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Atropisomeric Quinazolin-4-one Derivatives are Potent Noncompetitive α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptor Antagonists

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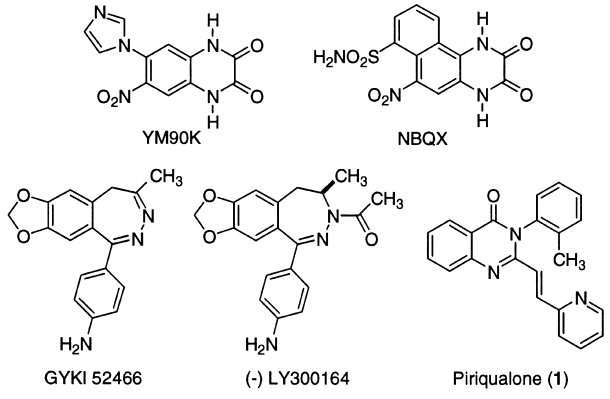
Abstract—Piriqualone (**1**) was found to be an antagonist of AMPA receptors. Structure–activity optimization was conducted on each of the three rings in **1** to afford a series of potent and selective antagonists. The sterically crowded environment surrounding the N-3 aryl group provided sufficient thermal stability for atropisomers to be isolated. Separation of these atropisomers resulted in the identification of (+)-**38** (CP-465,022), a compound that binds to the AMPA receptor with high affinity ($IC_{50} = 36$ nM) and displays potent anticonvulsant activity.

Introduction

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of glutamate receptors is associated with fast excitatory synaptic transmission in the central nervous system. During the course of neurodegenerative conditions including ischemic stroke and epilepsy, hyperactivation of glutamate receptors, and, in particular, *N*-methyl-D-aspartate (NMDA) and AMPA receptors, has been proposed as one of the processes that contributes to neuron death.[1] Consistent with this hypothesis, antagonists of both receptor subtypes have been protective in various anticonvulsant models.[2] Unfortunately, the prototype quinoxalindiones such as NBQX[3] and YM90K,[4] as well as other competitive AMPA receptor antagonist classes, are generally poorly soluble and thus difficult to formulate for clinical use.

Allosteric modulators of AMPA receptors based on the 2,3-benzodiazepine nucleus have recently been identified. Compounds from this class, such as GYKI 52466 and LY 300164, have also shown benefit as anticonvulsants and in stroke models.[5] However, poor solubility and modest potency remain potential issues. Attesting to these concerns, several new compounds (both competitive and noncompetitive) with improved solubility have now been reported as the area of AMPA receptor modulation continues to hold the interest of the research community.[6]



As part of ongoing studies into the pharmacology of glutamate receptors, we have identified a new antagonist template for AMPA receptors, the 3-aryl-2-(pyrid-2-ylvinyl)-quinazolin-4-ones, exemplified by the known anticonvulsant piriqualone.[7] Herein, we describe a

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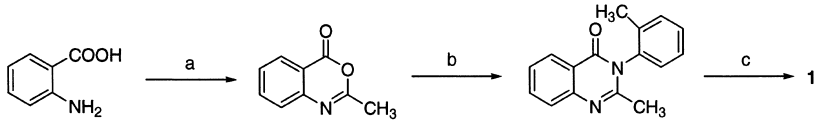


Scheme 1. Synthesis of quinazolin-4-ones: (a) Ac_2O, HOAc, reflux; (b) *o*-toluidine, HOAc, reflux; (c) 2-pyridinecarboxaldehyde, $ZnCl_2$, Ac_2O, dioxane, reflux.

succinct SAR program that led to the discovery of (*S*)-3-(2-chlorophenyl)-2-[2-(6-diethylaminomethyl-pyridin-2-yl)-vinyl]-6-fluoro-3*H*-quinazolin-4-one (CP-465,022), a potent antagonist that interacts with the receptor through an allosteric site.[8] This compound is highly selective for the AMPA receptor relative to NMDA or kainic receptors and displays potent anticonvulsant activity.

