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# Communications to the Editor

## Substituted 1,2-Dihydrophthalazines: Potent, Selective, and Noncompetitive Inhibitors of the AMPA Receptor

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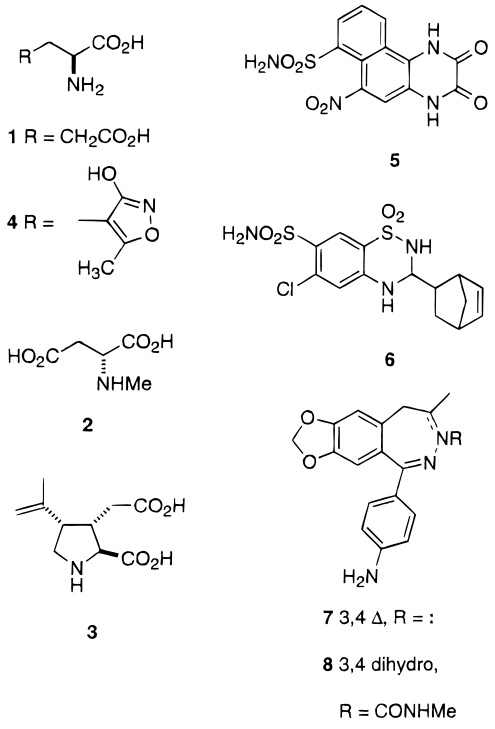
The amino acid L-glutamate **1** (Chart 1) is the major excitatory neurotransmitter of the central nervous system (CNS) and plays a key role in normal function of the CNS.[1] However, under certain pathological conditions, when extracellular concentrations of glutamate are excessive, neuronal damage and cell death can occur. This phenomenon has been termed excitotoxicity and there is increasing evidence that it plays a role in neurodegeneration associated with both acute (stroke, trauma) and chronic (Alzheimers disease, epilepsy) neurological disorders.[2] It is now recognized that agents which selectively antagonize certain ionotropic glutamate receptor subtypes reduce injury in animal models of stroke and epilepsy.[3] However, severe side effects, lack of solubility, and poor biodistribution profiles have hampered the clinical development of these agents.[2a,4] Therefore, valuable therapeutic potential exists for a new generation of centrally acting ionotropic glutamate receptor modulators.

Studies based on biochemistry and molecular biology have clearly shown that several subtypes of ionotropic glutamate receptors exist in the mammalian CNS.[5] The most widely studied receptor subtype is the *N*-methyl-D-aspartate (NMDA) receptor.[6] Two non-NMDA receptor subtypes, the kainate (KA) and α-amino-3-hydroxy-4-methylisoxazolepropionic acid (AMPA) receptors, have also been identified. The three receptor subtypes have been pharmacologically classified according to the ligands that selectively activate them (i.e. NMDA (**2**),[6] KA (**3**),[6] and AMPA (**4**)[7]). While numerous NMDA antagonists have been discovered, only a small number of selective AMPA antagonists have been reported.[8]

**Chart 1**




Modulation of the AMPA receptor can occur through several modes of action. Selective ligands such as AMPA (**4**) (agonist)[7] and NBQX (**5**) (antagonist)[8b] are competitive binders. As seen in whole cell electrophysiology assays the diuretic cyclothiazide (**6**), a noncompetitive agonist, potentiates AMPA receptor-mediated currents.[9] The 2,3-benzodiazepines, GYKI 52466 (**7**) and GYKI 53655 (**8**) (racemic mixture), noncompetitively inhibit AMPA-receptor currents.[10] The 2,3-benzodiazepines have also been shown to inhibit seizure activity[10b] as well as both focal and global ischemic damage *in vivo*.[11] The desirable effects of these latter agents prompted us to investigate other heterocycles with similar pharmacological properties. We now report preliminary studies on the preparation, electrophysiology, ancillary binding, and anticonvulsant activity of structurally novel 1,2-dihydrophthalazines, a new series

