Synthesis and Biological Properties of 4-Substituted Quinolin-2(1*H*)-one Analogues

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A variety of 4-substituted quinolin-2(1*H*)-ones were prepared and evaluated for *N*-methyl-D-aspartate (NMDA) receptor binding site activity and their abilities to inhibit neurotoxicity. The 4-(2-carbethoxyethanamino)-7-chloro-3-nitroquinolin-2(1*H*)-one (**9b**) exhibited favorable NMDA receptor binding site activity and 7-chloro-4-(benzylamino)-3-nitroquinolin-2(1*H*)-one (**9c**) showed the most potent neurotoxicity among them. The synthetic strategies involve the use of well known keto ester condensation and reductive ring cyclization of intermediates (**2a-d**) to afford 4-substituted quinolin-2(1*H*)-ones.

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Introduction.

Since the field had its origin in the discovery by Hayashi (1954) of the convulsive effects of L-glutamate and L-aspartate in mammalian brain and the demonstration later in the decade of the depolarizing and excitatory actions of these amino acids on single central neurons [1], the ionotropic *N*-methyl-D-aspartate (NMDA) receptor antagonists acting at the glycine site have shown broad therapeutic potential and offer a highly attractive target for central nervous system (CNS) drug development for the treatment of Alzheimer's disease, Parkinson's disease, stroke, head injury, epilepsy, and schizophrenia [2]. In comparison with NMDA receptor antagonists acting competitively at the glutamate site or noncompetitively as channel blockers, glycine antagonists may have significantly improved side-effect profiles [3]. Many classes of glycine antagonists with high affinity and selectivity have now been synthesized, and they can be categorized mainly as kynurenic acid, 2-carboxytetrahydroquinoline, quinoxalinedione, 2-carboxyindole and benzazepine-2,5-dione [4]. In spite of the many classes of compounds mentioned above, most of these lack activity in the central nervous system following systemic dosing. Evidently, significant improvements in blood-brain barrier permeability and bioavailability are important factors to be considered for good drug candidates.

Thus, the research of NMDA receptor glycine site antagonists mainly concentrated on increased *in vivo* activity observed with these compounds relative to carboxylic acid-containing glycine antagonists is probably a consequence of improved penetration of the blood-brain barrier [5].

The 4-hydroxyquinolones and their analogues are an important class of compounds, which possess widespread pharmacological properties [6,7] such as antibiotic activity [8] and platelet aggregation inhibition [9]. They are also useful intermediates for many industrial products such as dye stuffs [10] and herbicides [11,12]. Recent reports describe a novel class of 4-hydroxyquinolone derivatives, *i.e.*, 4-hydroxy-3-arylquinolin-2(1*H*)-one and 4-hydroxy-3-nitroquinolin-2(1*H*)-one, as potent and selective *N*-methyl-D-aspartate (NMDA) receptor glycine site antagonists after oral administration. Many of these compounds have been derived from the 4-hydroxyquinolin-2(1*H*)-one nucleus [13].

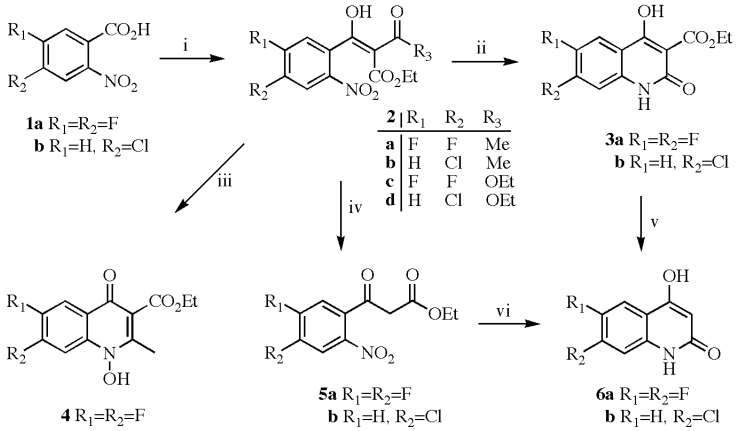
In a preliminary communication [14], we have reported the efficient synthesis of 4-hydroxyquinolones involving carbon-acylation in the presence of base, followed by internal ring cyclization. Our continued interest in synthesis and biological properties of 4-hydroxyquinolones has led us to the development of an efficient synthesis of 4-substituted quinolones. In this paper, we describe the preparation and NMDA rece]ptor binding site activity and inhibition effect on the neurotoxicity of 4-substituted quinolin-2(1*H*)-one analogues.

Chemistry.

The synthesis of the 4-hydroxyquinolin-2(1*H*)-ones is outlined in Scheme 1. We have used substituted benzoic acids as starting materials, which were reacted with thionyl chloride in toluene to give acyl chlorides in good yields. They were subsequently treated with the anion of ethyl acetoacetate, or diethyl malonate to give keto esters **2a-d** in excellent yields. In this condensation reaction, we found that the magnesium ethoxide was the most effective among bases such as sodium ethoxide, sodium hydride, sodium 3-aminoproylamide (NAPA), potassium 3-aminopropylamide (KAPA), and potassium *tert*-butoxide. It seems probable that the effectiveness of magnesium ethoxide can be explained in part by a magnesium chelation effect [15]. The keto diesters **2c,d** were smoothly transformed to 4-hydroxyquinolone compounds **3a,b** by reductive ring cyclization with sodium borohydride under alkaline conditions. Whereas, the diketo ester **2a** was converted to ethyl 6,7-difluoro-1-hydroxy-2-methyl-4-oxoquinoline-3-car-

boxylate (**4**) by mild catalytic hydrogenation over palladium-on-charcoal in ethanol at room temperature.

