Structure−Activity Relationships in a Series of 2(1*H*)-Quinolones Bearing Different Acidic Function in the 3-Position: 6,7-Dichloro-2(1*H*)-oxoquinoline-3-phosphonic Acid, a New Potent and Selective AMPA/Kainate Antagonist with Neuroprotective Properties

Patrice Desos, Jean M. Lepagnol, Philippe Morain, Pierre Lestage, and Alex A. Cordi*

Institut de Recherches Servier, 11, rue des Moulineaux, 92150 Suresnes, France

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Recently, we reported the synthesis of 3-(sulfonylamino)-2(1*H*)-quinolones, a new series of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate and *N*-methyl-D-aspartic acid (NMDA)/glycine antagonists. By exploring the structure−activity relationships (SAR) in this series, we were able to identify the 6,7-dinitro derivative **6** as a potent and balanced antagonist at both receptors. Unfortunately, compound **6** was devoid of *in vivo* activity in mice anticonvulsant testing. To overcome this critical limitation, new compounds bearing various acidic moieties at the 3-position of the quinolone skeleton were synthesized and evaluated. The SAR of these new analogues indicated that not all acidic groups are acceptable at the 3-position: A rank order of potency going from carboxylic ≈ phosphonic > tetrazole > mercaptoacetic > hydroxamic ≫ other heterocyclic acids was defined. In addition, the selectivity between the AMPA/kainate and NMDA/glycine sites is dependent on the nature of the substitution (nitro > chloro for AMPA selectivity), its position (5,7- > 6,7-pattern for glycine selectivity), and the distance between the quinolone moiety and the heteroatom bearing the acidic hydrogen (the longer the distance the more AMPA selective the compound). Among these new AMPA antagonists, we have identified 6,7-dichloro-2(1*H*)-oxoquinoline-3-phosphonic acid (**24c**) as a water soluble and selective compound endowed with an appealing pharmacological profile. Compared with the reference AMPA antagonist NBQX, the phosphonic acid **24c** is much less potent *in vitro* but almost equipotent *in vivo* in the audiogenic seizures model after intraperitoneal administration. Moreover, unlike NBQX, compound **24c** is also active after oral administration. In the gerbil global ischemia model, compound **24c** shows a neuroprotective effect at 10 mg/kg/ip, equivalent to the reference NBQX.

Introduction

L-Glutamic acid and L-aspartic acid are the major excitatory amino acid (EAA) neurotransmitters in the central nervous system.[1] Different receptors for glutamic acid have been characterized;[2,3] while some were shown to be associated with cationic channels, others were found to act through G protein-coupled secondary messenger systems. Pharmacologically, the more well-defined receptors are the ionotropic channels activated by either *N*-methyl-D-aspartic acid (NMDA) or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainic acid and the metabotropic receptors activated by *trans*-1-aminocyclopentane-1,3-carboxylic acid (ACPD).

Since 1982,[4] numerous competitive and noncompetitive NMDA antagonists have been discovered, and some were brought into the clinic to assess their potential as therapeutic agents in the acute treatment of stroke and head trauma. Unfortunately, these compounds appeared to be liable to strong psychotomimetic side effects which may impede their development.[5,6]

Therefore, there has been considerable, recent interest in selective antagonists of the AMPA receptor because of their relevance as therapeutic agents for stroke and other ischemic conditions where excessive EAA release has excitotoxic action on neurons.[7] The pharmacology of AMPA/kainate antagonists and their role in cerebral ischemia has been reviewed.[8]

The most potent selective AMPA/kainate antagonists belong to the quinoxalinedione class of compounds, such as NBQX (**1**), the first selective AMPA/kainate antagonist as reported by Sheardown,[9] and YM90K (**2**), disclosed more recently by Yamanouchi[10] (Figure 1). The structurally close isatine oxime NS 229[11] (**3**) is described as the first compound showing AMPA antagonism after oral administration. Recently, scientists at Merck disclosed a series of 3-nitro-3,4-dihydro-2(1*H*)-quinolones **4** as potent combined AMPA/kainate and NMDA/glycine antagonists,[12] and a group at Parke-Davis claimed *N*-sulfonyl derivatives of 3,4-dihydro-3-oxoquinoxalinecarboxylate **5** as antagonists at the NMDA-associated glycine site and AMPA receptor.[13]

Recently, we reported[14] the synthesis of 3-(sulfonylamino)-2(1*H*)-quinolones, a new series of AMPA/kainate and NMDA/glycine antagonists. In the respective publication, we speculated that the hydroxyl of one of the tautomeric forms of the quinoxalinedione could be mimicked by an acidic [(trifluoromethyl)sulfonyl]amino function at the 3-position of a quinolone. By exploring the structure−activity relationships (SAR) in this series, we were able to identify 6,7-dinitro derivative **6** (S 16678) as a potent and balanced antagonist at the two receptor types. However, the compound was devoid of any *in vivo* activity in a convulsion model in mice.

At that time, the structures of the known, selective AMPA antagonists **1**−**3** (Figure 1) were rather homogeneous, so the construction of a meaningful receptor model or the generation of predictive SAR could not be

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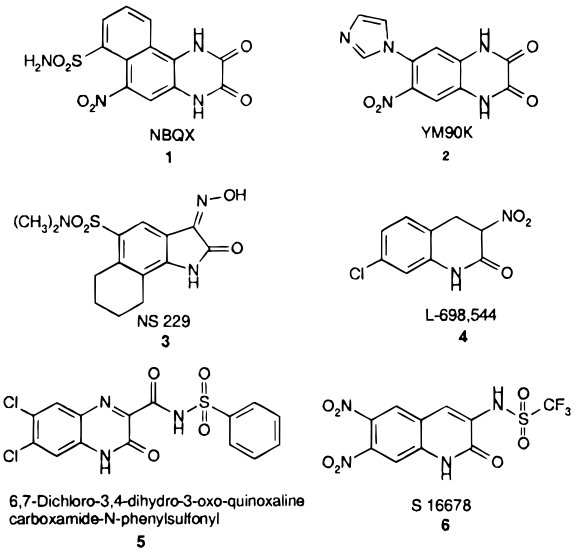
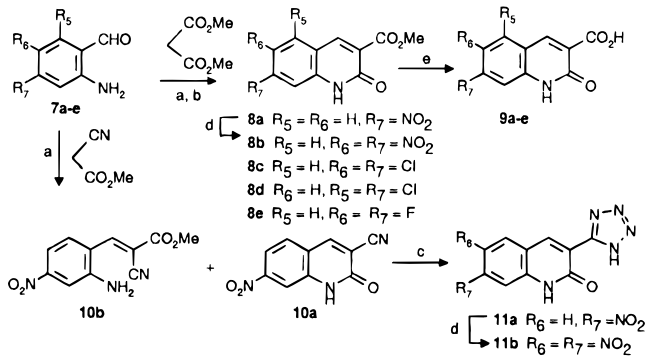


Figure 1.

Scheme 1[a]



[a] Reagents: (a) MeONa, MeOH; (b) 1 N HCl; (c) *n*-Bu_3SnN_3, toluene; (d) HNO_3, H_2SO_4; (e) 5 N HCl or 3 N NaOH.

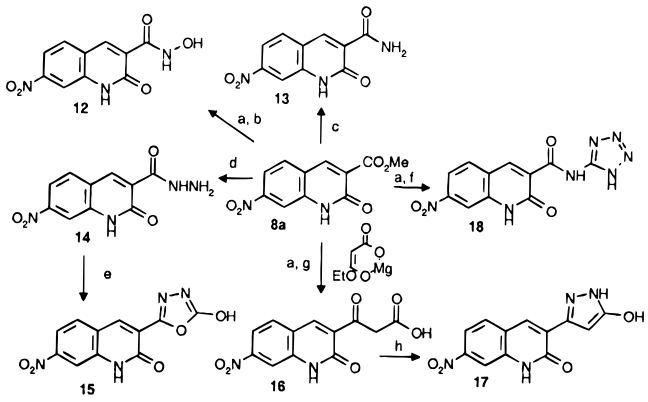
considered. Meanwhile the critical limitation of lack of *in vivo* activity needed to be overcome, and it was suspected that the physicochemical properties (solubility and lipophilicity) of the compounds were more to blame for this failure rather than their intrinsic activity at the level of the AMPA receptor. Therefore the hypothesis stated above was explored through the synthesis and biological evaluation of compounds bearing various acidic groups at the 3-position of the quinolone skeleton aiming at the discovery of compounds endowed with improved physicochemical properties. The SAR of these new analogs of **6** are described below.