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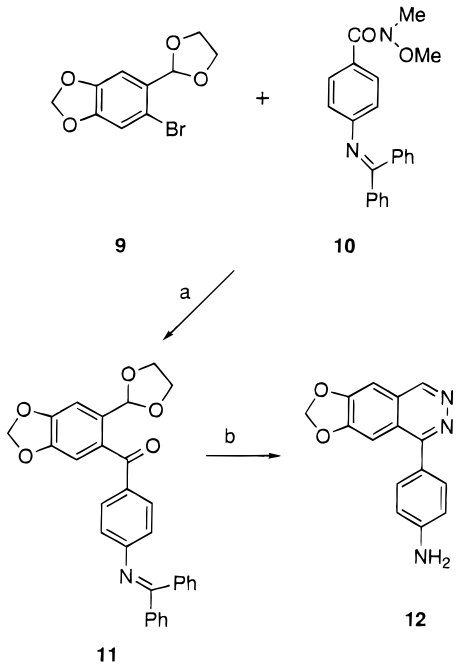
**Scheme 1**[a]




[a] (a) *n*-BuLi, THF, −78 °C; (b) $H_2NNH_2$·HCl, MeOH, $H_2O$.

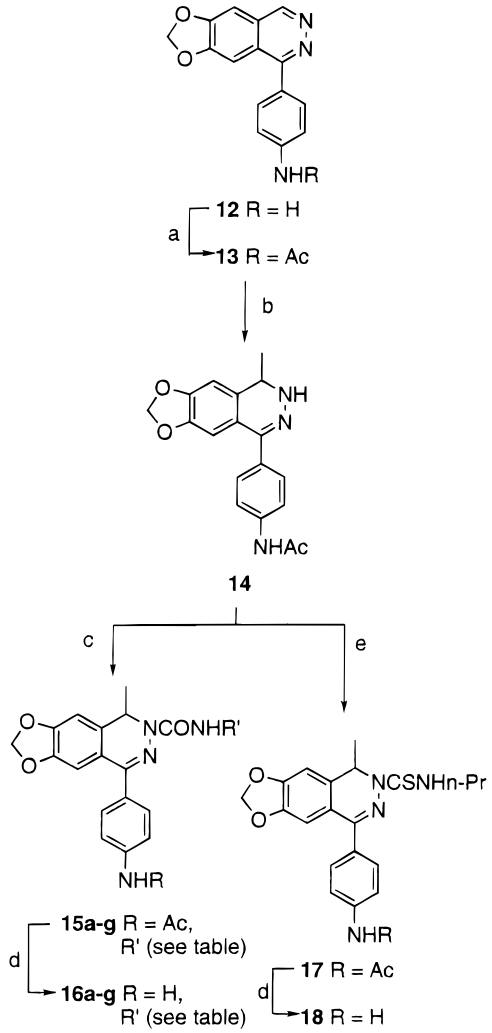
of compounds possessing selective, noncompetitive inhibitory properties at the AMPA receptor.

**Chemistry**. The fully aromatic phthalazine **12** was synthesized as shown in Scheme 1. Bromo acetal **9**[12] underwent rapid lithium halogen exchange upon treatment with *n*-butyllithium at −78 °C for 2 min. Subsequent treatment of this lithio species with the protected aminophenyl carboxamide **10**[13] provided the expected benzophenone derivative **11** (64%) which, when further reacted with hydrazine monohydrochloride (2 equiv),[13] resulted in a 94% yield of the fully deprotected (aminophenyl)phthalazine **12**.

Compound **12** also served as an intermediate to the dihydrophthalazines **16a**−**g** and **18** (Scheme 2). Hence, the amino group of **12** was reprotected as an acetanilide (acetic anhydride, 20 °C, 3 h, 85%) and the product **13** was treated with methyllithium which resulted in a 45−50% yield of the key intermediate **14**.[14] The final products **16a**−**g** were obtained in good yields via treatment of methyldihydrophthalazine **14** with various isocyanates (isocyanate, dichloromethane, 20 °C) followed by selective hydrolysis of the acetate. Refluxing **14** in toluene with *n*-propyl isothiocyanate with subsequent deprotection of the product **17** as above gave **18**.

