Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 539–542

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ALLOSTERIC MODULATORS OF THE AMPA RECEPTOR: NOVEL 6-SUBSTITUTED DIHYDROPHTHALAZINES

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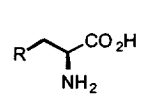
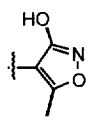
Received 25 June 1998; accepted 4 January 1999

Abstract: Novel analogs of the allosteric AMPA receptor modulator SYM 2206 have been prepared. Structure/activity correlations of these novel analogs and other dihydrophthalazines (DHPs) reveal the important contribution of the heteroatom-based aryl substituents in this class of noncompetitive inhibitors. One of the analogs (**6**, SYM 2189) is equipotent with the early series, but with reduced sedation.

The excitatory amino acid L-glutamate (**1**) is a major neurotransmitter in the central nervous system (CNS) and plays a key role in the normal function of the CNS.[1] However, under certain pathological conditions, excessive release of glutamate can lead to neuronal damage and cell death, a phenomenon that has been termed excitotoxicity.[2] There is abundant evidence that excitotoxic cell death plays a causative role in neurodegeneration associated with both acute (stroke, trauma) and chronic (Alzheimer's disease, Parkinson's disease, and epilepsy) neurological disorders in the CNS.[3]



1 R = CH_2CO_2H



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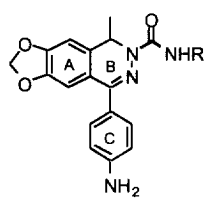
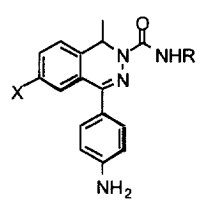


2 R=*n*-Pr (SYM 2206)
3 R=*n*-Bu (SYM 2207)



5 X = OMe, R =*n*-Bu
6 X = OMe, R =*n*-Pr (SYM2189)
7 X = OMe, R =Et
8 X = F, R =*n*-Pr

It is now recognized that agents that selectively antagonize ionotropic glutamate receptors can reduce injury in animal models of stroke and epilepsy.[4] However, severe side effects, lack of solubility, and poor biodistribution profiles have hampered the clinical development of these agents.[3a,5] Moreover, recent clinical studies have indicated that complete blockade of certain ionotropic glutamate receptors is a source of severe side effects. Therefore, valuable therapeutic potential exists for a new generation of centrally acting ionotropic glutamate receptor modulators. We have previously revealed a novel series of allosteric modulators **2** and **3** of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (**4**) (AMPA) receptors based upon the DHP framework which display highly encouraging *in vivo* efficacy in both anticonvulsion[6] and neuroprotection models[7] In our

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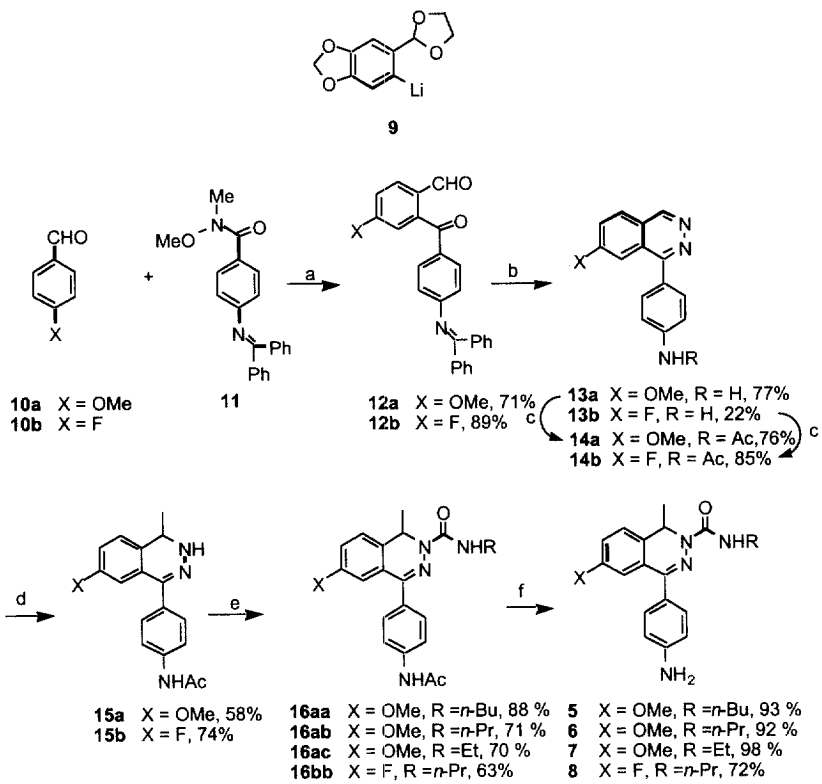
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00044-X

continuing structure-activity relationship (SAR) studies, we have now prepared a novel series of 1,2-DHPs **5-7** and **8** that explore the role of the oxygenated substituents on the A-ring of these species. We report herein the preliminary results of the synthesis, electrophysiology, and anticonvulsant activity of a family of structurally simplified DHPs.