Scheme 1



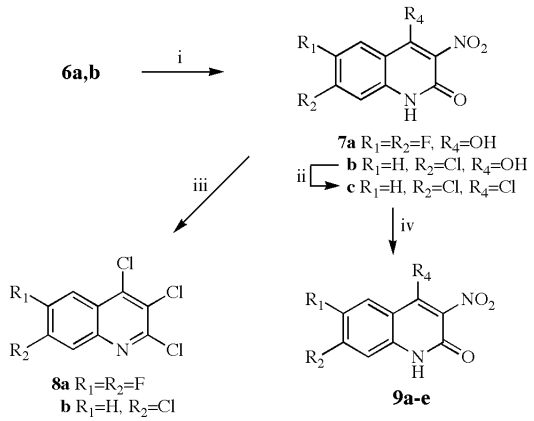
Reagents and reaction conditions: (i) $SOCl_2$, urea/toluene, 100°C , 3 h; CH_3COCO_2Et or $CH_2(CO_2Et)_2$, Mg, EtOH, CCl_4/toluene, room temperature, 30 min; (ii) $NaBH_4$, Pd-C, NaOH (*aq*), 1,4-dioxane, room temperature, 30 min; (iii) H_2, Pd-C, EtOH, 1 bar; (iv) *p*-TsOH/H_2O, reflux, 7 h; (v) 3N HCl (*aq*)/EtOH, reflux, 3 h; (vi) H_2, Pd-C, EtOH.

Treatment of diethyl 4,5-difluoro-2-nitrobenzoyl-malonate (**2c**) and diethyl 4-chloro-2-nitrobenzoyl-malonate (**2d**) with *p*-toluenesulfonic acid gave ethyl 3,4-difluoro-2-nitrobenzoylacetate (**5a**) and ethyl 4-chloro-2-nitrobenzoylacetate (**5b**), respectively, which were used without further purification for the next step. In this decarboxylation reaction, *p*-toluenesulfonic acid proved superior to 10% sulfuric acid, 20% acetic acid, or 15% sodium hydroxide.

Reductive ring cyclization of **5a** and **5b** with catalytic hydrogenation over palladium-on-charcoal in ethanol proceeded smoothly to afford 6,7-difluoro-4-hydroxyquinolin-2(1*H*)-one (**6a**), 7-chloro-4-hydroxyquinolin-2(1*H*)-one (**6b**), in 96% and 94% yields, respectively. The decarboxylation reaction of 4-hydroxyquinolone compounds **3a,b** were also performed in presence of 3 *N* hydrochloric acid to give 4-hydroxyquinolin-2(1*H*)-ones **6a,b** in good yields.

On the other hand, compounds **6a** and **6b** were nitrated with a mixture of nitric acid and acetic acid to produce 6,7-difluoro-3-nitro-4-hydroxyquinolin-2(1*H*)-one (**7a**) and 7-chloro-3-nitro-4-hydroxyquinolin-2(1*H*)-one (**7b**) in 77% and 70% yield, respectively. Chlorination of compound **7a,b** with excess phosphoryl chloride in the presence of triethylamine gave 6,7-difluoro-2,3,4-trichloroquinoline (**8a**) and 2,3,4,7-tetrachloroquinoline (**8b**). The hydroxyl group at the C-4 position of compound **7b** was exchanged with a chloride using a stoichiometric amount of phosphoryl chloride in dichloromethane to afford 4,7-dichloro-3-nitroquinolin-2(1*H*)-one (**7c**), which was reacted with hydroxyl amine, ethyl 3-aminopropionate, benzylamine, morpholine, or piperazine to yield NMDA receptor binding site antagonist candidate compounds (**9a-e**), (Table 1).

Scheme 2



Reagents and reaction conditions: (i) HNO_3/AcOH, 100 °C, 1 h; (ii) $POCl_3$/dichloromethane, room temperature, 16 h; (iii) $POCl_3$ (5.0 *eq*)/Et_3N, 100°C, 3 h; (iv) amines/pyridine, reflux, 2 h.

Table 1

Substitution of 3-Nitro-4-hydroxyquinolin-2(1*H*)-one (**7a**) or 4,7-Dichloro-3-nitroquinolin-2(1*H*)-one (**7c**)

Entry	R_1	R_2	R_4	Yield (%) [a]	Product
1	H	Cl	NHOH	71	**9a**
2	H	Cl	$NHCH_2CH_2CO_2Et$	68	**9b**
3	H	Cl	NH-Bn	79	**9c**
4	H	Cl	morpholinyl	72	**9d**
5	H	Cl	piperazinyl	77	**9e**

[a] Isolated yield.

Biological Activity.

4-Substituted quinolin-2(1*H*)-one analogues were evaluated for *in vitro* neurotoxicity and *N*-methyl-D-aspartate (NMDA) receptor binding site activity. In the first, for the inhibition effect on neurotoxicity, exposure of cortical cell cultures to 250μM NMDA resulted in a rapid swelling of the neuronal cell body within 2 h, and caused 90 to 100% neuronal death over the next day. These excitotoxic neuronal deaths were prevented by inclusion of 30 μM to 100 μM compound. We have found that **9b-d** show good anti neurotoxicity. Among these compounds, the 7-chloro-4-(benzylamino)-3-nitroquinolin-2(1*H*)-one (**9c**) showed the most activity (Figure 1).