Synthesis

The key intermediate carboxylic esters **8a**−**e** were readily prepared by the condensation of dimethyl malonate with variously substituted 2-aminobenzaldehydes **7a**−**e** (Scheme 1). The reaction proceeds smoothly at room temperature in methanol and in the presence of 2 equiv of sodium methoxide followed by acidic workup. Acid or base hydrolysis of **8a**−**e** gave carboxylic acids **9a**−**e**. Dinitro compound **8b** was obtained by nitration of **8a** at 80 °C in a mixture of HNO_3/H_2SO_4.

Similarly, the condensation of 2-aminobenzaldehyde **7a** with methyl cyanoacetate provided the nitrile **10a** plus a small quantity (16% by ^{1}H NMR) of the methyl (*E*)-2-amino-4-nitro-α-cyanocinamic ester isomeric intermediate **10b** which is unable to cyclize. The nitrile

Scheme 2[a]



[a] Reagents: (a) 3 N NaOH; (b) *N*,*N*′-carbonyldiimidazole (CDI), NH_2OH HCl, THF/DMF; (c) NH_3, MeOH/DMF; (d) NH_2NH_2, MeOH; (e) CDI, Et_3N, THF/DMF; (f) CDI, 5-aminotetrazole, THF; (g) CDI, magnesium enolate monoethyl ester malonic acid, THF/DMF; (h) NH_2NH_2, MeOH.

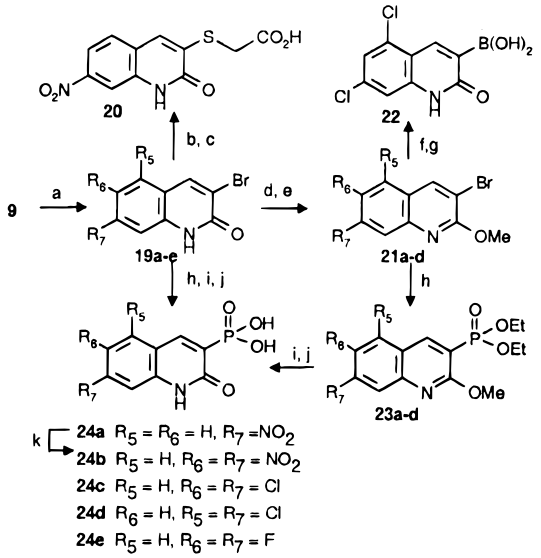
10a was purified by crystallization in DMF and transformed into the tetrazole **11a** through the action of tri-*n*-butyltin azide in refluxing toluene. The dinitro tetrazole **11b** was obtained through nitration of **11a** at 50 °C in a mixture of HNO_3/H_2SO_4.

Additional acidic compounds were prepared from ester **8a** and acid **9a** (Scheme 2). For example, the ester was substituted with ammonia and hydrazine to give the amide **13** and the hydrazide **14**, respectively. Condensation of **14** with *N*,*N*′-carbonyldiimidazole (CDI) afforded the hydroxyoxadiazole **15** in reasonable yield. In addition, the acid **9a** when activated by CDI in refluxing THF reacted with a variety of N and C nucleophiles such as hydroxylamine, 5-aminotetrazole, and the magnesium enolate of malonic acid monoethyl ester,[15] giving hydroxamate **12**, amidotetrazole **18**, and β-keto acid **16**, respectively. The latter compound afforded the pyrazole **17** by condensation with hydrazine.

Another key intermediate was generated from the acids **9a**−**e**. An unfruitful attempt of aromatic bromination of **9a** by bromine in pyridine led to the recovery of the 3-bromo derivative **19a** in essentially quantitative yield (Scheme 3). As shown in Scheme 4, the mechanism of this reaction could proceed through the addition of bromonium to the 3,4-double bond followed by deprotonation and decarboxylation−aromatization. Alternatively, as suggested by one referee, a possibility could be the bromination of the 3,4-double bond followed by a decarboxylation due to the presence of the β-carbonyl group. The double bond is then reformed by a base-catalyzed dehydrobromination which would proceed to give the 3-bromo product because of the greater acidity of the proton at the 3-position.

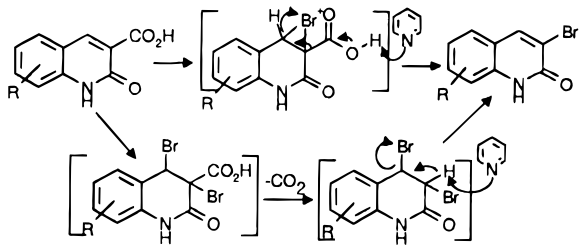
To our knowledge, there is no literature precedent of such halodecarboxylation reactions on a quinolone nucleus. Moreover, there are only a few literature reports on the synthesis of 3-bromo-2(1*H*)-quinolones. Earlier approaches started from the little accessible 3-unsubstituted-2(1*H*)-quinolone and proceeded by bromination of the heterocycle leading to a mixture of 6- and 3-bromo derivatives in moderate yields.[16−18] When the bromination is carried out on the quinoline *N*-oxide, prior to its rearrangement to 2(1*H*)-quinolone, again nonselective 6- and 3-bromination is observed,[19] and moreover, the subsequent transformation into the 2(1*H*)-

Scheme 3[a]



[a] Reagents: (a) Br_2, pyridine; (b) ethyl 2-mercaptoacetate, K_2CO_3, acetone; (c) LiOH, THF/H_2O; (d) $POCl_3$; (e) MeONa, MeOH; (f) $B(OEt)_3$, BuLi, THF; (g) HCl; (h) $HPO(OEt)_2$, $Pd[PPh_3]_4$, Et_3N, THF; (i) TMSBr, CH_3CN; (j) 3 N HCl, (k) HNO_3, H_2SO_4.

Scheme 4



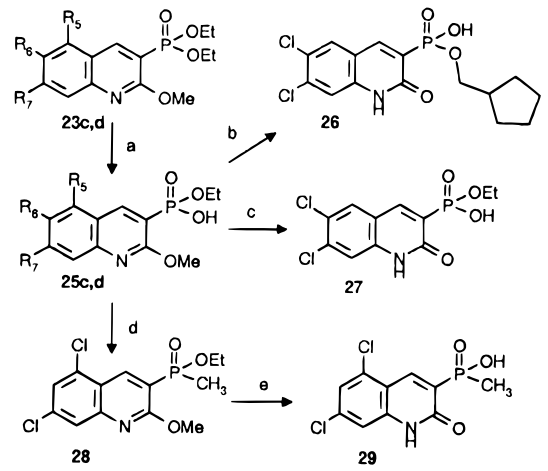
quinolone, mediated by various acylation agents, results with many side products.[20]

In our laboratory, we took advantage of this simple and unexpected accessibility of 3-bromo-2(1*H*)-quinolones to extend our SAR, introducing various acidic groups at the 3-position. Initially we used the bromine atom in position 3 as leaving group, allowing the introduction of a carboxylic side chain by direct nucleophilic substitution with the anion of ethyl 2-mercaptoacetate, providing **20** after saponification. Later, we used this functionality as a nucleophile via its metalation with BuLi or Pd(0). Initial attempts to generate *in situ* the lithio intermediate by addition of BuLi to a solution of **21d**, followed by addition of triethyl borate, failed to give the boronate **22** but led exclusively to the 3-dehalogenated 2(1*H*)-quinolone, thus indicating the instability of the organometallic species which must be quenched by proton abstraction from the solvent (THF). On the other hand, when BuLi was added to a THF solution of **21d** concomitant with triethyl borate, the boronate **22** was obtained with moderate yield after acid workup.

In contrast, palladium(0)-catalyzed coupling reaction of the 3-bromo derivatives with diethyl phosphite in the presence of triethylamine[21] (Heck conditions) allowed smooth preparation of the corresponding phosphonates **23c**,**d**. The latter were hydrolyzed in high yields by successive treatment with trimethylsilyl bromide and hydrochloric acid.

The phosphonate esters **23c**,**d** were subjected to selective, basic hydrolysis of one ethyl ester (Scheme 5),

Scheme 5[a]



[a] Reagents: (a) 1 N NaOH; (b) (i) PPh_3, diisopropyl azodicarboxylate (DIAD), cyclopentanemethanol, THF, (ii) TMSBr, (iii) 3 N HCl; (c) 3 N HCl; (d) (i) oxalyl chloride, DMF (catalytic), CH_2Cl_2, (ii) MeMgCl, THF; (e) (i) TMSBr, (ii) 3 N HCl.