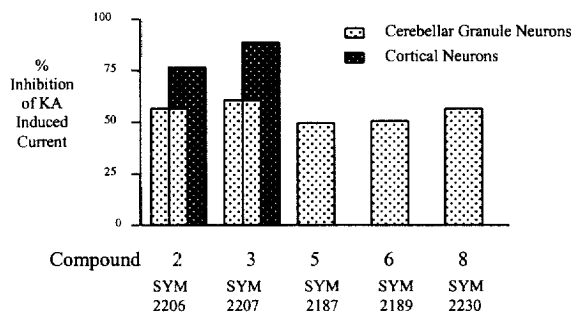
(a) TMEDA, *n*-BuLi, THF; (b) H_2NNH_2 2HCl, MeOH, H_2O; (c) Ac_2O, $CHCl_3$; (d) MeLi, THF; (e) RNCO, CH_2Cl_2; (f) 2 N NaOH, MeOH, reflux.

Scheme 1

Chemistry: Our previous preparation of **2** and **3** had employed piperonal as the origin of the A-ring. Aryl lithium **9** was generated *in situ* from the corresponding aryl bromide, which in turn was prepared in two steps from piperonal. In our hands, we have found **9** to not be robust. As a result of the complications and additional synthetic procedures inherent in the use of **9**, and the desire to evaluate the necessity of the two ring oxygens we have explored an alternative means to construct the DHP ring system. Comins has extensively documented the use of α-aminoalkoxides to direct lithiation adjacent to arylaldehydes.[8] Accordingly, DHPs **5-7**, and **8** were prepared from *p*-anisaldehyde (**10a**), and 4-fluorobenzaldehyde (**10b**) respectively (Scheme 1). The α-amino alkoxide formed from **10a** and lithium trimethylethylenediamine (TMEDA) in THF was treated with *n*-butyllithium. Subsquent treatment of this lithio species with the protected aminophenyl carboxamide (**11**).[9] gave

the expected benzophenone derivative **12a** (71%). Benzophenone **12a** was reacted with hydrazine hydrochloride with concomitant deprotection to yield phthalazine **13a** (77%). Acetylation of **13a** gave **14a**, which was treated with methyllithium to give 1-methyl-1,2-dihydrophthalazine **15a** (58%). Treatment with the appropriate isocyanate in dichloromethane followed by selective hydrolysis of the acetanilides gave **5-7** in good overall yield. Fluoro analog **8** was prepared in an analogous manner from **10b**. While coupling of **10b** with **11** proceeded much more cleanly than the methoxy series (89% vs 71%), the subsequent condensation to generate 4-arylphthalazine **13b** proceeded in low yield (22%). Since **13b** appeared to be as stable as **13a**, we feel that the greater electronegative nature of the fluorine substituent must result in increased side reactions of the aldehyde moiety of **12b**.

Figure 1: Inhibition of KA Induced Currents in Neuronal Preparations by Selected AMPA Modulators (10μm)



Biological Evaluation and Discussion: Previously we had characterised **2** and **3** employing patch-clamp electrophysiology on cultured cortical neurons.[6] Such cultured neurons are considered a reliable source of AMPA receptors. Kainic acid (KA) is a nondesensitizing agonist at AMPA receptors.[10] Thus, applying KA to cortical neuron preparations invokes an easily measurable steady state current. Application of either **2** or **3** (10 μm) resulted in a significant reduction in the observed current (77% and 89%, respectively). Since no reliable ligand-binding assay has been reported to date for allosteric modulators of the AMPA receptor, the electrophysiological assay has served as our primary screen. In this assay we chose to employ cerebellar granule neurons, an alternative source of AMPA receptors. However, as may be seen from the results in Figure 1, the potency of **2** and **3** is roughly comparable to that previously reported for cortical neurons. Interestingly, DHPs **5**, **6** and **8** appear to be approximately equipotent with **2** and **3**. The 6,7-dimethoxy-1,2-DHP and the corresponding 6,7-unsubstituted DHP analogs of **2** and **3** displayed very low potency compared to **2** against KA induced currents in cortical neurons.[11] This result had been interpreted to suggest that the A-ring substituents interact with the receptor in a highly volume restricted manner, wherein the additional spacial requirements of the two adjacent methoxy units are detrimental to efficient binding. The efficacy of **5** and **6** indicate that the important interaction of these substituents with the receptor is mediated through the 6-oxygenated appendage. Further, the activity of **8** indicates the presence of a positive electronic interaction of the 6-heteroatom substituent with the receptor surface rather than a simple steric requirement.

Previously we had shown that **2** and **3** were active in *in vivo* models of epilepsy.[6,12] Both compounds demonstrated efficient protection of both mice and rats in maximal electroshock (MES) assays of anticonvulsant activity (ED_{50}=35-50mg/kg). However, both compounds also demonstrated significant sedative effects at

slightly higher doses. Analysis of **6** (SYM 2189) in seizure models shows that this compound is equipotent with the earlier series with an ED_{50}=52 mg/kg against MES induced seizures in mice. However, SYM 2189 shows reduced sedation with an ED_{50}=150mg/kg in the rotarod test. The compound also shows to be effective for over two hours. Further pharmacological characterization of the anticonvulsant properties of **6** (SYM 2189) is currently underway and will be reported separately.

In summary, a new generation of DHPs without a 7-oxygenated substituent have been shown to inhibit KA induced currents associated with activation of the AMPA subtype of glutamate receptors. Compound **6** (SYM2189) was active in *in vivo* anticonvulsant assays and demonstrated diminished sedative properties. The potent and selective activity, and low sedative properties of this novel series of compounds coupled with their relatively simple synthesis make them promising antiepileptic/anticonvulsant drug candidates and valuable tools for the pharmacological study of glutamate receptors.

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