Chemistry

The synthetic route to piriqualone combines three structural fragments—anthranilic acid (A ring), *ortho* toluidine (B ring), and pyridine-1-carboxaldehyde (C ring).[9] Thus, SAR optimization was efficiently conducted through the combination of various commercial or readily accessible analogues of these simple components. In brief, anthranilic acid is converted to the benzoxazin-4-one with hot acetic anhydride in acetic acid (Scheme 1). Subsequent reaction with a suitable aniline fragment in refluxing acetic acid installed the B ring. Finally, condensation of this resultant 2-methyl-3-aryl-quinazolin-4-one with pyridine-2-carboxaldehydes provided the target compounds. The final condensation reaction was conveniently effected with acetic anhydride and anhydrous zinc chloride in refluxing dioxane.

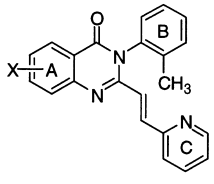
Pharmacology and SAR

To capture both competitive and noncompetitive antagonists in a high throughput screen, a functional assay of AMPA receptor activity was developed. We tested compounds for their ability to block kainate-induced $^{45}Ca^{+2}$ influx through AMPA receptors in rat cerebellar granule cells.[10] This method successfully identified the standard competitive and noncompetitive antagonists, NBQX and GYKI 52466, and was used exclusively for our primary SAR program. It is important to note that NBQX must compete with kainate for the binding site on the receptor. Thus, potency in this assay is much weaker than its IC_{50} for [^{3}H] AMPA binding displacement might suggest.[11] This assay was adapted to a 96-well format for rapid screening and piriqualone was identified as a lead. This compound had an IC_{50} of 0.50 μM and was found to inhibit AMPA receptor activity in a manner not competitive with agonist concentration. Subsequent experiments showed that the GYKI 52466 and piriqualone shared at least partial overlap at the binding site.[12]

Development of SARs followed from combination of the three ring fragments noted above. Optimization of each ring was conducted while generally maintaining the other two rings constant. In this fashion, a rapid SAR was established. Limited opportunity for substitution was found in the A ring (Table 1). Introduction of small substituents, especially halogen at C-6, gave about a 4-fold enhancement in activity. Since there was little difference between the various C-6 halogens, the 6-fluoro template was arbitrarily selected to explore structural modifications in the B and C rings.

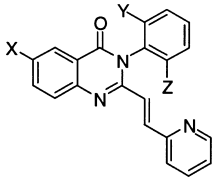
It soon became apparent that an *ortho* substituent in the B ring was essential for good AMPA receptor antagonist activity (Table 2). However, 2,6-disubstitution even with small fluorine atoms reduced potency. Substituents as large as bromine, methyl and trifluoromethyl were

Table 1. A ring SAR



Entry	X	IC_{50} (nM)
1	6-H	460±25
2	6-CH_3	1370±290
3	6-F	248±80
4	6-Cl	124±9
5	7-Cl	>3000
6	8-Cl	>1000
7	6,8-Cl_2	>1000
8	6-Br	162±34
9	8-OCH_3	>1000
10	6,7-$(OCH_3)_2$	>1000

Table 2. B ring SAR



Entry	X	Y	Z	IC_{50} (nM)
1	H	CH_3	H	460±25
11	H	F	H	116±31
12	H	Cl	H	433±65
13	H	Br	H	102±3
14	H	CF_3	H	146±9
15	H	OCH_3	H	1100±600
3	F	CH_3	H	248±80
16	F	F	H	180±30
17	F	Cl	H	96±8
18	F	Br	H	65±0.8
19	H	H	H	>1000
20	H	CH_3	CH_3	>1000
21	H	Cl	Cl	>1000
22	F	F	F	>300

well tolerated but did not lead to significant potency enhancement. However, a methoxy group reduced activity by 10- to 20-fold.