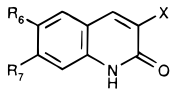
thus providing the monoesters **25c**,**d**. The lactams could be specifically deprotected by acid treatment affording **27**. In addition, the protected monoester **25c** reacted with cyclopentanemethanol under Mistunobu conditions[22] leading to the monocyclopentane methyl ester **26** after hydrolysis. Reaction of **25d** with oxalyl chloride in dichloromethane with DMF catalysis[23] gave the arylphosphonyl chloride intermediate which reacted with methylmagnesium chloride in THF leading to the corresponding arylmethylphosphinic acid **29**.

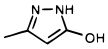
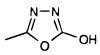
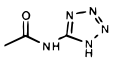
Results and Discussion

The electrophysiological data on kainate- and NMDA/glycine-induced currents of 3-substituted-2(1*H*)-quinolones are presented in Tables 1 and 2. Our previous investigations on quinoxalinedione derivatives[24] had shown that for *Xenopus* oocytes injected with rat cerebral cortex mRNA, the antagonism of AMPA and kainate responses is fairly parallel, indicating that both agonists activate the same non-NMDA type receptor in this preparation. Therefore, we used kainate-induced current in oocytes and membrane [^{3}H]AMPA binding as an initial evaluation of the AMPA/kainate antagonist potency of our compounds.

In the tables, the compounds have been organized so as to keep the substitution pattern constant (nitro vs chloro) while varying the 3-acidic functions. Initially considering those nine compounds bearing only a 7-nitro substituent, a rank order of activity regarding the kainate challenge could be deduced wherein **9a** $\approx$ **24a** > **11a** > **20** > **12** $\gg$ **17**, **18**, **15**, **13**. The equiactivity of **9a** and **24a** indicates that when attached to the 2(1*H*)-quinolone skeleton, carboxylic and phosphonic acid functions are bioisosteric with regards to the activity at the AMPA/kainate receptor and differ by 1 order of magnitude with regards to their efficacy at the level of the NMDA/glycine receptor, the phosphonate **24a** being more AMPA/kainate selective than the carboxylate **9a**. Tetrazole **11a**, hydroxamic acid **12**, and mercaptoacetic acid **20** appear to be marginally less active, while the other acidic heterocycles and the nonacidic amide **13** are devoid of any activity. The relative potency of the mercaptoacetic acid **20** as an antagonist at the AMPA/kainate site is interesting because in this structure the

Table 1. Influence of the Acidic Isosters in the 3-Position on *in Vitro* Pharmacological Data of the Nitro-Substituted 2(1*H*)-Quinolones[a]



compd	R_6	R_7	X	*Xenopus oocytes* IC_{50} (μM)		Selectivity NMDA/Gly Kainate
				Kainate	NMDA/Gly	
9a	H	NO_2	CO_2H	1.3	6.5	5
11a	H	NO_2		3	> 16	> 5
12	H	NO_2	CONHOH	10	> 100	> 10
24a	H	NO_2	PO_3H_2	1.4	57	41
17	H	NO_2		> 30	> 100	
15	H	NO_2		> 100	> 100	
13	H	NO_2	$CONH_2$	> 300	> 300	
18	H	NO_2		100	> 16	> 0.16
20	H	NO_2	SCH_2CO_2H	5	> 100	> 20
6	NO_2	NO_2	$NHSO_2CF_3$	0.16	0.16	1
9b	NO_2	NO_2	CO_2H	0.3	0.57	1.9
11b	NO_2	NO_2		0.6	20	33
24b	NO_2	NO_2	PO_3H_2	0.15	51.5	343
NBQX				0.09	255	2833
YM90K				0.3	30.5	102

[a] In the *Xenopus* oocyte assays, IC_{50} values were derived from inhibition curves constructed with data from four to eight experiments at each drug concentration. In all cases, enough experiments ($4 \leq n \leq 7$) were performed to calculate the corresponding standard deviation which never exceeded 20% of the mean values.

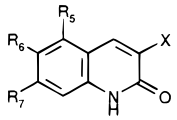
acidic function is substantially removed from the quinolone, indicating that the indispensable acidic function in that part of the molecule is interacting with as hypothetical cationic center on this receptor, which can not be very demanding in terms of distances.

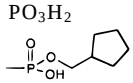
An additional nitro group in the 6-position, leading to compounds **24b** > **9b** > **11b**, confirms the validity of the phosphonic and carboxylic acid replacement hypothesis, and the efficacy of the three most potent compounds is increased as expected[25] from 4- to 10-fold.

When the same pattern of 6,7-disubstitution is applied with chloro substituents in place of nitro groups (Table 2), the same rank order **24c** > **9c** is obtained but the activity is lowered by more than 1 order of magnitude. Fluoro substitution (analogue **24e**) decreased the potency by 2 orders of magnitude.

In addition, the isomeric 5,7-dichloro pattern (Table 2) increases the affinity for the NMDA/glycine site while keeping the affinity for the AMPA/kainate receptor (see compounds **9d** and **24d**) more or less constant. By considering the ratio of IC_{50} between the kainate and NMDA/glycine assays, selectivity of the compounds can be defined (Tables 1 and 2). The phosphonic acid derivatives **24a**−**d** are more AMPA/kainate selective than their carboxylic acid counterparts **9a**−**d**. This

Table 2. Influence of the Acidic Isosters in the 3-Position on *in Vitro* Pharmacological Data of the Halogeno-Substituted 2(1*H*)-Quinolones[a]



compd	R_5	R_6	R_7	X	*Xenopus* oocytes IC_{50} (μM)		selectivity NMDA/Gly−kainate
					kainate	NMDA/Gly	
9c	H	Cl	Cl	CO_2H	10	0.81	0.08
24c	H	Cl	Cl	PO_3H_2	2.1	83.5	39.8
26	H	Cl	Cl		15	74	5
27	H	Cl	Cl	PO_3HEt	15	40	2,6
24e	H	F	F	PO_3H_2	65	111	1.71
9d	Cl	H	Cl	CO_2H	8	0.65	0.08
22	Cl	H	Cl	$B(OH)_2$	>100	>300	
24d	Cl	H	Cl	PO_3H_2	4	30	7.5
29	Cl	H	Cl	PO_2MeH	>100	51	<0.5

[a] In the *Xenopus* oocyte assays, IC_{50} values were derived from inhibition curves constructed with data from four to eight experiments at each drug concentration. In all cases, enough experiments ($4 \leq n \leq 7$) were performed to calculate the corresponding standard deviation which never exceeded 20% of the mean values.

could indicate that the steric hindrance around position 3 of the 2(1*H*)-quinolone is less well defined in the AMPA/kainate receptor than at the glycine site of the NMDA receptor. Moreover, the replacement of AMPA-favoring nitro substituents[25] by glycine-favoring chloro substituents[26] improves the IC_{50} of the carboxylic acid derivatives for the glycine site, while it has no effect, or the opposite effect, on the phosphonic acid-bearing compounds. This selectivity trend could be interpreted as reflecting the existence, at the level of the AMPA receptor, of a positively charged environment close to the positions 6 and 7 of the quinolone which tolerate sterically crowded polar groups such as NO_2, CN, or 1-imidazolyl.

Another way to consider the selectivity results is by analyzing the distance between the quinolone moiety and the heteroatom bearing the acidic hydrogen. In the 6,7-dinitro-substituted entities, the compound **6** (3-sulfonylamino) which has the shorter distance is not selective at all, while **24b** (3-phosphonic acid) which has the longest distance is the more AMPA/kainate selective compound. Thus, the increasing order of selectivity **6** < **9b** < **11b** < **24b** could be correlated with the distance increase between carbon 3 of the quinolone and the heteroatom bearing the negative charge in the conjugated base. These distances measured on Dreiding models gave the order: 15 < 24 < 25 < 25.5 nm, respectively. This trend is confirmed in the 7-mononitro-substituted series with the mercaptoacetic acid **20** as well as with the hydroxamic acid **12**, where the proton attached to the oxygen is considered the ionizable one.[27]

Attempts to expand these SARs to include other acidic groups such as the boronic (**22**) and phosphinic (**29**) acids led to inactive compounds. Phosphonic acid monoesters are expected to be better mimics of carboxylic acid functions as both are monobasic acids, while the phosphonic acids are dibasic acids. Hence, the synthesis of the monoesters of **24c** was undertaken, but the resulting products **26** and **27**, where the overall lipo-

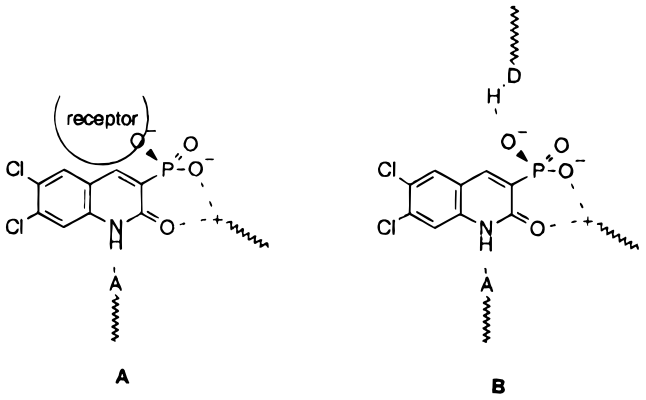


Figure 2. Tentative fitting of compound **24c** into the receptor models proposed in ref 12 for the AMPA (A) and NMDA/glycine (B) receptors, respectively.

philicity of the molecule was tentatively increased, were only marginaly active.