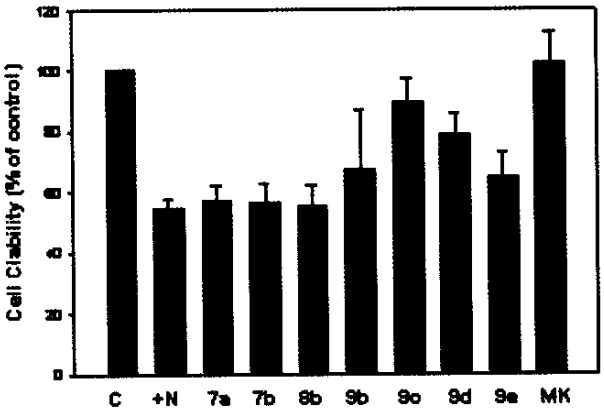


Figure 1. Inhibitory effects of 4-substituted quinoline analogues on neurotoxicity. Cortical cultures were exposed to excitotoxicant NMDA (500 μM) and tested compounds (100 μM). Neuronal death was analyzed 24 h later [C; control (without NMDA), +N; with NMDA, MK: MK-801 (10 μM)].

In the second, for the NMDA receptor binding site activity, the affinity for glycine binding site and pencyclidine binding on the NMDA receptor in rat brain cortex was evaluated using the binding ligands, [^{3}H]MPL for glycine and [^{3}H]MK-801 for pencyclidine, respectively, in prepared 4-hydroxyquinolones and their analogues. Among these compounds, **7a,b, 8b,** and **9b-d** exhibit fairly good activity for the NMDAR glycine binding site. Futhermore, **7a,b, 8a,** and **9b-e** with a chlorine atom in the aromatic ring showed good activity, while **3a**, **4**, **6a**, and **8a** with a fluorine atom in the aromatic ring exhibited poor NMDA receptor binding site activity due to an electronic effect in the aromatic ring. This seem to indicate that the presence of an electron withdrawing group in the benzene ring is not critical for high potency in 4-hydroxyquinolone and its analogues.

The presence of an electron withdrawing group in the aromatic ring is required for high activity in 4-hydroxy-3-nitroquinolin-2(1*H*)-ones, which is generally known for the glycine antagonists [16]. The 4-(2-carbethoxyethanamino)-7-chloro-3-nitroquinolin-2(1*H*)-one (**9b**) exhibited the most potent favorable NMDA receptor binding site activity among them. The *in vitro* NMDA receptor binding site activity was summarized in Table 2.

In conclusion, we report on the scope of a versatile synthesis and biological properties of 4-hydroxyquinolines and 4-substituted quinolone derivatives. The synthetic strategies involve the use of well known keto ester condensation and reductive ring cyclization of intermediates (**2a-d**) to afford 4-substitutedquinolin-2(1*H*)-ones. This synthesis is also applicable to large scale preparation.

We have found that **7a, 7b, 8b, 9b-d** exhibit fairly good activity for the NMDAR glycine binding site, but no activity for the NMDAR PCP binding site and **9b-d** also showed good activity for the inhibition neurotoxicity. Among these compounds, 4-(2-carbethoxyethanamino)-7-

Table 2

In vitro NMDA Receptor Binding Site Activity

Entry	Compound	Assay for NMDAR PCP binding site %-Inhibition			Assay for NMDAR Glycine binding site %-Inhibition		
		1 μM	100 μM	IC_{50} (μM)	1 μM	100 μM	IC_{50} (μM)
1	**3a**	-9.0	-2.5	ND [a]	-10.0	10.2	ND
2	**4**	-7.9	3.7	ND	-12.2	1.3	ND
3	**6a**	-3.3	25.1	ND	-24.5	-7.7	ND
4	**7a**	-6.0	40.5	ND	8.4	69.3	39.3
5	**7b**	<0.0	0.2	ND	12.3	71.4	24.2
6	**8a**	-9.0	16.0	ND	12.9	-4.6	ND
7	**8b**	<0.0	2.1	ND	7.0	45.3	40.5
8	**9b**	<0.0	2.1	ND	41.2	86.5	0.5
9	**9c**	<0.0	18.4	ND	13.0	83.1	10.5
10	**9d**	6.7	<0.0	ND	13.5	85.2	8.8
11	**9e**	<0.0	6.0	ND	20.8	89.6	5.6
12	MK 801 [b]	75.4	82.6	0.02	-	-	-
13	5,7-DCKA [c]	-	-	-	50.8	99.0	0.2

[a] Not determined; [b] The reference drug; [c]The reference drug; 5,7-dichlorokynurenic acid.

chloro-3-nitroquinolin-2(1*H*) -one (**9b**) exhibited favorable NMDA receptor binding site activity and 7-chloro-4-(benzylamino)-3-nitroquinolin-2(1*H*)-one (**9c**) showed the most potent anti neurotoxicity.

