Full Paper

Synthesis and Anti-HBV Activities Evaluation of New Ethyl 8-Imidazolylmethyl-7-hydroxyquinoline-3-carboxylate Derivatives in vitro

Yajing Liu[1], Yanfang Zhao[1], Xin Zhai[1], Xiuping Liu[2], Lixue Sun[1], Yanxia Ren[1], and Ping Gong[1]

[1] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, P. R. China
[2] Hebei University, Health Science Center, Baoding, P. R. China

Some new ethyl 8-imidazolylmethyl-7-hydroxyquinoline-3-carboxylate derivatives have been synthesized and evaluated for their anti-hepatitis B virus (HBV) activities and cytotoxicities in HepG2.2.15 cells stable transfection with HBV. Compounds **13a**, **11b**, **11c**, **12c**, **13c**, **11g**, and **12g** inhibited the expression of the viral antigens HBsAg or HBeAg in a low concentration, of which **11c** (IC_{50} = 12.6 μM, SI = 12.4), **12c** (IC_{50} = 3.5 μM, SI = 37.9), and **12g** (IC_{50} = 2.6 μM, SI = 61.6) showed more active abilities to inhibit the replication of HBV DNA than the positive control lamivudine (**3TC**, IC_{50} = 343.2 μM, SI = 7.0).

Keywords: Anti-HBV activities / 7-Hydroxyquinoline-3-carboxylates / Synthesis

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Introduction

Hepatitis B virus (HBV) infection remains a global public health problem, although an effective vaccine has been developed for humans. It is estimated that about 370 million people are chronically infected worldwide with roughly four million deaths annually from the resulting cirrhosis and hepatocellular carcinoma [1]. Currently, interferon-a and nucleoside inhibitors of HBV reverse transcriptase / polymerase, such as lamivudine, adefovir dipivoxil, entecavir, and telbivudine, are the only agents approved for the treatment of HBV infection [2]. However, the side effects of interferon-a and the emergence of resistant viruses under the application of nucleoside analogues prevent their long-term treatment [3–6]. Therefore, it is very important to develop drugs with novel molecular structures and optimal pharmacological profiles for the chemotherapy of HBV infection.

Quinoline derivatives (Fig. 1) have a variety of biological activities such as antimalarial, anti-rheumatoid arthritis, antimicrobial, anticancer, antivirus, etc. [7, 8]. In previous studies, we have synthesized a series of quinoline derivates and evaluated their antimicrobial, anticancer, and antivirus activities. Among them, compound ethyl 7-hydroxy-6-methoxy-2-((phenylthio)methyl)-8-((pyrrolidin-1-yl)methyl)quinoline-3-carboxylate (**QU-12**, Fig. 1) exhibited anti-HBV activity with an IC_{50} value of 36.8 μM and SI of 2.8, which increased our interest in ethyl 7-hydroxyquinoline-3- carboxylate derivatives. Zhao *et al*. recently disclosed a series of 1*H*-indole-3-car-

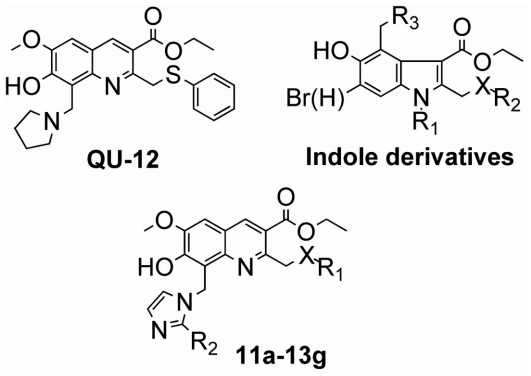
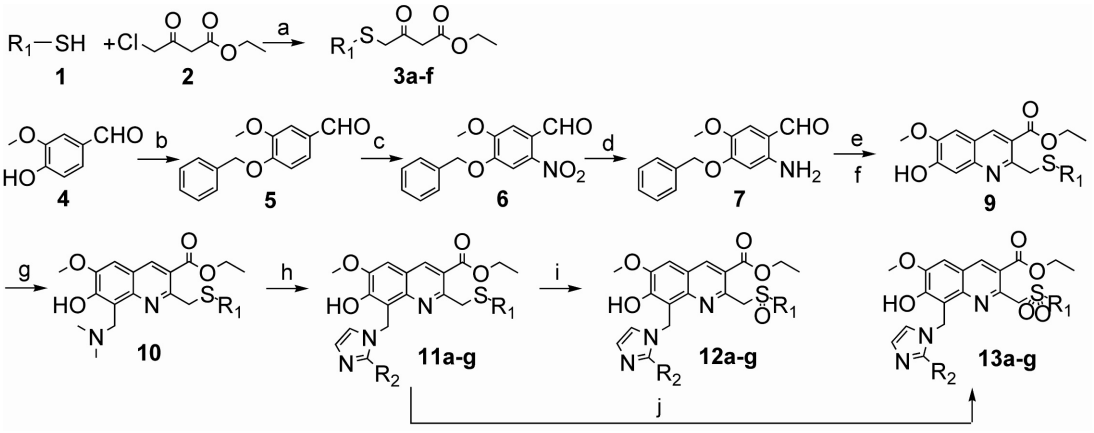