In the course of this work, a literature report was published[28] which described a close analogue of **24c**, 7-chloro-2(1*H*)-oxoquinoline-4-phosphonic acid, where the phosphonic acid function has been shifted from the 3- to the 4-position with a complete loss of activity. In the same publication, the homologue 5,7-dichloro-2(1*H*)-oxoquinoline-4-methylenephosphonic acid is described as a mild but selective AMPA/kainate antagonist. Overall, this result is difficult to reconciliate with the model of the AMPA receptor published recently,[12] where bulk intolerance at the 4-position is claimed to endow glycine selectivity to 4-substituted compounds. In contrast, **24c** would fit perfectly well in this proposed model although it will fit equally well in the proposed model of the NMDA/glycine receptor (Figure 2), therefore lacking the discriminative differences needed to explain the selectivity of the compound.

In addition to preliminary biological evaluations, our experience gained in the course of the chemical synthesis indicated that the phosphonic acid derivatives were surprisingly endowed with the better physicochemical properties. Indeed, in contrast to the very insoluble carboxylic acid derivatives **9a**−**d** and tetrazole derivatives **11a**,**b**, which could only be purified by recrystallization in DMF, the phosphonic acid derivatives **24a**−**d** were easily purified by recrystallization in ethanol or water. Moreover, the *in vitro* evaluation was dramatically facilitated through the ease of dissolving these phosphonate derivatives in aqueous solutions (disodium salt: 13 mg/mL), in contrast to the situations prevailing with the carboxylic acids (monosodium salt: <1 mg/mL) and, above all, with the tetrazoles, where sophisticated dissolution protocols employing cosolvents were necessary. These qualities are also in contrast with the solubility properties of the reference compound NBQX. Indeed, the *in vivo* evaluations and even the clinical experiments have been impeded by the poor water solubility and the bright yellow color which prevents blinding of the studies.[29]

The results obtained in the oocyte electrophysiology assay with **24c**,**d** were corroborated by binding experiments (Table 3) which confirms and expanded the difference in selectivity between the two compounds. An AMPA selective antagonist was at hand with **24c** and a balanced AMPA/kainate−NMDA/glycine antagonist with **24d**.

Compared with the reference AMPA antagonist NBQX, the phosphonic acid **24c** is much less potent *in vitro* but nearly equipotent *in vivo* in the audiogenic seizures model after intraperitoneal administration. Moreover, unlike NBQX, compound **24c** is also active when administered orally. Surprisingly, its isomer **24d**, resulting only from the shift of the 6-chloro substitution to the 5-position, is devoid of any *in vivo* activity. There is no explanation for this discrepancy, and one can only speculate that this may be due to either an unexpected better bioavailability of **24c** compared to **24d** or the above-mentioned difference in selectivity of AMPA versus glycine manifested by these two compounds.

In the gerbil global ischemia model, compound **24c** shows a neuroprotective effect at 10 mg/kg ip equivalent to the reference NBQX, and at 30 mg/kg ip a complete hippocampal neuronal protective effect was observed.

In conclusion, among these new AMPA antagonists, we have identified **24c** (S 17625) as a selective and water soluble compound which is endowed with an impressive pharmacological profile. More *in vivo* experiments on ischemia models are now in progress to validate the use of S 17625 as a potential therapeutic agent in the acute treatment of stroke. Moreover, at neuroprotective doses, the available AMPA/kainate receptor antagonists induce a number of side effects (depression of central glucose utilization, potentiation of anesthesia, ataxia, and/or nephrotoxicity) that may limit their clinical usefulness. Hence the side effect profile of **24c** will have to be clearly established so as to fully define its therapeutic potential, safety margin, and risk/benefit ratio.

Experimental Section

Biology. *Xenopus* Oocyte Experiments. Poly(A^+) messenger RNA isolated from rat cerebral cortex was injected in *Xenopus* oocytes,[30] and the oocytes were incubated for 2−3 days at 18 °C to allow expression. They were then stored at 6−8 °C until use (typically 1−2 weeks). Kainate-induced currents were recorded in "OR2" medium[31] of composition (in mM): NaCl, 82.5; KCl, 2.5; $CaCl_2$, 1; $MgCl_2$, 1; NaH_2PO_4, 1; HEPES, 5; pH = 7.4. For the recording of NMDA/glycine currents, $MgCl_2$ was omitted from the medium and the concentration of $CaCl_2$ was raised to 2 mM. Experiments were performed by means of a two-electrode voltage clamp using an Axoclamp 2A amplifier. Glass microelectrodes (1−3 MΩ) were filled with 3 M KCl. The holding potential was adjusted to −60 mV.

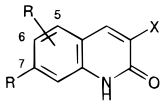
For the initial evaluation of the inhibitory potency of the compounds, the agonists were applied for 30 s at the following concentrations: kainate (100 μM) and glycine (3 μM)/NMDA (30 μM). The compounds were bath applied for 45 s before and 30 s after the application of agonists. Agonist responses were evaluated at the peak of the inward current. The IC_{50} values were calculated from inhibition curves using the relationship:

$$I = I_{max}/(1 + [\text{antagonist}]/IC_{50})^{n_H}$$

(where n_H represents the Hill slope coefficient) by a nonlinear curve-fitting program.

Binding Experiments. Membrane Preparation. Rat cerebral cortices were homogenized in ice cold 0.32 M sucrose buffer (AMPA binding) or 50 mM Tris-acetate (glycine binding) and centrifuged at 1000*g* for 10 min. The resulting supernatant was centrifuged at 37000*g* for 25 min or at 40000*g* for 15 min, respectively. For AMPA binding the pellet was then washed in 30 mM Tris-HCl containing 2.5 mM $CaCl_2$, centrifuged as described above, lysed in distilled water, and centrifuged again. The resulting pellet was then incubated with

Table 3. Comparison of *in Vitro* and *in Vivo* Pharmacological Data of Selected 2(1*H*)-Quinolones and NBQX[a]



compd	R	X	rat cortical membrane binding, IC_{50} (μM) [^{3}H]AMPA	[^{3}H]Gly	audiogenic seizures in DBA/2 mice, ED_{50} (mg/kg) ip	po	transient global ischemia in gerbil protection (%) 10 mg/kg ip ($n = 10$)	30 mg/kg ip ($n = 6$)
6	6,7-di-NO_2	$NHSO_2CF_3$	0.09	1.38	>50	nt	nt	nt
9b	6,7-di-NO_2	CO_2H	0.15	1.8	>50	nt	nt	nt
24d	5,7-di-Cl	PO_3H_2	3.5	5.1	>50	nt	nt	nt
24c	6,7-di-Cl	PO_3H_2	0.9	111	19 (13−26)	45 (21−97)	40	100
NBQX			0.06	>500	8 (4−17)	na	40	50

[a] In the binding assays, the IC_{50} values were determined from inhibition curves fitting five to six points, each point representing the mean value of duplicates. Enough experiments ($n \geq 5$) were conducted to calculate the corresponding standard deviations which were in the ±8% range of the mean values. In the DBA/2 mice audiogenic seizure assay, the ED_{50}s were determined from dose−effect curves fitting four points (10 mice/dose). Values in parentheses are 95% confidence intervals. In the gerbil transient forebrain ischemia study, the protection index was determined through comparison of the mean injury score of treated animals ($n = 10$) with the score, measured in the same experiment, of sham-operated gerbils ($n = 5$, score = 0) and untreated ischemic gerbils ($n = 10$, score = 4), respectively. nt = not tested. na = not active.