EXPERIMENTAL

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Tetrahydrofuran and diethyl ether were distilled from sodium benzophenone ketyl prior to use. Thin layer chromatography (tlc) was performed on precoated silica gel 60 F_{254} plates from EM reagents and visualized with 254-nm UV light or ceric sulfate-ammoniummolybdate-sulfuric acid spray. Flash chromatography was carried out on silica gel 60 (E. M. Merck, particle size 0.040 0.063 mm, 230 400 mesh ASTM). ^{1}H nmr and ^{13}C nmr spectra were recorded on a Bruker DPX 300 at 300 MHz and 76 MHz, respectively. The chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane, and *J*-values were in Hz. Infrared spectra (ir) were obtained on a Jasco FT/IR-300E spectrometer. Mass spectra (ms) were recorded on a Shimadzu-LKB 9000 GC/MS system. High resolution mass spectra (hrms) were obtained on a JEOL JMS-HX-110A/110A high resolution mass spectrometer. Elemental analyses were performed on a CE instruments Model 1110 elemental analyzer. All compounds have analytical results within ±0.4% of their theoretical values. All mps were uncorrected. When necessary, chemicals were purified according to the reported procedure [17].

NMDA Receptor Binding Site Activity.

Synaptic membranes for receptor studies were prepared from forebrains of male SD rats as described [18]. [^{3}H]MDL 105,519 binding assays were carried out in 96-well plates [19]. In brief, synaptic membranes (50 μg/well) were incubated at 25 °C for 30 min in a final volume of 0.25 ml reaction mixture containing 4 nM [^{3}H]MDL 105,519 in 50 mM Tris-acetate (pH 7.1). After incubation, the reaction mixture was washed 2 times with 0.2 ml of ice-cold 50 mM Tris-acetate buffer by rapid filtration using a Harvester 96® (Tomtec, CT, USA) through Whatman GF/A glass fiber filter presoaked in the assay buffer. Nonspecific binding was determined in the presence of 1 mM glycine. [^{3}H]MK-801 binding assays were conducted in 24-well plates. Synaptic membranes (300 μg/well) were incubated at 30°C for 60 min in 50 mM Tris-acetate (pH 7.1) containing 5 nM [^{3}H]MK-801, 0.1 μM glutamate, 1 μM glycine, and 100 μM MK-801 for nonspecific binding. The reaction was terminated by rapid filtration using an Inotech cell harvester (Inotech, Switzerland) through Whatman GF/A glass fiber filter. Data were obtained from three separate experiments performed in duplicate, and analyzed by nonlinear regression using Prism (Graphpad Software Inc., USA) for the determination of IC_{50} values.

Inhibition Effect on Neurotoxicity.

Cerebral corteces were removed from the brains of 15-day-old fetal mice. The neocortices were triturated and plated on 24-well plates (with approximately 10^5 cells/culture well) precoated with 100 μg/ml poly-D-lysine and 4 μg/ml laminine, in Eagle's minimal essential media (Earle's salts, supplied glutamine-free), and supplemented with horse serum (5%), fetal bovine serum (5%), 2mM glutamine, and 21 mM glucose. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. After 7 days *in vitro* (div), the cultures were shifted to the plating media containing 10 μM cytosine arabinoside without fetal serum. Cultures were then fed twice per week. Mixed cortical cell cultures containing neurons and glia (div 16-22) were exposed to excitotoxin, N-methyl-D-aspartate (NMDA), in Eagle's minimal essential media supplemented with 21 mM glucose and 26.5 mM bicarbonate. The morphology of the degenerating neurons was observed under a phase contrast microscope over the next 24 h. Neuronal death was analyzed 24 h later by measuring how much lactate dehydrogenase (LDH) had been released into the bathing medium. The percentage of neurons undergoing actual neuronal death was normalized to the mean LDH value that is found after a 24-h exposure to 500 μM NMDA (defined as 100) or a sham control (defined as 0). The levels of neuronal injury by LDH assay were routinely confirmed by counting viable neurons excluding trypan blue.

General Procedure for the Preparation of Keto Esters (**2a-d**).

Thionyl chloride (60.3 mmol) was added to a well-stirred suspension of compound **1a** or **1b** (50.2 mmol) and urea (0.3 g) in anhydrous toluene (40 ml). The reaction mixture was heated in an oil bath at 100°C for 3 h, and then cooled to room temperature. A mixture of ethyl acetoacetate or diethyl malonate (50.2 mmol), magnesium (53.4 mmol), ethanol (165 mmol), carbon tetrachloride (1.1 ml) and 80 ml of anhydrous toluene was stirred at room temperature for 1 h, and refluxed for 1 h. The reaction mixture was cooled to 5 °C. The former solution was cannulated into the latter. The resulting reaction mixture was stirred at room temperature for 30 min, and then 20 mL of 10% hydrochloric acid was added. The mixture was extracted with ether, and the combined organic extracts were washed with brine, dried and concentrated at reduced pressure.

Ethyl 2-(4,5-Difluoro-2-nitrobenzoyl)-3-oxobutanoate (**2a**).

This compound was obtained in 97% yield as a yellow liquid, R_f = 0.30 (20% ethyl acetate in hexanes); ir (ν_{max}, chloroform) 3405, 1712, 1639, 1437, 1014 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 13.55 (br s, 1H), 8.47 (dd, 1H, *J*=7.0 Hz, *J*=7.0 Hz), 7.83 (dd, 1H, *J*=7.8 Hz, *J*=7.8 Hz), 3.90 (q, 2H, *J*=7.1 Hz), 2.45 (s, 3 H), 0.86 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (deuteriochloroform): δ 192.3, 173.7, 165.9, 157.2, 155.0, 149.8, 119.6, 118.6, 113.5, 100.4, 58.9, 24.3, 13.4; ms (FAB$^+$): 316 (M$^+$+1, base peak), 270, 186, 137; hrms calcd. for $C_{13}H_{11}F_2NO_6$: 316.0633 (M$^+$+1), found 316.0632.

