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SYNTHESIS AND GLUTAMATE ANTAGONIST ACTIVITY OF 4-PHOSPHONOALKYLQUINOLINE DERIVATIVES: A NOVEL CLASS OF NON-NMDA ANTAGONIST

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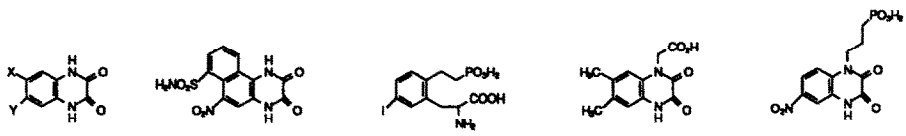
Abstract: The synthesis of ten 4-phosphonoalkylquinoline derivatives and the evaluation of their glutamate antagonist activity is described. Several compounds demonstrate significant antagonism at both 6-cyano-7-nitroquinoxalin-2,3-dione (CNQX) binding sites and the glycine regulatory site of the NMDA receptor. These compounds have also been shown to act as functional antagonists of kainic acid in *Xenopus* oocytes.

It is now commonly accepted that the amino acids L-aspartate and L-glutamate mediate a substantial portion of the excitatory neurotransmission in the vertebrate central nervous system.[1] Hyperactivity at EAA neurons has been associated with neurodegenerative disorders such as status epilepticus, Huntington's chorea, and Parkinson's and Alzheimer's diseases;[2] in addition, neuronal degeneration observed as a result of cerebral ischemic insult following stroke, hypoxia or hypoglycemia may be due to overstimulation at EAA receptors.[2] These observations have resulted in considerable research effort being focused on the development of selective and potent antagonists of excitatory amino acid neurotransmission, particularly compounds acting act the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazol-propanoic acid (AMPA) and kainic acid (KA) receptor subtypes.[1] The quinoxalinediones described by Honore et al. (1-3)[3] are antagonists at AMPA receptors as well as the glycine coagonist site of the NMDA receptor, and have been demonstrated to possess neuroprotective properties in vitro and in vivo.

We recently disclosed that appropriately substituted 2-(phosphonoalkyl)phenylalanines, exemplified by **4**, were selective and potent antagonists at non-NMDA ionotropic receptors.[4] As part of our program aimed at developing novel and selective antagonists for the various EAA receptor subtypes, we used our phenylalanine compounds and the quinoxalinediones reported by Honore et al. as templates to design a new class of non-NMDA antagonist. These compounds comprise a quinolin-2-one nucleus substituted at the 4-position with a phosphonoalkyl chain and bearing various substituents in the benzenoid ring. Subsequent to the completion of this study, structurally related compounds were described by Epperson et al., exemplified by **5**,[5] and Huth et al., exemplified by **6**.[6]

A total of ten compounds were prepared for the present study. Compounds **7-9** were prepared from 7-chlorolepidine[7] as shown in Schemes I and II. Compounds **10-14** were prepared by carefully treating the appropriately substituted acetoacetanilides with one equivalent of bromine to provide bromoacetanilides



1: DNQX: X = Y = NO_2
2: CNQX: X = CN, Y = NO_2

3: NBQX

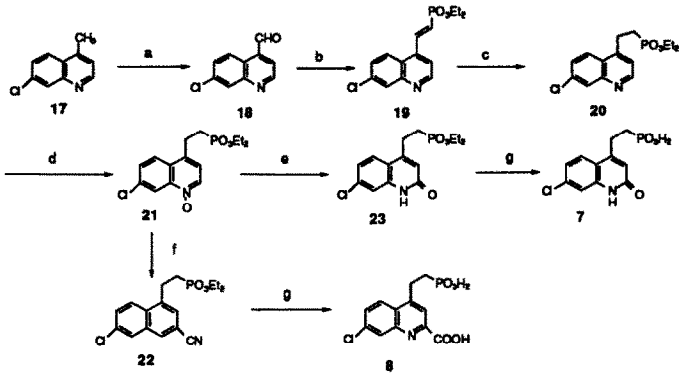
4

5

6

28 a-e[8] (Scheme III), which were cyclized to the corresponding 4-bromomethylquinolin-2-ones in con. H_2SO_4. Reaction of **30** a-e with triethylphosphite followed by acid hydrolysis delivered the final products. Nitro compound **15** was prepared by nitrating 2-methoxy-4-(diethylphosphono)methyl-7-chloroquinoline (**36**), followed by successive deprotection with bromotrimethylsilane and hydrobromic acid (Scheme IV). Compound **16** was prepared via the palladium-catalyzed coupling of 4-bromo-7-chloroquinoline[9] with diethylphosphite followed by oxidation to the N-oxide, rearrangement to the quinolin-2-one, and acid hydrolysis (Scheme V).