Triton TX-100 for 30 min at 25 °C, centrifuged, and rinsed twice with Tris buffer and then stored at −80 °C. For glycine binding, the pellet was washed three times with Tris-acetate buffer, suspended in 0.32 M sucrose buffer, and frozen at −80 °C until further use.

Binding Assay.[32] The thawed membrane suspension (200 μL) was incubated in the presence of 25 μL of the ligand [^{3}H]-AMPA (4 °C, 30 min, 20 nM) or [^{3}H]glycine (4 °C, 60 min, 50 nM) and 25 μL of a known solution of the investigated compound. Nonspecific binding was defined in the presence of 100 μM quisqualic acid or 100 μM glycine. The specific binding was assayed over the range 0.1−300 nM in the absence (control) or presence of the drug to be tested. The membranes were collected by filtration using a Skatron cell harvester. Data were analyzed using Lundon software.

Audiogenic Seizures in DBA/2 Mice. Audiogenic seizures were performed on 22−25 day old DBA/2 mice according to literature procedures.[33,34] Compounds were administered, to groups of 10 mice/dose, intraperitoneally (ip) 30 min before or orally (po) 1 h before an auditory stimulation (14 kHz, 100 dB, 60 s) generated by an amplifier (Brueel and Kjaer 2706) delivered through loud speakers in a sound chamber. Mice were placed singly in the sound chamber and observed before, during, and after the auditory simulation. The criterion for a positive response was the occurrence of a running response within 20 s of the auditory stimulation. Doses which protected 50% of animals from clonic seizure (ED_{50} value with 95% confidence limits) were calculated by the method of Lichtfield and Wilcoxon.[35]

Transient Forebrain Ischemia in Gerbils. Transient complete forebrain ischemia was produced in adult Mongolian gerbils (60−80 g) anesthetized with 2% isoflurane according to the method of Kirino.[36] Cerebral ischemia was induced by bilateral occlusion of the carotid arteries for 5 min. Before and during ischemia, body temperature was monitored and maintained at an average of 37 °C using a rectal probe and thermostatically controlled heating blanket. After ischemia, temperature control was accomplished with the aid of a heating lamp and kept at around 37 °C until thermal homeostasis was restored. After 4 days, the animals were killed and the brain was removed and fixed. Paraffined coronal sections (7 μm) were cut at the hippocampal level and stained with luxol fast blue. The CA_1 subfield was assessed for neuronal death on a scale ranging from 0 for a normal hippocampus to 4 for an almost total loss of neuronal cells (>90%). This evaluation was carried out by two independent technicians who were blind to treatment given to the specific sample (sham, control, or drug treated). Compounds were administered ip at doses of 10 and 30 mg/kg at 60 min before and 30 and 90 min after the onset of occlusion. In each experiment, controls ($n = 10$) received the compound vehicle only following the same time schedule.

Chemistry. Reagents were commercially available and of synthetic grade. ^{1}H NMR spectra were recorded on Bruker 200 or 400 MHz spectrometers and are given in ppm relative to TMS. Infrared spectra were recorded on a Bruker Fourier transform spectrometer as Nujol emulsion. All new substances were monospot by TLC and exhibited spectroscopic data consistent with the assigned structures. Elemental analyses (C, H, N) were performed on a Carlo Erba 1108 instrument and agree with the calculated values within the ±0.4% range. Melting points were obtained on a Reichert hot stage microscope and are uncorrected. Silica gel 60, Merck 230−400 mesh, was used for both flash and medium pressure chromatography. TLC were performed on precoated 5 × 10 cm, Merck silica gel 60 F254 plates (layer thickness 0.25 mm).

7-Nitro-2(1*H*)-oxoquinoline-3-carboxylic Acid Methyl Ester (8a). To a solution of 4-nitro-2-aminobenzaldehyde (**7a**) (500 mg, 3.01 mmol) and dimethyl malonate (0.51 mL, 4.5 mmol) in methanol (50 mL) was added a solution of 5.25 M sodium methoxide in methanol (2.6 mL, 11.37 mmol). The mixture was stirred at room temperature overnight. The yellow precipitate was filtered, washed with methanol, and stirred in 1 N HCl (75 mL). The white solid was filtered and washed with ether to yield **8a** (390 mg, 49%): mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 12.4−12.5 (bs, 1H), 8.65 (s, 1H), 8.1 (d, 1H), 8.05 (d, 1H), 8.0 (dd, 1H), 3.85 (s, 3H). Anal. ($C_{11}H_8N_2O_5$) C, H, N.

6,7-Dinitro-2(1*H*)-oxoquinoline-3-carboxylic Acid Methyl Ester (8b). To a solution of the above compound (330 mg, 1.33 mmol) in 96% H_2SO_4 (0.5 mL) was added 87% HNO_3 (0.5 mL) dropwise, and the reaction mixture was stirred at 80 °C for 4 h. The mixture was cooled to 0 °C, ice added, and the precipitate filtered, washed with water, and dried under vacuum to yield **8b** as a yellow solid (300 mg, 77%): mp >260 °C; ^{1}H NMR (DMSO-*d6*) δ 12.9 (bs, 1H), 8.9 (s, 1H), 8.7 (s, 1H), 7.8 (s, 1H), 3.85 (s, 3H).

6,7-Dinitro-2(1*H*)-oxoquinoline-3-carboxylic Acid (9b). A suspension of ester **8b** (200 mg, 0.68 mmol) was refluxed in 5 N HCl (20 mL) for 4 h. The reaction was cooled to room temperature, and the solid was filtered, washed with water, and crystallized from DMF to yield **9b** as a yellow solid (90 mg, 47%): mp 298 °C; ^{1}H NMR (DMSO-d_6) δ 13 (bs, 2H), 9.0 (s, 1H), 8.9 (s, 1H), 7.9 (s, 1H). Anal. ($C_{10}H_5N_3O_7$) C, H, N.

7-Nitro-2(1*H*)-oxoquinoline-3-carboxylic Acid (9a). A suspension of ester **8a** (2.0 g, 8.05 mmol) in 5 N HCl (40 mL) was stirred at reflux for 6 h. The precipitate was filtered, washed with water, and recrystallized from DMF to yield **9a** (1.83 g, 97%): mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 12.5−15.0 (bs, 2H), 9.0 (s, 1H), 8.25 (d, 1H), 8.20 (d, 1H), 8.1 (dd, 1H). Anal. ($C_{10}H_6N_2O_5$) C, H, N.

6,7-Dichloro-2(1*H*)-oxoquinoline-3-carboxylic Acid (9c). **9c** was obtained from the condensation of 4,5-dichloro-2-aminobenzaldehyde[14] and dimethyl malonate as described for

8a, followed by hydrolysis as described for **9a**, and recrystallized from DMF: 89% overall yield; mp 230 °C; ^{1}H NMR (DMSO-d_6) δ 12.0−16.0 (bs, 2H), 8.9 (s, 1H), 8.4 (s, 1H), 7.6 (s, 1H). Anal. ($C_{10}H_5Cl_2NO_3$) C, H, N, Cl.

5,7-Dichloro-2(1*H*)-oxoquinoline-3-carboxylic Acid (9d). **9d** was obtained from the condensation of 4,6-dichloro-2-aminobenzaldehyde[14] and dimethyl malonate as described for **8a**, followed by hydrolysis as described for **9a**, and recrystallized from DMF: 78% overall yield; mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 12.0−15.5 (bs, 2H), 8.8 (s, 1H), 7.7 (d, 1H), 7.45 (d, 1H). Anal. ($C_{10}H_5Cl_2NO_3$) C, H, N, Cl.

6,7-Difluoro-2(1*H*)-oxoquinoline-3-carboxylic Acid (9e). **9e** was obtained from the condensation of 4,5-difluoro-2-aminobenzaldehyde[37] and dimethyl malonate as described for **8a**, followed by hydrolysis as described for **9a**: 31% overall yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.0−16.0 (bs, 2H), 8.95 (s, 1H), 8.2 (dd, 1H), 7.4 (dd, 1H).