Ethyl 2-(4-Chloro-2-nitrobenzoyl)-3-oxobutanoate (**2b**).

This compound was obtained in 94% yield as a yellow liquid, R_f = 0.33 (20% ethyl acetate in hexanes); ir (ν_{max}, chloroform) 3386, 1710, 1641, 1266 cm^{-1}; ^{1}H nmr (deuteriochloroform, ref [20]): δ 13.32 (br s, 1H), 8.42 (d, 1H, *J*=7.1 Hz), 8.06 (d, 1H, *J*=6.9 Hz), 7.38 (d, 1H, *J*=7.4 Hz), 4.01 (q, 2H, *J*=7.1 Hz), 2.42 (s, 3H), 0.96 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (deuteriochloroform): δ 192.8, 174.2, 165.7, 158.0, 155.4, 135.2, 119.0, 118.1, 113.5, 101.4, 58.9, 24.3, 13.5.

Diethyl 2-(4,5-Difluoro-2-nitrobenzoyl)malonate (**2c**).

This compound was obtained in 95% yield as a yellow liquid, R_f = 0.30 (33% ethyl acetate in hexanes); ir (ν_{max}, chloroform): 3390, 1703, 1687, 1460, 1080, cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 13.26 (br s, 1H), 8.11 (dd, 1H, *J*=7.1 Hz, *J*=6.9 Hz), 7.33

(dd, 1H, *J*=8.7 Hz, *J*=7.9 Hz), 4.24 (q, 2H, *J*=7.1 Hz), 4.16 (q, 2H, *J*=7.1 Hz), 1.26 (t, 3H, *J*=7.1 Hz), 1.18 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (deuteriochloroform): δ 169.4, 169.0, 166.2, 158.1, 152.9, 148.2, 120.3, 118.6, 113.8, 95.7, 62.0, 61.9, 14.0, 13.9; ms (FAB$^+$): 346 (M$^+$+1), 274, 186 (base peak), 154, 137; hrms calcd. for $C_{14}H_{13}F_2NO_7$: 346.0738 (M$^+$+1), found 346.0734.

Diethyl 2-(4-Chloro-2-nitrobenzoyl)malonate (**2d**).

This compound was obtained in 98% yield as a yellow liquid, R_f = 0.31 (33% ethyl acetate in hexane); ir (ν_{max}, chloroform) 3289, 1730, 1687, 1460, 1080, cm^{-1}; ^{1}H nmr (deuteriochloroform, ref [21]): δ 14.02 (br s, 1H), 8.45 (d, 1H, *J*=7.0 Hz), 8.22 (d, 1H, *J*=8.0 Hz), 7.51 (d, 1H, *J*=7.0 Hz), 4.21 (q, 2H, *J*=7.1 Hz), 4.18 (q, 2H, *J*=7.1 Hz), 1.24 (t, 3H, *J*=7.1 Hz), 1.18 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (deuteriochloroform): δ 170.5, 168.9, 167.3, 158.2, 149.3, 135.1, 121.4, 118.7, 114.8, 96.7, 62.1, 62.1, 14.0, 13.9.

Ethyl 6,7-Difluoro-4-hydroxyquinolin-2(1*H*)-one-3-carboxylate (**3a**).

To a solution of keto diester **2c** (3.5 g, 10 mmol) in dioxane (45 ml) was added 20% aqueous sodium hydroxide (10 ml) and 10% palladium-on-charcoal (0.4 g) at room temperature. The mixture was stirred for 20 min, and then added dropwise to a solution of sodium borohydride (0.7 g, 18.5 mmol) in water (5 ml). The reaction mixture was stirred at room temperature for 30 min, and filtered through Celite. The filtrate was concentrated to remove dioxane, and the residue was acidified with 10% hydrochloric acid to give a pale yellow solid, which was recrystallized from ethanol to give 1.9 g (72%) of **3a**, R_f = 0.26 (15% methanol in chloroform); mp 280 °C (dec); ir (ν_{max}, potassium bromide): 3270-3030, 1649, 1579, 1188 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.86 (br s, 1H), 8.10 (dd, 1H, *J*=9.3 Hz, *J*=9.2 Hz), 7.71 (dd, 1H, *J*=7.0 Hz, *J*=6.8 Hz), 4.44 (q, 2H, *J*=7.1 Hz), 1.40 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 168.2, 165.7, 162.9, 152.1, 148.6, 143.9, 117.2, 112.9, 109.9, 101.9, 61.9, 14.4; ms (m/e): 269 (M$^+$), 239 (base peak), 171, 143, 115.

Anal. Calcd. for $C_{12}H_9F_2NO_4$: C, 53.54; H, 3.37; N, 5.20. found C, 53.36; H, 3.51; N, 5.39.

Ethyl 7-Chloro-4-hydroxyquinolin-2(1*H*)-one-3-carboxylate (**3b**).