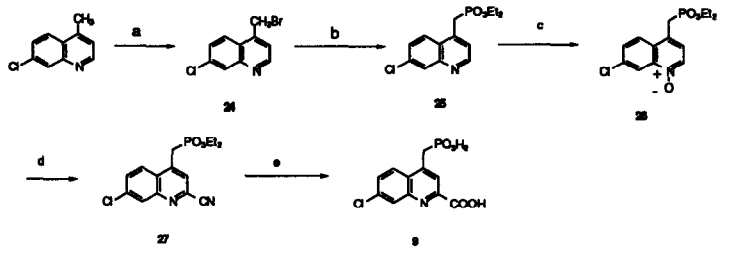
SCHEME I



Legend: a) SeO_2, dioxane/H_2O; b) Sodio tetraethylmethylene bisphosphonate, THF, 0°C; c) H_2, 5% Pd/C; d) MCPBA, $CHCl_3$; e) MsCl, NaOH, tetrabutylammonium hydrogen sulfate, phase transfer conditions; f) N-dimethylcarbabamyl chloride, TMS cyanide, CH_2Cl_2; g) 6N HCl.

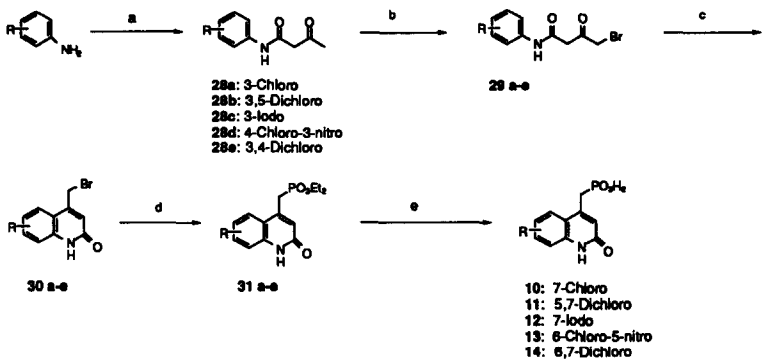
Test compounds were evaluated for their ability to inhibit kainic acid (KA)-induced inward currents in *Xenopus* oocytes injected with poly (A+) mRNA from rats, as previously described.[10] KA was chosen as the agonist since responses to KA are large and non-desensitizing, whereas responses to AMPA are much smaller in magnitude because they rapidly desensitize. Potencies were determined from dose-response curves and IC_{50} values were converted to K_i values using the Cheng-Prusoff equation.

SCHEME II



Legend: a): NBS, CCl_4, hv; b): $P(OEt)_3$; c): MCPBA, $CHCl_3$; d): TMS cyanide, N,N-dimethylcarbamyl chloride, CH_2Cl_2; e): 6N HCl, reflux.

SCHEME III



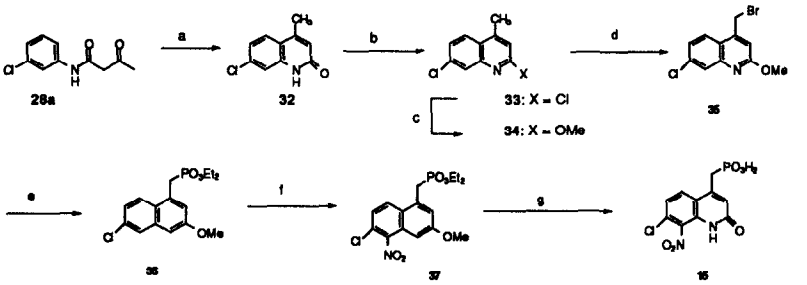
Legend: a): Diketene, toluene, reflux 2 hrs; b): Br_2, $CHCl_3$; c): Con. H_2SO_4, 120°C, 1 hr (for **34**, PPA instead of H_2SO_4); d): $P(OEt)_3$, reflux, overnight; e): 6N HCl, reflux overnight.

[^{3}H]CNQX was used to label KA/AMPA receptors in rat cortical membranes,[11] and the new compounds were evaluated for their ability to displace radioligand. Compounds were also evaluated for their binding affinity at the glycine co-agonist site of the NMDA receptor,[12] as well as their ability to inhibit NMDA-sensitive L-[^{3}H]glutamate binding.[12] Electrophysiological and receptor binding data for compounds **7-16** are tabulated in Table I.