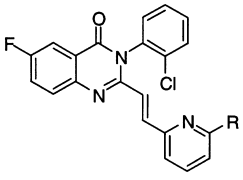
Up to this point, our SAR development in the A and B rings showed very limited opportunity to modify the structure and retain or improve on the activity profile. Equally important, these changes produced compounds that had relatively poor solubility (observed in handling but not quantitatively measured). Therefore, we turned to C ring modifications as we sought a means to optimize SAR and solubility.

Modification of the pyridyl C ring was carried out on the template containing the 6-fluoroquinazolin-4-one A ring and the *ortho* chlorophenyl B ring. Though many changes were tolerated, C-6 proved to be an optimal substitution site (Table 3). Thus a wide range of substituents including neutral and H-bond donors and acceptors generally led to products with sub 100 nM potency. We were particularly attracted to the aminomethyl analogue. Though somewhat less potent (270 nM), it offered a handle to enhance solubility.

Mono- and disubstitution of the aminomethyl group modestly improved potency and yielded several compounds with attractive profiles. In particular, the diethylaminomethyl analogue **38** (CP-392,110) was selected for further profiling. In patch clamp electrophysiology experiments, the compound selectively blocked AMPA receptor mediated inward currents with an IC_{50} of 25 nM. No effect was observed on NMDA receptor mediated currents at concentrations up to 1 μM. However, at 10 μM, a 36% current reduction was observed suggesting a minimum 400-fold separation in potencies at these two glutamate receptors.[13] In addition, no nonglutamate receptor affinity was identified at concentrations up to 1 μM.[14]

In vivo, **38** was very effective at blocking seizures in mice induced by pentalenetetrazol (administered sc) or AMPA (administered icv, $ID_{50} = 3.8$ and 3.6 mg/kg, sc, respectively, Table 4).[15] In anesthetized rat, **38** reduced the population spike amplitude in the CA3 region of the hippocampus (a measure of in vivo synaptic transmission). At a dose of 2 mg/kg iv, 65.9±9.1% blockade was observed.[16]

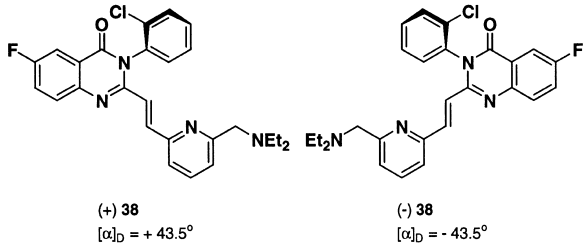
Table 3. C ring SAR



Entry	R	IC_{50}, nM
17	H	96±8
23	CH_3	36±3
24	OCH_3	31±6
25	CHO	97±30
26	CN	244±28
27	$COOCH_3$	400
28	COOH	>1000
29	CH_2OH	58±9
30	CH_2OAc	45±3
31	CH_2OCH_3	42±2
32	$CH_2OCH_2CH_3$	82±3
33	CH_2F	56±13
34	CH_2NH_2	340±70
35	CH_2NHCH_3	103±18
36	$CH_2N(CH_3)_2$	178±31
37	$CH_2NHCH_2CH_3$	81±11
38	$CH_2N(CH_2CH_3)_2$	74±4
39	-Pyrrolidine-	31±4
40	-Piperidine-	>300
41	$CH_2NCH(CH_3)_2$	59±10
42	$CH_2N(CH_3)CH_2CH_2N(CH_3)_2$	150±60

Atropisomer Characterization

Restricted rotation generally due to the presence of bulky substituents, especially in *ortho* substituted biphenyls and related compounds, can result in stable isolable chiral structures known as atropisomers.[17] Perhaps the best known atropisomers are the BINAP and BINOL compounds which have found great utility as catalysts for asymmetric synthetic processes.[18]