Figure 1. Structure of indole derivatives and ethyl 7-hydroxyquinoline-3-carboxylates.

Correspondence: Ping Gong, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, 110016 Shenyang, Liaoning, P. R. China.
E-mail: gongpinggp@126.com
Fax: +86 24 2398-6429



Reaction conditions: (a) KOH, methanol, r.t., 6 h; (b) K_2CO_3, DMF, 80°C, 3 h; (c) HNO_3, 40°C, 5 h; (d) $HCONH_2$, Zn, absolute alcohol, reflux, 8 h; (e) compound **3**, piperidine, isopropanol, reflux, 12-15 h; (f) AcOH/HCl = 2/1, 80°C for 6 h; (g) 33% dimethylamine, 37% formaldehyde, acetic acid, 50°C, 3 – 5 h; (h) 1*H*-imidazole or 2-methyl-1*H*-imidazole, HCl, 1,4-dioxane, 60 – 80°C, 2-3 h; (i) sodium perborate, acetic acid, 40°C, 3-5 h; (j) H_2O_2, Na_2WO_4, CH_3OH, 20°C, 3 – 7 h.

Scheme 1. Synthesis of target compounds.

boxylic esters as the potent inhibitors of HBV replication [9, 10], and their chemical structures were similar to **QU-12**. The structure-activity relationship (SAR) of 1*H*-indole-3-carboxylic esters indicated that heterocyclic groups such as pyrrolidinyl and imidazolyl at 4-position were more in favor of enhancing anti-HBV effects than other groups, and the oxidation state of sulfur and the substituent on the phenyl ring at 2-position would influence the anti-HBV activity.

In view of these observations, we designed and synthesized a new series of ethyl 8-imidazolylmethyl-7-hydroxy quinoline-3-carboxylate derivatives referring to the SAR of 1*H*-indole-3-carboxylic esters. Imidazolmethyl and 2-methylimidozolmethyl were introduced into the 8-position of quinoline, and other modifications were mainly focused on the 2-position. Arylthiomethyl, arylsulfinylmethyl, or arylsulfonylmethyl were incorporated at position-2, and various substituents were introduced into the phenyl ring to investigate their influence on anti-HBV activities.

Results and discussion

Chemistry

The synthesis pathway for ethyl 8-imidazolylmethyl-7-hydroxy quinoline-3-carboxylate derivatives is presented in Scheme 1. The substituents of compounds **11a – 13g** are listed in Table 1. The commercially available vanillin **4** was treated with benzyl chloride to give the compound **5** [11], which was nitrated with typical nitration reagent (fuming nitric acid) to provide 2-nitrobenzaldehyde **6** [12]. Activated zinc powder with a catalytic amount of formamide, was found to be an efficient reagent to reduce the nitro group prior to the aldehyde group to get 2-aminobenzaldehyde **7**. The thioether **3** was prepared via etherification of ethyl 4-chloroacetoacetate **2** and corresponding thiophenol **1** [10]. Then, treatment of *o*-aminobenzaldehyde **7** with **3** in the presence of piperidine afforded the ethyl-3-quinoline carboxylate **8** [13], which was deprotected by mixed acid (acetic acid/hydrochloric acid, 2 : 1) to produce compound **9**. Because imidazole or methylimidazole could not be directly introduced into position-8 by standard Mannich reaction as pyrrolidyl (**QU-12**), **10** was obtained first by Mannich reaction of **9** with dimethylamine and formaldehyde in acetic acid. Then, treatment of **10** with imidazole or 2-methylimidazole in 1,4-dioxane yielded the target compounds **11a – g**, which reacted with sodium perborate alone or hydrogen peroxide catalyzed with sodium wolframate to obtain the target compounds **12a – g** and **13a – g**, respectively.