According to the same procedure, compound **3b** was obtained in 72% yield from **2d**, R_f = 0.33 (20% methanol in chloroform); mp 287°C (dec); ir (ν_{max}, potassium bromide) 3346, 1725, 1684, 1181, cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6, ref [22]): δ 13.54 (br s, 1H), 8.23 (dd, 1H, *J*=8.4 Hz, *J*=8.3 Hz), 7.86 (dd, 1H, *J*=7.6 Hz, *J*=6.6 Hz), 7.13 (d, 1H, *J*=6.9 Hz), 4.31 (q, 2H, *J*=7.1 Hz), 1.37 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 167.8, 166.5, 163.8, 156.2, 147.5, 143.4, 116.3, 113.9, 111.0, 102.0, 62.0, 14.1;

Ethyl 6,7-Difluoro-1-hydroxy-2-methyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (**4**).

A solution of the diketo ester **2a** (3.2 g, 10.2 mmol) in ethanol (80 ml) was hydrogenated over 10% palladium-on-charcoal (0.6 g) under atmospheric pressure at room temperature for 3 h. The reaction mixture was filtered through Celite and evaporated to give a pale yellow solid, which was recrystallized from ethanol to give 2.2 g (78%) of **4**, R_f = 0.33 (10% methanol in chloroform); mp 202 °C; ir (ν_{max}, potassium bromide): 3450, 1717, 1605, 1475, 1110 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.33 (br s, 1H), 8.04 (dd, 1H, *J*=10.1 Hz, *J*=8.4 Hz), 7.91 (dd, 1H, *J*=7.1 Hz, *J*=6.8 Hz), 4.26 (q, 2H, *J*=7.1 Hz), 2.42 (s, 3H), 1.30 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 168.0, 164.9, 152.5, 149.1, 147.4, 143.7, 135.2, 119.9, 110.9, 102.4, 59.0, 13.7, 12.2; ms (m/e): 283 (M$^+$), hrms calcd. for $C_{13}H_{11}F_2NO_4$: 284.0707 (M$^+$ + 1), found 284.0727.

Ethyl 3,4-Difluoro-2-nitrobenzoylacetate (**5a**).

A solution of diethyl 4,5-difluoro-2-nitrobenzoylmalonate (**2c**, 7.0 g, 20.3 mmol) and *p*-toluenesulfonic acid (0.2 g) in 80 ml of water was refluxed for 12 h. The reaction mixture was cooled to room temperature and extracted with 120 ml of ethyl acetate. The organic layer was washed with 2% sodium bicarbonate and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give a yellow oil 5.0 g (91%) of **5a**, R_f = 0.35 (50% ethyl acetate in hexane); ir (ν_{max}, chloroform); 3280-3045, 1738, 1605, 1070 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 8.07 (dd, 1H, *J*=6.7 Hz, *J*=6.7 Hz), 7.45 (dd, 1H, *J*=7.4 Hz, *J*=7.4 Hz), 4.18 (q, 2H, *J*=7.1 Hz), 3.88 (s, 1H), 2.54 (s, 1H), 1.28 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (deuteriochloroform): δ 182.9, 168.3, 159.4, 158.7, 153.6, 133.3, 117.7, 115.9, 61.5, 44.8, 14.0; ms (FAB$^+$) 274 (M$^+$+1, base peak), 186, 137, 89; hrms calcd. for $C_{11}H_9F_2NO_5$: 274.0527 (M$^+$+1), found 274.0528.

Ethyl 4-Chloro-2-nitrobenzoylacetate (**5b**).

According to the same procedure, compound **5b** was obtained in 72% yield from **2d**, R_f = 0.40 (50% ethyl acetate in hexane); ir (ν_{max}, chloroform) 3258-3022, 1726, 1669, 1242 cm^{-1}; ^{1}H nmr (deuteriochloroform, ref [23]): δ 8.41 (d, 1H, *J*=7.9 Hz), 8.08 (d, 1H, *J*=7.1 Hz), 6.97 (d, 1H, *J*=7.4 Hz), 4.20 (q, 2H, *J*=7.1 Hz), 3.68 (s, 1 H), 2.59 (s, 1 H), 1.21 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (deuteriochloroform): δ 189.3, 170.8, 150.1, 140.2, 135.6, 129.8, 128.4, 122.9, 60.1, 42.3, 13.7.

6,7-Difluoro-4-hydroxyquinolin-2(1*H*)-one (**6a**).

The keto ester **5a** (5.5 g, 20.1 mmol) in ethanol (120 ml) was hydrogenated over 10% palladium-on-charcoal (1.0 g) at 36 psi for 1 h. The mixture was filtered through Celite and evaporated. A dark yellow solid was obtained, which was recrystallized from ethanol to give 3.8 g (96%) of **6a**, R_f = 0.35 (20% methanol in chloroform); mp 310 °C (dec); ir (ν_{max}, potassium bromide); 3200-2230, 1685, 1486, 1173 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 11.65 (br s, 1H), 11.34 (br s, 1H), 7.69 (dd, 1H, *J*=8.7 Hz, *J*=8.7 Hz), 7.21 (dd, 1H, *J*=7.0 Hz, *J*=7.0 Hz), 5.76 (s, 1H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 163.4, 152.8, 149.5, 146.3, 143.1, 136.4, 110.5, 103.3, 98.6; ms (m/e) 197 (M$^+$); hrms: calcd. for 197.0288 (M$^+$), found 197.0291.

