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SYNTHESIS AND CHARACTERIZATION OF N-(ACENAPHTH-5-YL)-N'-(4-METHOXYNAPHTH-1-YL)GUANIDINE AS A GLUTAMATE RELEASE INHIBITOR AND POTENTIAL ANTI-ISCHEMIC AGENT

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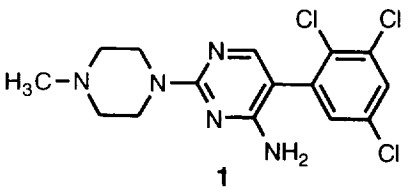
Abstract. Structure-activity relationship (SAR) studies of N,N'-diarylguanidines as glutamate release inhibitors led to the synthesis of N-(acenaphth-5-yl)-N'-(4-methoxynaphth-1-yl)guanidine (**6**). Compound **6**, with IC_{50} values of 1.3 and 2.2 μM in ^{14}C-guanidine flux and $^{45}Ca^{2+}$- flux assays respectively, effectively inhibited both veratridine and K^+-evoked glutamate release in rat brain synaptosomal preparations.

There is a great deal of interest in developing drug treatments which limit the extent of nerve cell death following an ischemic insult to the brain. Nerve cell death following ischemia is thought to result from the actions of excessive glutamate released from metabolically compromised nerve terminals.[1-3] Glutamate binds to both N-methyl-D-aspartate (NMDA) and non-NMDA receptor sites to elicit its neurotoxic effects. NMDA antagonists are highly effective in animal models of focal cerebral ischemia,[4] but are reportedly less effective in models of global cerebral ischemia.[5,6] Non-NMDA antagonists such as NBQX are highly effective in rat models of global cerebral ischemia,[7] but are apparently less effective in focal cerebral ischemia.[8] However, blockers of glutamate release prevent excessive activation of both NMDA and non-NMDA receptor subclasses by acting at an earlier stage of the cascade of events leading to cell death. Although the release of neurotransmitters other than glutamate may also be affected, glutamate release blockers have recently been shown to be effective in animal models of both focal and global cerebral ischemia.[9,10] Presynaptic voltage-activated Ca^{2+} channels and Na^+ channels each play an important role in regulating the release of glutamate from presynaptic nerve terminals, and specific subtypes of these channels may play differential roles in the release of particular transmitters.[11] Accordingly, blockade of the voltage-activated ion channels which control glutamate release is considered as an approach toward developing potential neuroprotective agents.

Compounds that have previously been reported to block glutamate release (**Chart 1**) include both Na^+ channel blockers, such as 4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl)pyrimidine (**1, BW 619C89**)[12] and analogues,[10] and riluzole (**2, PK 26124**),[13] as well as Ca^{2+} channel blockers, such as 2-(4-(*p*-fluorobenzoyl)-piperidin-1-yl)-2'-acetonaphthone (**3, E 2001**),[14] cone snail peptide toxins (e.g. **w-CgTX-MVIIC**)[15] and spider venom peptide toxins (e.g., **w-Aga-IVA**).[16]

In Honor of A. V. Rama Rao's 60th Birthday



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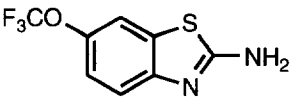

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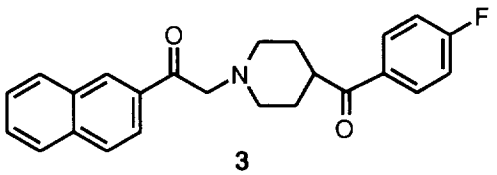

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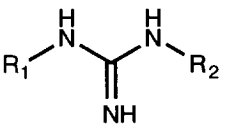

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The guanidinium ion is permeant for many ion channels, including voltage-activated sodium channels, and many substituted guanidines are effective ion channel blockers. The degree of selectivity for ion channel classes ranges from the very broad spectrum of the monosubstituted acyl guanidine amiloride[17] to the extremely selective blockade of neuronal Na^+ channels by tetrodotoxin (TTX) and related toxins.[18] Lipkind and Fozzard have recently proposed a structural model of the tetrodotoxin and saxitoxin binding site based on the primary amino acid sequence of Na^+ channels and the observed alterations of tetrodotoxin affinity induced by specific site-directed mutations.[19] Furthermore, it is proposed that the high-affinity block is imparted by interaction of the positively-charged guanidinium group of tetrodotoxin with negatively charged residues at a site known as the "ion-selectivity-filter domain," in addition to interactions of other groups of the toxin with adjacent residues on the channel. Differences in ion selectivity among ion channel classes may result, at least in part, from structural differences in the ion-selectivity-filter domain, and this may account for the high degree of specificity exhibited by tetrodotoxin and other guanidine derivatives.[20]

Chemistry

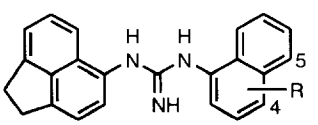
A synthetic chemistry program was initiated to design and synthesize novel guanidines of type **4** that selectively inhibit glutamate release through interactions at the voltage-gated ion channels. The first compound that showed potent activity in the glutamate release assay was N,N'-bis(acenapth-5-yl)guanidine (**5**, **CNS 1145**), which has an IC_{50} of 0.8 μM in K^+–evoked glutamate release assay. However, *in vivo* studies were hampered by poor solubility (0.37 mg/mL in 0.3M mannitol). Analogues of compound **5** were therefore investigated for improved solubility with retained or improved activity. During the course of SAR studies,[21] we have synthesized N-(acenaphth-5-yl)-N'-(4-methoxynaphth-1-yl)guanidine hydrochloride (**6, CNS 1237**) which has an IC_{50} of 1.0 μM for glutamate release inhibition activity. Compound **6** also possessed the appropriate solubility (1.69 mg/mL in 0.3M mannitol) required for further pharmacological investigations. Synthesis of compound **6** involved reflux of a mixture of acenaphth-5-yl-cyanamide [1.71 g, 8.8 mmol; prepared from 5-aminoacenaphthene and cyanogen bromide (**caution: toxic !**) in ethyl acetate] and 4-methoxy-1-naphthylamine hydrochloride (1.76 g, 8.4 mmol) in chlorobenzene (35 mL). After 5 h, the reaction mixture was allowed to cool to 20 °C and then let stand at RT for 10 h. The precipitated solid was collected by filtration and washed with dichloromethane (4 X 20 mL) and the solid was crystallized from acetonitrile containing few drops of isopropanol-HCl at pH 1 to give compound **6** • HCl (1.85 g, 52 %) as a