Assay for anti-HBV activity

The anti-HBV activity of the synthesized compounds **11a – 13g** was evaluated in HepG2.2.15 cells, and the results were summarized in Table 1. As shown, using IC_{50} and SI as the results expression, most of them exhibited an overall inhibition of virion from the secretion of the HBV antigens to the replication of HBV DNA, in which compound **12g** exhibited the best activity of anti-HBV *in vitro* (IC_{50} = 2.6 μM, SI = 61.5).

Comparing **12a** with **12b**, there were no significant differences between imidazolyl and 2-methylimidazolyl, which were introduced into 8-position on the quinoline

Table 1. The substituents, anti-HBV activity and cytotoxicity of the targets compounds **11a**–**13g**.

NO.	X	R_1	R_2	TC_{50}[a] (μM)	HBsAg		HBeAg		HBV DNA replication	
					IC_{50}[b] (μM)	SI[c]	IC_{50} (μM)	SI	IC_{50} (μM)	SI
11a	S	Phenyl	H	205.5	48.1	4.3	–	–	–	–
12a	SO	Phenyl	H	238.9	27.7	8.6	–	–	59.1	4.0
13a	SO_2	Phenyl	H	179.2	13.5	13.3	–	–	–	–
11b	S	Phenyl	CH_3	13.4	3.7	3.6	11.4	1.2	–	–
12b	SO	Phenyl	CH_3	250.7	36.5	6.9	–	–	52.8	4.7
13b	SO_2	Phenyl	CH_3	155.7	18.8	8.3	21.4	7.3	–	–
11c	S	4-Fluorophenyl	H	156.7	7.9	19.8	–	–	12.6	12.4
12c	SO	4-Fluorophenyl	H	132.7	11.2	11.8	–	–	3.5	37.9
13c	SO_2	4-Fluorophenyl	H	24.8	6.0	4.1	–	–	13.6	1.8
11d	S	4-Methoxyphenyl	CH_3	21.7	–	–	–	–	–	–
12d	SO	4-Methoxyphenyl	CH_3	84.7	–	–	–	–	31.4	2.7
13d	SO_2	4-Methoxyphenyl	CH_3	152.6	–	–	–	–	–	–
11e	S	4-Chlorobenzyl	CH_3	36.1	15.4	2.3	–	–	–	–
12e	SO	4-Chlorobenzyl	CH_3	60.8	–	–	–	–	57.8	1.1
13e	SO_2	4-Chlorobenzyl	CH_3	147.2	48.3	3.0	–	–	–	–
11f	S	3-Methylphenyl	H	23.1	–	–	–	–	–	–
12f	SO	3-Methylphenyl	H	501.9	–	–	–	–	362.2	1.4
13f	SO_2	3-Methylphenyl	H	134.7	–	–	–	–	61.2	2.2
11g	S	3,4-Difluorophenyl	H	114.6	7.8	14.6	4.1	27.8	21.2	5.4
12g	SO	3,4-Difluorophenyl	H	159.9	14.2	11.3	–	–	2.6	61.6
13g	SO_2	3,4-Difluorophenyl	H	24.8	–	–	15.3	1.6	–	–
3TC				2402.4	–	–	–	–	343.2	7.0

[a] 50% cytotoxic concentration in HepG2.2.15 cells.
[b] 50% inhibitory concentration.
[c] Selectivity index (SI: TC_{50}/IC_{50}); – no antiviral activity at the concentration lower than its TC_{50}.