Anal. calcd. for $C_9H_5F_2NO_2$: C, 54.83; H, 2.56; N, 7.10. found C, 54.89; H, 2.51; N, 7.23.

7-Chloro-4-hydroxyquinolin-2(1*H*)-one (**6b**).

According to the same procedure, compound **6b** was obtained in 88% yield from **5b**, R_f = 0.32 (20% methanol in chloroform); mp 300 °C (dec), {(ref [24], mp 279 °C (dec)}; ir (ν_{max}, potassium bromide) 3327, 1694, 1516, 1072 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.85 (br s, 1H), 11.22 (br s, 1H), 7.69 (d, 1H, *J*=8.0 Hz), 7.35 (d, 1H, *J*=7.2 Hz), 5.83 (s, 1 H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 168.3, 154.2, 151.0, 145.4, 141.3, 135.3, 109.1, 104.9, 99.6.

6,7-Difluoro-3-nitro-4-hydroxyquinolin-2(1*H*)-one (**7a**).

A solution of **6a** (1.1 g, 5.6 mmol), nitric acid (70%, 12 ml, 8.0 mmol) and glacial acetic acid (8 ml) was heated at 90 °C

for 1 h. The reaction mixture was cooled to room temperature and then diluted with 12 ml of ice water. The product was filtered and washed with water, and dried to give 1.0 g (77%) of **7a** as yellow crystals, R_f = 0.30 (10% methanol in chloroform); mp 193-194 °C; ir (ν_{max}, potassium bromide); 3280-2160, 1658, 1420, 1188 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.93 (br s, 1H), 7.80 (dd, 1H, *J*=8.4 Hz, *J*=8.4 Hz), 7.11 (dd, 1H, *J*=6.9 Hz, *J*=6.9 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 156.4, 154.7, 151.5, 147.6, 144.3, 135.9, 127.1, 112.8, 104.5; ms (m/e) 242 (M^+); hrms: calcd. for 242.0139 (M^+), found 242.0142.

Anal. Calcd. for $C_9H_4F_2N_2O_4$: C, 44.65; H, 1.67; N, 11.57. found C, 44.73; H, 1.71; N, 11.70.

7-Chloro-3-nitro-4-hydroxyquinolin-2(1*H*)-one (**7b**).

According to the same procedure, compound **7b** was obtained in 70% yield from **6b**, R_f = 0.33 (10% methanol in chloroform); mp 204-205 °C (ref [24]); ir (ν_{max}, potassium bromide) 3236, 1696, 1652, 1247 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 12.43 (br s, 1H), 8.12 (d, 1H, *J*=7.9 Hz), 7.61 (d, 1H, *J*=7.3 Hz) 7.06 (d, 1H, *J*=6.8 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 168.1, 155.5, 150.1, 148.7, 143.2, 134.5, 128.6, 112.9, 105.5; ms (m/e) 240 (M^+).

4,7-Dichloro-3-nitroquinolin-2(1*H*)-one (**7c**).

To a solution of 7-chloro-3-nitro-4-hydroxyquinolin-2(1*H*)-one (**7a**, 0.8 g, 3.3 mmol) in dichloromethane (12 ml) was added phosphoryl chloride (1.2 g, 7.8 mmol) in dichloromethane (5 ml), and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was cooled to 0 °C and the solid was collected by filtration. Recrystallization from chloroform gave (1.2 g, 70%) of **7c** as a beige solid. R_f = 0.40 (10% methanol in chloroform); mp 106-107 °C; ir (ν_{max}, potassium bromide) 3032, 1665, 1467, 1236 cm^{-1}; ^1H nmr (deuteriochloroform): δ 7.89 (dd, 1H, *J*=8.0 Hz, *J*=8.0 Hz), 7.61 (d, 1H, *J*=7.3 Hz), 7.36 (dd, 1H, *J*=6.9 Hz, *J*=6.9 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 157.4, 155.7, 154.9, 144.7, 135.7, 130.2, 125.0, 113.1, 107.2.

6,7-Difluoro-2,3,4-trichloroquinoline (**8a**).

A solution of **7a** (2.5 g, 10.3 mmol), phosphoryl chloride (15.3 g, 100 mmol) and triethylamine (1.6 g, 15.6 mmol) was heated at 100 °C for 3 h. The excess solvent was evaporated *in vacuo*, and the residue was poured into 33 ml of ice water. The solution was brought to pH 6 with 2 *N* sodium hydroxide and the precipitated solid was filtered, washed with water, and dried to afford 2.4 g (86%) of **8a** as a white solid, R_f = 0.33 (ethyl acetate, neat); mp 96°C; ir (ν_{max}, potassium bromide); 3390, 1509, 1252, 1196, 660 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.03 (dd, 1H, *J*=8.0 Hz, *J*=8.0 Hz), 7.00 (dd, 1H, *J*=7.4 Hz, *J*=7.3 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 156.1, 153.9, 152.9, 150.5, 134.1, 130.8, 122.2, 116.5, 111.8; ms (m/e) 268 (M^+), 232, 220, 197 (base peak), 171, 136, 112.

2,3,4,7-Tetrachloroquinoline (**8b**).