3-Cyano-7-nitro-2(1*H*)-quinolone (10a). To a solution of 4-nitro-2-aminobenzaldehyde (**7a**) (1.0 g, 6.0 mmol) in methanol (50 mL) were added methyl cyanoacetate (0.634 mL, 7.2 mmol) and a solution of 5.25 M sodium methoxide in methanol (1.64 mL, 8.61 mmol). The mixture was stirred at room temperature overnight. The yellow precipitate was collected and washed with methanol before being stirred in 1 N HCl (75 mL). The solid was filtered and recrystallized from DMF to yield **10a** (750 mg, 58%): mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 12.0−14 (bs, 1H), 8.95 (s, 1H), 7.9−8.15 (m, 3H). Anal. ($C_{10}H_5N_3O_3$) C, H, N.

7-Nitro-3(1*H*-tetrazol-5-yl)-2(1*H*)-quinolone (11a). Tri-*n*-butyltin azide (1.53 g, 4.64 mmol), 3-cyano-7-nitro-2(1*H*)-quinolone (**10a**) (500 mg, 2.32 mmol), and toluene (50 mL) were combined and refluxed for 12 h. The solvent was removed in vacuo, and the residue was stirred in 1 N NaOH for 30 min. The medium was acidified with 4 N HCl. The solid which precipitated was collected and after crystallization in DMF gave 180 mg (30%) of **11a**: mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 11.5−14 (bs, 1H), 9.1 (s, 1H), 8.25 (d, 1H), 8.2 (bs, 1H). Anal. ($C_{10}H_6N_6O_3$) C, H, N.

6,7-Dinitro-3-(*N*-(1*H*)-tetrazol-5-yl)-2(1*H*)-quinolone (11b). The above compound (1 g, 3.87 mmol) was stirred at 50 °C for 1.5 h in a mixture of 96% H_2SO_4 (2 mL) and 87% HNO_3 (2 mL). The reaction mixture was cooled to 0 °C, ice added, and the precipitate collected, washed with water, dried, and recrystallized from DMF to give **11b** as a yellow solid (736 mg, 63%): mp >320 °C; ^{1}H NMR (DMSO-d_6) δ 9.15 (s, 1H), 9.0 (s, 1H), 7.95 (s, 1H). Anal. ($C_{10}H_5N_7O_5$) C, H, N.

7-Nitro-3-(*N*-(1*H*)-tetrazol-5-ylcarbamoyl)-2(1*H*)-quinolone (18). To a suspension of acid **9a** (600 mg, 2.56 mmol) in THF (40 mL) was added CDI (456 mg, 2.81 mmol), and the mixture was heated to reflux for 1 h. The reaction mixture was cooled to room temperature, and 5-aminotetrazole (264 mg, 2.56 mmol) was added. The mixture was stirred at reflux for 3 h and then cooled to room temperature and 1 N HCl (20 mL) added. The precipitate was collected, washed with 1 N HCl, and crystallized from DMF to yield **18** as a yellow solid (420 mg, 54%): mp >320 °C; ^{1}H NMR (DMSO-d_6) δ 13.25−13.0 (bs, s, 2H), 9.05 (s, 1H), 8.35 (d, 1H), 8.25 (s, 1H), 8.10 (d, 1H). Anal. ($C_{11}H_7N_7O_4$) C, H, N.

7-Nitro-2(1*H*)-quinolone-3-hydroxamic Acid (12). To a solution of acid **9a** (400 mg, 1.71 mmol) in a mixture of THF (40 mL) and DMF (3 mL) was added CDI (416 mg, 2.56 mmol), and the mixture was heated to reflux for 1 h. The reaction mixture was cooled to room temperature and hydroxylamine hydrochloride (238 mg, 3.42 mmol) added. The reaction mixture was heated to reflux for 3 h and then cooled to room temperature and 3 N HCl (40 mL) added. The yellow precipitate was collected, washed with 1 N HCl, and crystallized from DMF to yield **12** as a yellow solid (177 mg, 42%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.8 (s, 1H), 11.45 (s, 1H), 9.55 (s, 1H), 8.9 (s, 1H), 8.25 (d, s, 2H). Anal. ($C_{11}H_7N_7O_4$) C, H, N.

7-Nitro-2(1*H*)-quinolone-3-hydrazinecarboxylic Acid (14). Hydrazine monohydrate (98%, 300 μL, 6.04 mmol) was added to a suspension of methyl ester **8a** (300 mg, 1.21 mmol) in methanol (30 mL) and the reaction mixture heated to reflux, with stirring, for 24 h. The reaction mixture was cooled to room temperature, and the yellow precipitate was collected, washed with MeOH, and recrystallized from DMF to yield **14** as a yellow solid (233 mg, 77%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 13.5−12.0 (s, 1H), 10.5 (s, 1H), 8.9 (s, 1H), 8.25 (d, 1H), 8.20 (d, 1H), 8.05 (dd, 1H), 4.5−5.2 (s, 1H). Anal. ($C_{10}H_8N_4O_4$) C, H, N.

7-Nitro-3-(2-hydroxy-1,3,4-oxadiazol-5-yl)-2(1*H*)-quinolone (15). CDI (260 mg, 1.57 mmol) and triethylamine (290 μL, 2.09 mmol) were added to a suspension of **14** (260 mg, 1.05 mmol) in a mixture of THF (10 mL) and DMF (1 mL). The reaction mixture was heated to reflux, with stirring, for 20 h. After cooling to room temperature, 1 N HCl (10 mL) was added. The yellow precipitate was collected, washed with MeOH, and crystallized from DMF to yield **15** as a yellow solid (70 mg, 16%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.6 (s, 1H), 8.65 (s, 1H), 8.10 (m, 2H), 8.0 (d, 1H). Anal. ($C_{11}H_6N_4O_5$) C, H, N.

7-Nitro-2(1*H*)-oxoquinoline-3-carboxamide (13). A suspension of methyl ester **8a** (500 mg, 2.02 mmol) in a mixture of methanol (30 mL) and DMF (30 mL) was saturated with ammonia gas and the mixture stirred at 60 °C overnight. The solvents were evaporated under vacuum, and the residue was recrystallized in DMF to yield **13** as a yellow solid (373 mg, 79%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 13.0−12.5 (bs, 1H), 9.0−8.9 (bs, 2H), 8.95 (s, 1H), 8.2 (d, 1H), 8.0 (dd, 1H), 7.95 (d, 1H). Anal. ($C_{10}H_7N_3O_4$) C, H, N.

3-(1-Carboxy-2-oxo-2-ethyl)-7-nitro-2(1*H*)-quinolone (16). CDI (2.1 g, 12.95 mmol) was added to a suspension of acid **9a** (2.0 g, 8.54 mmol) in a mixture of THF (200 mL) and DMF (20 mL) and the mixture heated to reflux, with stirring, for 1 h. The reaction mixture was cooled to room temperature, and the magnesium enolate of monoethyl ester malonic acid[15] (1.98 g, 12.8 mmol) was added. The reaction mixture was heated to reflux for 1.5 h and then cooled to room temperature. HCl (1 N) was added and the precipitate collected to yield **16** (1.75 g, 67%): mp >320 °C; ^{1}H NMR (DMSO-d_6) δ (keto form) 8.7 (s, 1H), 8.3−7.9 (m, 3H), 4.15 (s, 2H), 4.1 (q, 2H), 1.15 (t, 3H), (enol form) 12.4 (s, 1H), 9.0 (s, 1H), 8.3−7.9 (m, 3H), 6.8 (s, 1H), 4.25 (q, 2H), 1.3 (t, 3H). Anal. ($C_{11}H_7N_7O_4$) C, H, N.

3-(5-Hydroxy-1*H*-pyrazol-3-yl)-7-nitro-2(1*H*)-quinolone (17). Hydrazine monohydrate (98%, 30 μL, 0.64 mmol) was added to a suspension of **16** (100 mg, 0.32 mmol) in ethanol (10 mL) and the reaction mixture heated to reflux, with stirring, for 12 h. The reaction mixture was cooled to room temperature; the precipitate was collected and washed with ethanol to yield **17** (58 mg, 67%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.5 (s, 1H), 12.0 (s, 1H), 9.8 (s, 1H), 8.5 (s, 1H), 8.2 (s, 1H), 8.05 (d, 1H), 7.95 (d, 1H), 6.3 (s, 1H). Anal. ($C_{12}H_8N_4O_4$) C, H, N.