Based on previous reports,[19] it was highly likely that the rotational barrier in these compounds would be sufficient to allow isolation of the distinct atropisomers. In combination with the quinazolin-4-one C-2 side chain and the C-4 carbonyl, the *ortho* B ring SAR strongly suggested that only one of the potential atropisomers of our new AMPA receptor antagonists had the appropriate topological profile to fit into a putative binding site. Thus, we investigated whether a single atropisomer would have sufficient thermal stability for isolation. The atropisomers of **38** were readily separated by chiral HPLC.[20] The individual isomers were further characterized by optical rotation and X-ray crystallography

Table 4. Pharmacological profile of **38** and its atropisomers

	38	(+)-**38**	(−)-**38**
Inhibition of AMPA receptor mediated $^{45}Ca^{+2}$ uptake in rat cerebellar neurons	74±4 nM	38±3 nM	>300 nM
Blockade of pentalenetetrazol-induced seizures in mice, sc ID_{50} (95% CI)	3.8 mg/kg (2.1–6.8)	1.5 mg/kg (0.31–3.2)	>10 mg/kg
Blockade of ivc AMPA-induced seizures in mice, sc ID_{50} (95% CI)	3.6 mg/kg (3.2–4.1)	3.1 mg/kg (2.1–4.5)	Not tested
Inverted grid, mice, MED, sc	10.0 mg/kg	5.6 mg/kg	Not tested

and had the absolute configuration illustrated below. To evaluate thermal stability, solutions of (+)-**38** were maintained at various temperatures and monitored by HPLC.[21] At 37 °C, no equilibration was detected over a 7 day period. At higher temperatures, thermal equilibration was observed. For example, at 70 °C, 10% racemization was noted after 90 h. Importantly, no equilibration was seen over 1 week in pH 7.4 phosphate buffer at 37 °C, suggesting good in vivo stability.

Consistent with our previous SAR which taught that a single *ortho* substituent was essential for activity, only one of the atropisomers, (+)-**38**, blocked AMPA receptor activity in the primary assay (Table 4). Likewise, all the anticonvulsant activity resided in this compound. AMPA receptor antagonists had been reported to have CNS depressant side effects. Thus, we further evaluated **38** and its atropisomers for inverted grid performance in mice.[22] Minimum effective doses (MEDs) for behavioral disruption in these experiments were 2- to 4-fold higher than the anticonvulsant ID_{50}s. This separation from side effects was consistent with the previous report.[23]

Finally, poor solubility has been a recurring issue for most previously described classes of AMPA receptor antagonists. It was assumed that the basic C ring side chain would endow this new series with an improved solubility profile. This has proven to be the case. Preparation of (+)-**38** as its highly crystalline mesylate salt provided a product with excellent aqueous solubility (180 mg/mL at pH 4.7). Thus formulation, even for iv administration, should be feasible.

Conclusion

In summary, the quinazolin-4-ones represent a new and highly selective class of AMPA receptor antagonists. These quinazolin-4-ones exist as stable isolable atropisomers and all the antagonist activity resides in a single antipode (*S* isomer in the case of **38**). Within the context of SAR development, introduction of a basic amine side chain on the pyridyl C ring produced an optimal combination of potency and aqueous solubility. The end result of this effort was the identification of (+)-**38** as an ideal probe to explore the pharmacology of the AMPA receptor. Consistent with previous findings, (+)-**38** was a potent anticonvulsant when evaluated against pentalenetetrazol and AMPA induced seizures while retaining a modest safety profile.

Beyond epilepsy, AMPA receptor antagonists may have therapeutic benefit in the treatment of ischemic stroke (both focal and global),[24] CNS trauma,[25] pain,[26] and Parkinson's disease[27] based on activity in various in vivo disease models. However, recently, the selectivity of many of the early prototype AMPA antagonists, particularly relative to the kainate ionotropic glutamate receptors, has been called into question.[28] Thus, the true spectrum of activity of a pure AMPA receptor blocker may be less clear cut than the original in vivo studies might suggest. Therefore, highly selective compounds such as (+)-**38** will likely play a critical role in defining the role of the AMPA receptor in the treatment of neurodegenerative and other diseases of the central nervous system.[29]

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