**Biological Results and Discussion**. Since no reliable ligand-binding assay has been reported for noncompetitive agonists and antagonists affecting the AMPA receptor, attention was focused on an electrophysiological assay to serve as the primary screen. Voltage-clamped cortical neurons, which exhibit robust AMPA receptor-mediated currents, have previously been used to assess the potency of substituted 2,3-benzodiazepines. AMPA receptor responses were stimulated by brief applications of kainic acid, an agonist that induces large, non-desensitizing (steady-state) currents in these cells.[10a,d] Using this assay, compounds **5**, **7**, **12**, **16a**−**g**, and **18** were screened for their ability to inhibit AMPA receptor currents using initial concentrations of 100 or 10 $\mu$M. Concentrations providing 50% inhibition ($IC_{50}$'s) were determined from current traces

**Scheme 2**[a]




[a] (a) $Ac_2O$; (b) MeLi (4 equiv), TMEDA, THF; (c) R′NCO, $CH_2Cl_2$; (d) 1 N NaOH, MeOH, reflux; (e) *n*-PrNCS, toluene reflux.

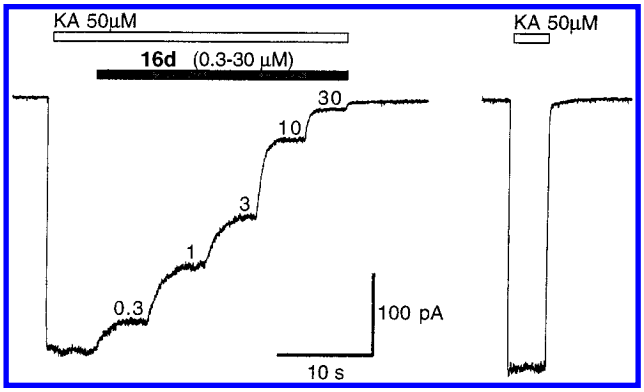



**Figure 1.** Inhibition of current in a voltage-clamped neuron isolated from rat cerebral cortex by increasing concentrations of **16d**. Application of 50 $\mu$M kainic acid (hollow bar) to a cell elicits a current date to stimulation of AMPA receptors.[10a,d] Subsequent addition of 16d (filled bars) at concentrations ranging from 0.3 to 30 mM causes a stepwise progressive inhibition of the current. After a 30 s wash in control saline, the kainic acid-stimulated current recovers completely (right-hand trace).

similar to that shown for dihydrophthalazine **16d** (Figure 1). The resulting data are shown in Table 1. The 2,3-benzodiazepine **7** displayed an $IC_{50}$ value similar to that found in the literature (10 $\mu$M)[10b] while (aminophenyl)phthalazine **12** provided minimal activity

**Table 1.** Inhibition of AMPA Currents in Rat Cortical Cells Stimulated with 50 $\mu$M Kainic Acid

| no.[a] | R′ | % inhibn at 10 $\mu$M (100 $\mu$M) | $IC_{50}$[b](±SE) |
|---|---|---|---|
| **12** | | (38) | |
| **16a** | $CH_3$ | 13(88) | 23(5.9) |
| **16b** | $C_2H_5$ | 55 | 7.2(0.7) |
| **16c** | *i*-$C_3H_7$ | 45 | |
| **16d** | *n*-$C_3H_7$ | 77 | 2.8(0.4) |
| **16e** | *n*-$C_4H_9$ | 89 | 1.8(0.2) |
| **16f** | *t*-$C_4H_9$ | 70 | 5.4(1.5) |
| **18** | | 79 | |
| **16g** | $C_6H_5$ | (35) | |
| **7** | | 51 | 10(0.8) |
| **5** | | 98[c] | 0.14 |

[a] Compounds **16a**−**g** and **18** are racemic mixtures. [b] $IC_{50}$ ($\mu$M) values represent an average of at least four cells. [c] Percent inhibition at 3 $\mu$M.