3-Bromo-6,7-dichloro-2(1*H*)-quinolone (19c). Bromine (3.6 mL, 69.8 mmol) was added dropwise to a suspension of acid **9c** (9.0 g, 34.9 mmol) in pyridine (90 mL) at 0 °C, and the mixture was stirred at 100−110 °C for 1.5 h. The dark brown solution was cooled to room temperature and poured into 1 N HCl (400 mL). The precipitate was collected, washed with 1 N HCl followed by water, and then dried to yield **19c** (8.5 g, 83%): mp > 260 °C; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H), 7.65 (s, 1H), 7.3 (s, 1H). Anal. ($C_9H_4BrCl_2NO$) C, H, N. Br.

3-Bromo-5,7-dichloro-2(1*H*)-quinolone (19d). **19d** was prepared from **9d** according to the method described for **19c**: 98% yield; mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 8.5 (s, 1H), 7.5 (s, 1H), 7.35 (s, 1H). Anal. ($C_9H_4BrCl_2NO$) C, H, N, Br.

3-Bromo-7-nitro-2(1*H*)-quinoline (19a). **19a** was prepared from **9a** according to the method described for **19c** and recrystallized from DMF: 64% yield; mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 12.6 (bs, 1H), 8.7 (s, 1H), 8.10 (s, 1H), 8.0 (d, 1H), 7.9 (d, 1H). Anal. ($C_9H_5BrN_2O_3$) C, H, N, Br.

3-Bromo-6,7-difluoro-2(1*H*)-quinolone (19e). **19e** was prepared from **9e** according to the method described for **19c** and recrystallized from DMF: 87% yield; mp 265−271 °C; ^{1}H NMR (DMSO-d_6) δ 12.5−12.2 (bs, 1H), 8.5 (s, 1H), 7.8 (dd, 1H), 7.25 (dd, 1H). Anal. ($C_9H_4BrF_2NO$) C, H, N, Br.

3-Bromo-6,7-dichloro-2-methoxyquinoline (21c). A solution of **19c** (8.5 g, 29 mmol) in phosphorus oxychloride (100 mL) was stirred at reflux overnight. Most of the excess of phosphorus oxychloride was removed in vacuo, and the residue was carefully hydrolyzed at 0 °C by adding crushed ice. The

precipitate was collected, washed several times with water, and then dried at 80 °C under vacuum to give 7.9 g (88%) of the chloroquinoline: mp 184 °C; ^{1}H NMR (DMSO-d_6) δ 8.9 (s, 1H), 8.35 (s, 1H), 8.25. Anal. ($C_9H_3BrCl_3N$) C, H, N.

To a suspension of the chloro intermediate (4.0 g, 12.8 mmol) in methanol (100 mL) was added a solution of 5.25 M sodium methoxide in methanol (2.8 mL, 14.7 mmol). The mixture was stirred at 65 °C for 10 h. After cooling, the precipitate was collected to yield **21c** (3.60 g, 92%): mp 176−179 °C; ^{1}H NMR (DMSO-d_6) 8.65 (s, 1H), 8.2 (s, 1H), 8.0 (s, 1H), 4.10 (s, 3H). Anal. ($C_{10}H_6BrCl_2NO$) C, H, N.

3-Bromo-5,7-dichloro-2-methoxyquinoline (21d). 21d was prepared from **19d** according to the method described for **21c**: 77% overall yield; mp 134−138 °C; ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 7.85 (s, 1H), 7.75 (s, 1H), 4.05 (s, 3H). Anal. ($C_{10}H_6BrCl_2NO$) C, H, N.

3-Bromo-7-nitro-2-methoxyquinoline (21a). 21a was prepared from **19a** according to the method described for **21c**: 69% overall yield; mp 163−170 °C; ^{1}H NMR (DMSO-d_6) δ 8.9 (s, 1H), 8.55 (s, 1H), 8.25 (d, 1H), 8.10 (d, 1H), 4.10 (s, 3H). Anal. ($C_{10}H_7BrN_2O_3$) C, H, N.

6,7-Dichloro-2-methoxyquinoline-3-phosphonic Acid Diethyl Ester (23c). Tetrakis(triphenylphosphine)palladium (1.85 g, 1.6 mmol) and **21c** (10.0 g, 32.1 mmol) were added under nitrogen atmosphere to a stirred mixture of diethyl phosphite (8.30 mL, 64.2 mmol) and triethylamine (8.95 mL, 64.2 mmol) in THF (15 mL). The resultant mixture was stirred at reflux for 12 h. After cooling to room temperature, the mixture was taken up in ethyl acetate (450 mL) and washed with 1 N HCl and brine. After evaporation in vacuo, the residue was purified by chromatography on silica gel (cyclohexane/ethyl acetate, 50/50, and then ethyl acetate) to give the diethyl phosphonate **23c** (7.3 g, 62%): mp 119−120 °C; ^{1}H NMR (DMSO-d_6) δ 8.75 (d, 1H), 8.5 (s, 1H), 8.1 (s, 1H), 4.15 (m, 4H), 4.1 (s, 3H), 1.3 (m, 6H). Anal. ($C_{14}H_{16}Cl_2NO_4P$) C, H, N, Cl.

5,7-Dichloro-2-methoxyquinoline-3-phosphonic Acid Diethyl Ester (23d). 23d was prepared from **21d** according to the method described for **23c**: 49% yield; mp 52−54 °C; ^{1}H NMR (DMSO-d_6) δ 8.85 (d, 1H), 7.7 (d, 1H), 7.4 (d, 1H), 4.15 (m, 4H), 4.1 (s, 3H), 1.3 (t, 6H). Anal. ($C_{14}H_{16}Cl_2NO_4P$) C, H, N, Cl.

7-Nitro-2-methoxyquinoline-3-phosphonic Acid Diethyl Ester (23a). 23a was prepared from **21a** according to the method described for **23c**: 39% yield; 83−85 °C; ^{1}H NMR (DMSO-d_6) δ 8.75 (d, 1H), 9.0 (d, 1H), 8.6 (d, 1H), 8.35 (dd, 1H), 8.3 (dd, 1H), 4.3 (q, s, 7H), 1.3 (m, 6H). Anal. ($C_{14}H_{17}N_2O_6P$) C, H, N.

6,7-Dichloro-2(1*H*)-oxoquinoline-3-phosphonic Acid (24c). Bromotrimethylsilane (40 mL) was added to a suspension of phosphonate diester **23c** (17.1g, 47 mmol) in acetonitrile (300 mL) and the mixture stirred at 80 °C for 1.5 h. The reaction mixture was evaporated to dryness under vacuum and the resulting residue stirred at 80 °C in 3 N HCl (100 mL) for 3 h. The precipitate was collected and recrystallized from ethanol to give **24c** (11.5 g, 83%): mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 11.5−12.5 (m, 3H), 8.35 (d, 1H), 8.2 (s, 1H), 7.5 (s, 1H). Anal. ($C_9H_6Cl_2NO_4P$) C, H, N, Cl.

5,7-Dichloro-2(1*H*)-oxoquinoline-3-phosphonic Acid (24d). 24d was prepared from **23d** according to the method described for **24c** and recrystallized from ethanol: 62% yield; mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 11.5−13.0 (m, 3H), 8.4 (d, 1H), 7.5 (s, 1H), 7.3 (s, 1H). Anal. ($C_9H_6Cl_2NO_4P$) C, H, N, Cl.

7-Nitro-2(1*H*)-oxoquinoline-3-phosphonic Acid (24a). 24a was prepared from **23a** according to the method described for **24c** and recrystallized from water: 59% yield; mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 11.5−13.0 (m, 3H), 8.5 (d, 1H), 8.1 (d, 1H), 8.1 (d, 1H), 7.95 (dd, 1H). Anal. ($C_9H_7N_2O_6P$) C, H, N.

6,7-Dinitro-2(1*H*)-oxoquinoline-3-phosphonic Acid (24b). The above compound (150 mg, 0.55 mmol) was stirred at 80 °C for 2.5 h in a mixture of 96% H_2SO_4 (0.3 mL) and 87% HNO_3 (0.3 mL). The reaction mixture was cooled to 0 °C and ice added. The precipitate was stirred for 30 min, filtered, washed with water, and recrystallized from ethanol to yield **24b** as a light yellow solid (100 mg, 57%): mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 9.15 (s, 1H), 9.0 (s, 1H), 7.95 (s, 1H). Anal. ($C_{10}H_5N_7O_5$) C, H, N.

6,7-Difluoro-(1*H*)-oxoquinoline-3-phosphonic Acid (24e). 24e was prepared from the **19e** according to the method described for **23c**, followed by treatment with bromotrimethylsilane as for the preparation of **24c**, and recrystallized from ethanol: 18% overall yield; mp 291−294 °C; ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H), 8.0 (dd, 1H), 7.25 (dd, 1H). Anal. ($C_9H_6F_2NO_4P$) C, H, N.