According to the same procedure, compound **8b** was obtained in 75% yield from 7b, R_f = 0.45 (10% methanol in chloroform); mp 89-90 °C; ir (ν_{max}, potassium bromide) 3305, 1668, 1412, 1203 cm^{-1}; ^1H nmr (deuteriochloroform): δ 8.08 (dd, 1H, *J*=7.8 Hz, *J*=7.8 Hz), 7.64 (d, 1H, *J*=7.1 Hz), 7.35 (d, 1H, *J*=7.5 Hz); ^{13}C nmr (deuteriochloroform): δ 156.2, 148.7, 139.7, 137.6, 129.3, 128.7, 127.6, 121.4, 120.2.

General Procedure for the Preparation of 4-Substituted 3-Nitroquinolin-2(1*H*)-ones (**9a-e**).

To a solution of **7a** or **7b** (1.5 mmol) in pyridine (10 ml) was added several amines (2.0 mmol), and the reaction mixture was heated under reflux for 2 h. The reaction mixture was cooled to 10°C and a solid was collected by filtration to afford the product, which was recrystallized from chloroform to give pale yellow crystals.

7-Chloro-4-(hydroxyamino)-3-nitroquinolin-2(1*H*)-one (**9a**)

This compound was obtained in 71% yield as pale yellow solid, R_f = 0.45 (10% methanol in chloroform); mp 223-224 °C; ir (ν_{max}, potassium bromide) 3466-2986, 1689, 1535, 1153 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 10.04 (br s, 2H), 7.91 (dd, 1H, *J*=7.8 Hz, *J*=7.8 Hz), 7.74 (d, 1H, *J*=7.4 Hz), 7.57 (dd, 1H, *J*=7.1 Hz, *J*=7.1 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 165.2, 156.4, 149.7, 145.3, 134.5, 133.3, 126.3, 118.1, 112.5; hrms calcd. for $C_9H_6ClN_3O_4$: 256.0047 (M^++1), found 256.0069.

4-(2-Carbethoxyethanamino)-7-chloro-3-nitroquinolin-2(1*H*)-one (**9b**).

This compound was obtained in 68% yield as pale yellow solid, R_f = 0.31 (20% methanol in chloroform); mp 205-206 °C; ir (ν_{max}, potassium bromide) 3310, 1728, 1662, 1514, 1206 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.11 (dd, 1H, *J*=8.0 Hz, *J*=8.0 Hz), 7.81 (d, 1H, *J*=7.5 Hz), 7.46 (dd, 1H, *J*=7.4 Hz, *J*=7.4 Hz), 4.32 (q, 2H, *J*=7.1 Hz), 4.09 (s, 2 H), 1.43 (t, 3H, *J*=7.1 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 169.8, 157.7, 155.7, 152.6, 147.3, 134.1, 130.5, 126.4, 116.2, 115.2, 61.5, 43.5, 14.7; hrms calcd. for $C_{14}H_{14}ClN_3O_5$: 340.0622 (M^++1), found 340.0614.

7-Chloro-4-(benzylamino)-3-nitroquinolin-2(1*H*)-one (**9c**).

This compound was obtained in 79% yield as a pale yellow solid, R_f = 0.34 (10% methanol in chloroform); mp 176-177 °C; ir (ν_{max}, potassium bromide) 3287, 1677, 1434, 1210 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 7.94 (d, 1H, *J*=7.9 Hz), 7.68 (d, 1H, *J*=7.1 Hz), 7.41 (dd, 1H, *J*=7.9 Hz, *J*=7.9 Hz), 7.37-7.18 (m, 5 H), 4.28 (s, 2 H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 157.6, 154.7, 153.9, 147.6, 138.8, 134.7, 130.3, 129.4, 127.5, 116.5, 115.5, 44.2; hrms calcd. for $C_{16}H_{12}ClN_3O_3$: 330.0567 (M^++1), found 330.0578.

7-Chloro-4-(morpholinyl)-3-nitroquinolin-2(1*H*)-one (**9d**).

This compound was obtained in 72% yield as a pale yellow solid, R_f = 0.40 (20% methanol in chloroform); mp 208-209 °C; ir (ν_{max}, potassium bromide) 3316, 1674, 1510, 1208 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 7.78 (d, 1H, *J*=7.6 Hz), 7.54 (d, 1H, *J*=7.3 Hz), 7.38 (d, 1H, *J*=7.2 Hz), 3.64-3.51 (m, 4H), 3.43-3.25 (m, 4H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 165.7, 157.9, 155.4, 144.8, 135.5, 129.7, 127.3, 117.2, 112.5, 67.5, 66.5, 47.1, 46.9; hrms calcd. for $C_{13}H_{12}ClN_3O_4$: 310.0516 (M^++1), found 310.0532.

7-Chloro-4-(piperazinyl)-3-nitroquinolin-2(1*H*)-one (**9e**).

This compound was obtained in 77% yield as a pale yellow solid, R_f = 0.36 (20% methanol in chloroform); mp 221-223 °C; ir (ν_{max}, potassium bromide) 3362, 1668, 1485, 1212 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 7.80 (d, 1H, *J*=7.8 Hz), 7.52 (d, 1H, *J*=7.5 Hz), 7.26 (d, 1H, *J*=7.4 Hz), 3.12-2.85 (m, 4H), 2.76-2.59 (m, 4H); ^{13}C nmr (dimethyl sulfoxide-d_6): δ 165.0, 159.8, 140.3, 137.4, 127.5, 125.6, 121.7, 119.3, 110.2, 54.7, 54.1, 52.5,

51.7; hrms calcd. for $C_{13}H_{13}ClN_4O_3$: 309.0676 (M^++1), found 309.0684.

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