The electrophysiological data demonstrate that 4-phosphonoquinoline derivatives are capable of antagonizing KA-induced currents in *Xenopus* oocytes. The two quinolinic acids (**8** and **9**; K_is of 450 and 132 μM, respectively) were less potent than the corresponding quinolin-2-ones **7** and **10** (K_is 51 and 32

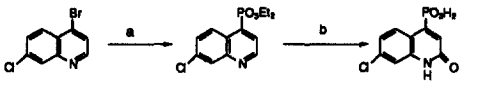
μM, respectively). Phosphonomethyl compound **10** was comparable in potency to its ethyl analogue **7**. Attachment of the phosphonic acid moiety directly to the aromatic ring abolished activity (**16**; K_i >> 1000 μM).

SCHEME IV



Legend: a): Con. H_2SO_4, 120°C, 1 hr ; b): $POCl_3$, reflux, 2 hrs; c): NaOMe/MeOH, reflux, overnight; d): NBS, CCl_4, hv; e): $P(OEt)_3$, reflux, overnight; f) HNO_3/H_2SO_4; g) TMS bromide, then 48% HBr.

SCHEME V



Legend: a): $P(OEt)_2(OH)$, $Pd(Ph_3P)_4$, toluene, 90°C, 18 hrs; b): MCPBA, Ac_2O/HOAc, 18 hrs, then 6N HCl.

A larger halogen substituent in the 7-position (**12**, K_i = 15.6 μM) increased potency in the oocyte assay, as did 5,7- and 6,7-disubstitution (**11** and **14**, Ki = 18.2 and 9.6 μM, respectively). The most potent compound examined in this assay was 6,7-dichloro compound **14**; this compound is comparable in potency to our previously described phosphonoethylphenylalanines (e.g., **4**). Compound **12**, which lacks a 7-substituent, showed little activity (K_i > 300 μM). Interestingly, 7-chloro-8-nitro compound **15** was also quite ineffective in blocking KA-induced currents (Ki > 379 μM), indicating that the placing of a nitro group in the 8-position of **10** vitiated the activity. It is noteworthy that the effect of ring substitution on the present compounds parallels that of the previously described phenylalanine derivatives.

The ability of the test compounds to displace radiolabeled CNQX roughly paralleled their ability to block agonist-induced currents in oocytes, as did their ability to inhibit the binding of labeled glycine at the strychnine-insensitive glycine site. Compounds **8**, **13**, **15** and **16** were the least potent in any of these

receptor assays. Phosphonoethyl compound **7** manifested a twenty-fold decrease in affinity for NMDA and GLY sites relative to phosphonomethyl compound **10**; the CNQX affinities are similar. Dichloro compounds **11** and **14** have lost affinity at the NMDA site relative to **10**, with a lesser attenuation in glycine site affinity.

It is instructive to compare the present compounds with the related examples **4** and **5**. Compound **4** has reported K_i's of 1.21 μM and 5.28 μM against tritiated AMPA and glycine, respectively; **5** has an IC_{50} of 1.8 μM against AMPA. These results are consistent with the trend of electronegative substituents in the 7-position being favorable for activity, as is 6,7-disubstitution. In addition, Epperson et al. noted that the ratio of AMPA and GLY site affinities of their compounds could be affected by the acidic side chain length.

TABLE I. BIOLOGICAL ACTIVITY OF COMPOUNDS

COMPOUND	INHIBITION OF KA-INDUCED CURRENTS IN OOCYTES K_i (μM)	RECEPTOR BINDING POTENCY IC_{50} (μM)		
		GLU	GLY	CNQX
4	3.5	>300	81	7.3
7	51	600	231	91
8	450	> 1000	> 1000	384
9	132	nd	69	61
10	32	30	10	59
11	18.2	> 100	29	26
12	15.6	35	37	35
13	> 300	>1000	> 1000	> 1000
14	9.6	> 100	79	15
15	379	>1000	>1000	300
16	>> 1000	>300	> 1000	nd
CNQX	nd	nd	7.9	0.13

In summary, we have described a new class of non-NMDA receptor antagonist which demonstrates functional antagonism. Modulation of receptor affinity was shown to result from structural modification. The parallels in observed substituent SAR between the quinolone, quinoxalinedione, and amino acid (phenylalanine) non-NMDA antagonist structures may indicate that these diverse structures interact at closely related sites on the receptor complexes. It is hoped that the present compounds, together with others

recently described,[4-7, 13] will prove to be useful tools for unraveling the complexities of glutamate receptor subtypes and their physiological relevance.

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