cycle. All of the compounds, except for **12d**, with electron-withdrawing fluoro on the phenyl at 2-position were superior to those with electron-donating methoxy, methyl, or no substitution. For example, compound **12g** with 3,4-difluoro substituent (IC_{50} = 2.6 μM) and **12c** with 4-fluoro substituent (IC_{50} = 3.5 μM) were 132 and 98-times more active than lamivudine (**3TC**, IC_{50} = 343.2 μM), and their activities were comparable to indole derivative 10_L (IC_{50} = 3.1 μM) [10]. The anti-HBV activities were abolished by inserting methylene into the arylsulfur moiety at 2-position (**11e**, **12e**, and **13e**). With the conversion of sulfur into sulfinyl, the ability to inhibit the replication of HBV DNA was improved obviously (compare **11a** with **12a**, **11b** with **12b**, **11c** with **12c**, **11d** with **12d**, and **11g** with **12g**). Nevertheless, there were no significant distinctness between the sulfur and sulfonyl. In addition, compounds **13a**, **11b**, **11c**, **12c**, **13c**, **11g**, and **12g** displayed good suppressant properties on the production of HBsAg and HBeAg.

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The authors have declared no conflict of interest.

Experimental

Chemistry

All chemicals were obtained from commercial suppliers and used without purification. Melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Glass-backed sheets of silica gel 60 F254 (EM Science) were used for TLC. ^{1}H-NMR spectra were performed using Bruker 300 or 600 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Compounds **3a**–**g**, **5**, and **6** were synthesized according to the literature [10, 11, 12].

*2-Amino-4-(benzyloxy)-5-methoxybenzaldehyde **7***

Compound **6** (24.6 g, 0.085 mol), absolute ethanol (55 mL) and formamide (2 mL) were stirred vigorously and heated until the solution boiled gently. The heating was turned off, and 5-g portions of 28.0 g (0.43 mol) of activated zinc dust were added frequently enough to keep the solution boiling and refluxed for 8 hours. The hot mixture was filtered and the solvent was cooled to room temperature. The yellow crystals was filtered, washed with ethanol and dried to give **7** (13.5 g, 62%). M.p. 198–200°C; MS [MH^+] (m/z): 258.1; ^{1}H-NMR ($CDCl_3$), δ: 10.2 (s, 1H, *C*HO), 7.2–7.1 (m, 5H, Ph-H), 7.0 (s, 1H, C^6-H), 6.1 (s, 1H,C^3-H), 4.9 (s, 2H, CH_2O), 3.8 (s, 3H, OCH_3).

General procedure for the synthesis of ethyl 7-hydroxy-6-methoxy-2-((arylthio)methyl)quinoline-3-carboxylate ***9a – 9f***

To a solution of *o*-aminobenzaldehyde **7** (13.5 g, 0.053 mol) and ethyl 3-oxo-4-(arylthio)butanoate **3** (0.053 mol) in isopropanol (10 mL) was added piperidine (0.5 mL, 0.005 mol). The solution was stirred at reflux for 12 – 15 hours. The mixture was cooled down to room temperature. The solvent was removed under vacuum, and then 20 mL water was added. The water solution was extracted with methylene chloride, and the organic phase was washed with saturated sodium chloride solution and water. The organic phase was dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield the crude compounds **8** as red oil. The mixed acid (AcOH/HCl = 2/1, 25 mL) was poured into the red oil **8** directly, and the solution was heated at 80°C for 6 h. The reaction mixture was cooled to room temperature, and the isolated solids were filtered off, dried to give compounds **9** as a buff to yellow powder.

9a: Yield 92%, m.p.: 182 – 184°C, MS [MH$^+$] (m/z): 369.4, ^{1}H-NMR (DMSO-d$_6$) δ: 8.3 (s, 1H, C^4-H), 7.2 (s, 1H, C^8-H), 7.2 – 7.0 (m, 6H, Ph-H + C^5-H), 4.3 (s, 2H, C*H*$_2$S), 4.2 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.5 (s, 3H, OC*H*$_3$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