white solid: mp 249-51 °C; ^{1}H NMR (DMSO) δ 3.45-3.35 (m, 4H), 4.02 (s, 3H), 7.05-8.25 (m, 11H). HPLC (C_{18} Reverse Phase, CH_3CN:H_2O with 0.1% TFA) 99% (Rt = 11 min). Anal. ($C_{24}H_{22}N_3ClO$) C, H, N.

Biology

Four biological assays were used to characterize the inhibition of calcium and sodium channels and of glutamate release by the compounds.[22] The block of synaptosomal calcium channels was determined by inhibition of $^{45}Ca^{2+}$ flux into synaptosomes depolarized by elevated potassium concentrations, and the block of sodium channels was determined by inhibition of the flux of ^{14}C-guanidinium ion into cultured CHO cells transfected with and stably expressing the neuronal Type II sodium channel. For synaptosomal glutamate release experiments, purified rat brain synaptosomes isolated from whole brain were loaded with ^{3}H-glutamate utilizing the Na^+-dependent glutamate transporters present in the synaptosomal membranes. The labeled synaptosomes were depolarized with a continuous stream of high K^+-buffer (55 mM K^+) with or without Ca^{2+} (2.4 mM), and the resulting ^{3}H-glutamate release was collected in 0.3 sec fractions. Alternatively, 50 μM veratridine was used to depolarize the synaptosomes by activating the voltage-dependent sodium channels. Ca-dependent glutamate release was calculated for each experiment, and reflects the release due to exocytosis.

Table 1. Biological data[a]



Compd[b]	R	mp °C[c]	K-evoked Glu. Rel. IC50 (μM)[d]	Ver. evoked Glu. Rel. (% block @ 3 μM)[e]	$^{45}Ca^{2+}$ Flux IC50(μM)[f]	^{14}C-Guanidine Flux IC50 (μM)[g]
1		168-70[h]	[7][i]	nd	3.86	2.55 ±0.05
2		119-20[j]	[0][i]	49	>30	2.20 ±0.96
5	4, 5-CH_2-CH_2-	288-90	0.8	nd	1.13 ±0.04	2.03 ±0.33
6	4-OCH_3	249-51	1.0	87	2.24±0.38	1.31 ±0.17

[a]Each biological experiment was run in triplicate. The biological experiments for Compounds **5** and **6** were repeated more than 3 times and the SEM is ≤10%. [b]All new compounds were analyzed for C, H and N analyses. [c]The melting points are uncorrected. [d]See ref. 23 and Turner, T. J.; Pearce, L. B.; Goldin, S. M. *Analytical Biochemistry* **1989,** 178, 8. [e]See Blaustein, M. P. *J. Physiol.* **1975,** 247, 617. [f]See ref. 23 and Nachsen D. A. and Blaustein M. P. *J. Physiol.* **1985,** 361, 251. [g]See Tamkun, M. M and Catterall, W. A. *Mol. Pharmacol.* **1981,** 19, 78. [h]Lit. mp 162-64 °C; see ref. 12. [i]Percent block at 3 μM drug concentration. [j]Lit. mp 117-19 °C; see ref.13. nd=not determined.

Compound **6** inhibited K^+-evoked glutamate release (through block of voltage-gated Ca^{2+}-channels) and veratridine evoked glutamate release (through block of voltage-gated Na^+-channels) in the synaptosomal preparations more effectively than compounds **1** and **2.** Further characterization of this compound series indicated that these guanidines blocked both the voltage-gated Ca^{2+}-channels and Na^+-channels more potently than compounds **1** and **2.** For example, compound **6** has an IC_{50} of 2.24 and 1.31 μM in $^{45}Ca^{2+}$ flux and ^{14}C-guanidine flux assays respectively compared to 3.86 and 2.55 μM for compound **1** or >30 and 2.20 μM for compound **2.** We hypothesize that the dual actions of our compounds are due to interactions at a

common site of shared homology between presynaptic Ca^{2+}-channels and neuronal Type II Na^{+}-channels. Further compound **6** was reported to show efficacy in the *in vivo* rat middle cerebral artery occlusion (MCAO) model of focal ischemia, with intravenous doses of 6, 12 or 24 mg/kg over 4 hours resulting in about a 35% decrease in cerebral infarct volumes, with no significant reduction in blood pressure and a modest dose-dependent reduction in heart rate.[23] The above biological results clearly demonstrate that compounds which block both voltage-gated Na^{+} and Ca^{2+}-channels can be effective glutamate release inhibitors and potential neuroprotective agents.

In summary, we have identified novel guanidine derivatives that inhibit excess glutamate release in adult rat brain synaptosomes, by a unique mechanism involving blockade of both voltage-gated Na^{+}-channels and Ca^{2+}-channels. *In vivo* activity has been reported for compound **6** in a model of focal ischemia. Further studies are focused on developing the therapeutic potential of this compound series as anti-ischemic agents.

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