at 100 $\mu$M (<40% inhibition). When **12** was converted to the 1,2-dihydrophthalazines **16a**−**f** (racemic mixtures), a dramatic increase in AMPA receptor inhibition was observed (Table 1). Antagonism increased with the length of the pendant alkyl chain. The trend is apparent for dihydrophthalazines with pendant groups bearing normal alkyl functionality ($IC_{50}$'s of 23, 7.2, 2.8, and 1.8 $\mu$M for R = methyl, ethyl, *n*-propyl, and *n*-butyl, respectively), a pattern that contrasts with the data reported for the 2,3-benzodiazepine analogues.[15] Particularly notable is dihydrophthalazine **16e** (SYM 2207) which had an $IC_{50}$ value of 1.8 $\mu$M and was similar to the literature value of the most potent noncompetitive AMPA antagonist GYKI 53655 (**8**, $IC_{50}$ = 1.0 $\mu$M).[10b] Size limitations of the side chain are evident with the phenyl derivative **16g** (35% inhibition at 100 $\mu$M). Interestingly, the thio derivative **18** showed approximately the same level of activity as its oxygenated counterpart **16d** (79% inhibition at 10 $\mu$M for **18** vs 77% inhibition at 10 $\mu$M for **16d**).

Numerous ligands for the AMPA receptor show activity at the kainate receptor subtype as well.[16] In order to define a selectivity profile for the dihydrophthalazines (AMPA vs kainate receptor activity), selected compounds were tested in the voltage-clamp assay for modulation of kainate receptor currents. Human embryonic kidney (HEK) cells were used to express the GluR6 receptor.[17] This homomeric ligand gated ion channel is known to have properties similar to the native kainate receptor.[17] When **16b** and **16d** were tested for inhibition of GluR6 currents, neither compound showed greater than 10% inhibition at a concentration of 100 $\mu$M (data not shown).

Further selectivity data was obtained by screening compounds **16d** and **8** for their ability to displace tritiated ligands in a variety of binding assays.[18] Dihydrophthalazine **16d** showed very weak binding in a nonselective adenosine assay (32% inhibition at 10 $\mu$M) while displaying no binding in all others tested (<10% inhibition of tritiated ligand binding at 10 $\mu$M). The 2,3-benzodiazepine **8**, however, showed approximately 62% inhibition of binding (at 10 $\mu$M) to the central benzodiazepine receptor while remaining relatively inactive at the other receptors assayed (data not shown). None of the compounds bound competitively to glutamate receptors (NMDA, AMPA, kainate, and strychnine-insensitive glycine assays).

Both competitive and noncompetitive AMPA antagonists such as NBQX and GYKI 52466 have been reported to be active in *in vivo* electrical seizure models.[19] 1,2-Dihydrophthalazine **16d** was tested in the maximum electroshock (MES) test in mice. The $ED_{50}$ was determined to be 30 mg/kg (ip dose). The results clearly indicate that **16d** penetrates the blood-brain barrier and inhibits the onset of electrically stimulated seizure activity.[20]

In summary, novel heterocycles consisting of a 1,2-dihydrophthalazine core have been shown to selectively and noncompetitively inhibit currents associated with activation of the AMPA subtype of the glutamate receptor. The activity of the most potent compound, **16e** (SYM 2207), was similar to the literature value reported for GYKI 53655 (2,3-benzodiazepine, **8**) in the same electrophysiology assay. Unlike **8**, the 1,2-dihydrophthalazine **16d** was shown to be inactive at the central benzodiazepine binding site. Furthermore, **16d** was active in an *in vivo* anticonvulsant assay. The potent and selective activity of the 1,2-dihydrophthalazines coupled with their relative ease of synthesis and improved solubility properties should make them valuable tools for the pharmacological study of glutamate receptors. Further structure activity and *in vivo* studies are in progress.

**Supporting Information Available:** Experimental details with spectral data (6 pages). Ordering information is available on any current masthead page.

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