating a restricted optimal position for the aromatic side chain in the pharmacophore model.

Table 2: IC_{50} values [μM] for the AMPA and NMDA receptors for the derivatives **1**, **8**, **10**, **11** and, **12** (Scheme 1). The IC_{50} values are means of 2 to 4 separate experiments, obtained from 6 or 12 concentrations o feach compound, run in triplicate. Variation between the experiments were less than 50%.



	subst. R	X	MDL	AMPA
1a		Br	0.01	11.3
1b		Br	0.01	> 10
1c		Cl	0.01	~ 10
1d		H	0.09	>> 10
1e		NO_2	0.04	28.8
1f		PO_3H_2	> 10	>> 10
1g		Br	0.007	> 10
1h		Br	0.04	>> 10
1i		Br	20	>> 10
1j		Br	0.03	> 10
1k		Br	0.02	> 10
1l		Br	0.04	> 10
1m		Br	1.2	> 10

	subst. R	X	MDL	AMPA
1n		Br	0.05	> 10
1o		Br	3.9	> 10
8a		NO_2	0.9	4.34
8b		NO_2	0.03	0.63
8c		NO_2	0.03	0.30
8d		NO_2	0.35	1.1
10a		Br	0.01	~ 10
10b		Br	0.04	> 10
10c		Br	0.05	~ 10
10d		Br	4.0	>> 10
11a		Br	1.5	> 10
11b		Br	0.11	~ 10
12		Br	0.86	> 10

The best compounds of these series were tested i.p. in the electroshock model in mice[14] with a pretreatment time of 30 min. at a dose of 50 mg/kg. Disappointingly, they displayed no significant effect in this test paradigm. This might be explained by a very poor permeability across the blood-brain barrier for this class of substances, or by the generally low solubility of amide derivatives of quinoxaline-2,3-diones in water. However, some experimental evidence showed that a few tested compounds did actually enter the central nervous system

after peripheral application. **1a** was inactive in the electroshock test, yet proved to be neuroprotective in the *in vivo* quinolinic acid-induced excitotoxicity model[15] and moderately active in the metrazole test[14] (50mg/kg, 1h, 30 % effect). These results suggest that strong and selective glycine site antagonists which are capable of penetrating into the brain do not necessarily display anticonvulsant activity in the electroshock model. This observation was corroborated by the fact that a water soluble, very potent and selective glycine site antagonist, belonging to a different class of compounds[16] not described here, was found to be inactive in the electroshock model even after i.c.v. application.

In **summary**, the derivatization and optimization of the original lead compound **9a** led to a series of very potent and selective glycine site antagonists, resulting in a relatively clear SAR. The compounds represented in Table 1 seem to fit into a tightly defined binding pocket at the receptor. The optimal binding geometry for the series summarized in Table 2 is also very restricted. Modifications in the 7-position (**1b-e**) and various changes in the 5-aminomethyl substituent (e.g. **1b** vs. **8a**, **11a**, **12** and **1g** vs. **1i**; **1k** vs. **1m**) proved to have strong effects on the binding affinity.

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