3-[(Carboxymethyl)thio]-7-nitro-2(1*H*)-quinolone (20). A suspension of **19a** (800 mg, 2.97 mmol), potassium carbonate (452 mg, 3.27 mmol), and ethyl 2-mercaptoacetate (0.36 mL, 3.27 mmol) in a mixture of acetone (25 mL) and DMF (2 mL) was refluxed under nitrogen for 2 h. After cooling to room temperature, the mixture was diluted with water and the suspension filtered to give 3-[(carbethoxymethyl)thio]-7-nitro-2(1*H*)-quinolone (800 mg, 87%): mp 254−257 °C; ^{1}H NMR (DMSO-d_6) δ 12.5 (bs, 1H), 8.15 (s, 1H), 8.0 (d, 1H), 7.8 (m, 2H), 4.15 (qd, 2H), 4.05 (s, 2H), 1.2 (t, 2H).

To a suspension of this compound (720 mg, 2.4 mmol) in a mixture of THF (10 mL) and water (10 mL) was added lithium hydroxide monohydrate (300 mg, 7.2 mmol), and the mixture was refluxed for 4 h. After cooling to room temperature, the reaction mixture was acidified with 1 N HCl and the solid collected, washed several times with water, and then dried at 80 °C under vacuum to give **20** (585 mg, 83%): mp 290−294 °C; ^{1}H NMR (DMSO-d_6) δ 13.05 (bs, 1H), 12.45 (bs, 1H), 8.15 (s, 1H), 8.0 (d, 1H), 7.8 (d, s, 2H), 3.95 (s, 2H). Anal. ($C_{11}H_8N_2O_5S$) C, H, N, S.

5,7-Dichloro-2(1*H*)-oxoquinoline-3-boronic Acid (22). Triethyl borate (2.24 mL, 13.2 mmol) followed by 1.6 M *N*-butyllithium in hexane (4.95 mL, 7.9mmol) was added dropwise at −70 °C via syringe to a solution of **21d** (2.0 g, 6.6 mmol) in THF (40 mL). The solution was stirred for 45 min at −70 °C and allowed to warm to room temperature over 3 h; 1 N HCl (50 mL) was added and the solution extracted with ethyl acetate (150 mL). The organic extract was washed with brine, dried over magnesium sulfate, filtered, and concentrated. The residue was taken up in methylene chloride (30 mL) and the precipitated product was collected to give 5,7-dichloro-2-methoxyquinoline-3-boronic acid (650 mg, 36%): ^{1}H NMR (DMSO-d_6) δ 8.2−8.4 (bs, 2H), 8.5 (s, 1H), 7.8 (d, 2H), 7.7 (d, 2H), 4.0 (s, 3H).

This compound (300 mg, 1.10 mmol) was refluxed in 6 N HCl for 4 h, the suspension filtered, and the solid recrystallized from DMF to give **22** (248 mg, 88%): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.4 (bs, 1H), 8.7−8.9 (bs, 2H), 8.55 (s, 1H), 7.5 (d, 1H), 7.35 (d, 1H). Anal. ($C_9H_6BCl_2NO_3$) C, H, N, Cl.

6,7-Dichloro-2-methoxyquinoline-3-phosphonic Acid Monoethyl Ester (25c). A suspension of diethyl ester **23c** (200 mg, 0.55 mmol) was stirred at 100 °C in 3 N NaOH (2 mL) for 3 h. The reaction mixture was acidified with 1 N HCl and extracted with ethyl acetate to give an oil which crystallized (180 mg, 97%): mp 166−169 °C; ^{1}H NMR (DMSO-d_6) δ 8.7 (d, 1H), 8.45 (s, 1H), 8.05 (s, 1H), 4.05 (s, 3H), 4.0 (m, 2H), 1.25 (t, 3H). Anal. ($C_{12}H_{12}Cl_2NO_4P$) C, H, N, Cl.

6,7-Dichloro-2(1*H*)-oxoquinoline-3-phosphonic Acid Monoethyl Ester (27c). The above compound (100 mg, 0.29 mmol) was stirred at 80 °C for 1 h in 3 N HCl (1 mL), and the suspension was collected by filtration to give pure **27** (88 mg, 92%): mp 268−276 °C; ^{1}H NMR (DMSO-d_6) δ 11.5−12.5 (m, 1H) 8.45 (d, 1H), 8.25 (s, 1H), 7.5 (s, 1H), 4.05 (q, 2H), 1.2 (t, 3H). Anal. ($C_{11}H_{10}Cl_2NO_4P$) C, H, N, Cl.

5,7-Dichloro-2-methoxyquinoline-3-phosphonic Acid Monoethyl Ester (25d). 25d was prepared from **23d** according to the method described for **25c**: 92% yield; mp 153−157 °C; ^{1}H NMR (DMSO-d_6) δ 8.75 (d, 1H), 7.9 (d, 1H), 7.7 (d, 1H), 4.05 (s, 3H), 4.0 (q, 2H), 1.25 (t, 3H).

5,7-Dichloro-2(1*H*)-oxoquinoline-3-phosphonic Acid Monoethyl Ester (27d). 27d was prepared from **25d** according to the method described for **27c**: 89% yield; mp 225−230 °C; ^{1}H NMR (DMSO-d_6) δ 13.0−12.0 (bs, 1H), 8.5 (d, 1H), 7.55 (d, 1H), 7.35 (d, 1H), 4.05 (q, 2H), 1.2 (t, 3H). Anal. ($C_{11}H_{10}Cl_2NO_4P$) C, H, N, Cl.

5,7-Dichloro-2-methoxyquinoline-3-methylphosphinic Acid Ethyl Ester (28). A solution of oxalyl chloride (0.155 mL, 1.77 mmol) in anhydrous methylene chloride was added dropwise to the monoester **25d** (500 mg, 1.49 mmol) dissolved in a mixture of anhydrous methylene chloride (3 mL) and DMF (20 μL). Upon complete addition of the oxalyl chloride, the solution was heated to reflux for 1 h. The solvent was removed in vacuo and the residue taken up in anhydrous THF (25 mL). Methylmagnesium chloride (3 M solution in THF, 0.58 mL, 1.74 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 1.5 h; 1 N HCl (1 mL) was added dropwise and the reaction mixture diluted with ethyl acetate (150 mL) and washed with 1 N HCl and 1 N NaOH. After evaporation, the residue was purified by chromatography on silica gel (methylene chloride/methanol, 98/2) to give pure **28** (240 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 9.1 (d, 1H), 7.8 (d, 1H), 7.5 (d, 1H), 4.15 (s, 3H), 4.1 (m, 1H), 3.85 (m, 1H), 1.85 (d, 3H), 1.3 (t, 3H).

5,7-Dichloro-2(1*H*)-oxoquinoline-3-methylphosphinic Acid (29). The above compound was hydrolyzed according to the method described from **24c**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.0−12.5 (bs, 1H), 8.55 (d, 1H), 7.5 (d, 1H), 7.35 (d, 1H), 1.65 (d, 3H). Anal. ($C_{10}H_8Cl_2NO_3P$) C, H, N, Cl.

6,7-Dichloro-2(1*H*)-oxoquinoline-3-phosphonic Acid Monocyclopentylmethyl Ester (26). Cyclopentanemethanol (49 μL, 0.42 mmol) and triphenylphosphine (109 mg, 0.42 mmol) were added to a solution of monoester **25c** (100 mg, 0.28 mmol) in anhydrous THF (2 mL). The solution was stirred for 10 min at room temperature, and diisopropyl azodicarboxylate was added. Upon completion of the condensation reaction, bromotrimethylsilane (92 μL, 0.7 mmol) was added and stirring continued for an additional 1 h. The reaction mixture was evaporated to dryness, 3 N HCl (4 mL) was added, and the mixture was stirred at 90 °C for 5 h. After cooling to room temperature, ethyl acetate (25 mL) was added. The biphasic system was stirred vigorously, and the white solid which precipitated was collected to give **26** (89 mg, 85%): mp 286−288 °C; ^{1}H NMR (DMSO-d_6) δ 12.5−11.5 (bs, 1H), 8.4 (d, 1H), 8.25 (s, 1H), 7.5 (s, 1H), 3.8 (t, 2H), 2.15 (m, 1H), 1.75−1.15 (m, 8H). Anal. ($C_{15}H_{16}NO_4Cl_2P$) C, H, N, Cl.

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