9b: Yield 85%, m.p.: 174 – 176°C, MS [MH$^+$] (m/z): 387.2, ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.4 (s, 1H, C^8-H), 7.3 (t, 2H, Ph-H), 7.1 (s, 1H, C^5-H), 7.0 (q, 2H, J = 5.1 Hz, Ph-H), 4.4 (s, 2H, C*H*$_2$S), 4.3 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.5 (s, 3H, OC*H*$_3$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

9c: Yield 87%, m.p.: 186 – 189°C, MS [MH$^+$] (m/z): 399.2, ^{1}H-NMR (DMSO-d$_6$) δ: 8.4 (s, 1H, C^4-H), 7.3 (s, 1H, C^8-H), 7.2 (d, 2H, J = 8.8 Hz, Ph-H), 7.3 (s, 1H, C^5-H), 7.0 (d, 2H, J = 8.8 Hz, Ph-H), 4.3 (s, 2H, C*H*$_2$S), 4.2 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.7 (s, 3H, OC*H*$_3$), 3.5 (s, 3H, OC*H*$_3$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

9d: Yield 68%, m.p.: 162 – 164°C, MS [MH$^+$] (m/z): 417.0, ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.3 (s, 1H, C^8-H), 7.2 (s, 1H, C^5-H), 7.1 (d, 2H, J = 8.4 Hz, Ph-H), 7.0 (d, 2H, J = 8.4 Hz, Ph-H), 4.3 (q, 2H, J = 7.0 Hz, C*H*$_2$CH$_3$), 4.0 (s, 2H, C*H*$_2$S), 3.7 (s, 3H, OC*H*$_3$), 3.4 (s, 2H, SC*H*$_2$), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$C*H*$_3$).

9e: Yield 90%, m.p.: 185 – 188°C, MS [MH$^+$] (m/z): 383.2, ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.3 (s, 1H, C^8-H), 7.2 – 7.1 (m, 4H, Ph-H + C^5-H), 6.8 (s, 1H, Ph-H), 4.5 (s, 2H, C*H*$_2$S), 4.3 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.6 (s, 3H, OC*H*$_3$), 2.2 (s, 3H, CC*H*$_3$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

9f: Yield 82%, m.p.: 187 – 190°C, MS [MH$^+$] (m/z): 405.1, ^{1}H-NMR (DMSO-d$_6$) δ: 8.6 (s, 1H, C^4-H), 7.3 (s, 1H, C^8-H), 7.2 (s, 1H, C^5-H), 6.9 – 6.8 (m, 2H, Ph-H), 6.8 (d, 1H, J = 8.9 Hz, Ph-H), 4.4 (s, 2H, C*H*$_2$S), 4.3 (q, 2H, J = 7.0 Hz, C*H*$_2$CH$_3$), 3.6 (s, 3H, OC*H*$_3$), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$C*H*$_3$).

General procedure for the synthesis of ethyl 7-hydroxy-8-((dimethylamino)methyl)-6-methoxy-2-((arylthio)methyl)-quinoline-3-carboxylate ***10a – 10f***

A solution of 33% dimethylamine (3.8 mL, 0.025 mol) in acetic acid was cooled to 10°C, and then 37% formaldehyde (1.1 mL, 0.011 mol) was added and stirred at 10°C for 30 min. Compound **9** (0.01 mol) was added to the reaction mixture, then heated to 50°C for 3 – 5 hours. The solvent was evaporated *in vacuo,* and poured into water (100 mL). The pH of the resulting mixture was adjusted to 8 with 15% sodium hydroxide solution, and the solid product was collected by filtration, washed with water. The crude product was recrystallized from acetone to obtain compound **10a – g**.

10a: Yield 95%, m.p.: 176 – 178°C, MS [MH$^+$] (m/z): 427.2, ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.4 (d, 2H, Ph-H), 7.3 – 7.2 (m, 3H, Ph-H + C^5-H), 7.1 (t, 1H, Ph-H), 4.7 (s, 2H, C*H*$_2$S), 4.4 (s, 2H, C*H*$_2$N), 4.3 (q, 2H, J = 7.0 Hz, C*H*$_2$CH$_3$), 3.8 (s, 3H, OC*H*$_3$), 2.4 (s, 6H, N(C*H*$_3$)$_2$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

10b: Yield 90%, m.p.: 149 – 151°C, MS [MH$^+$] (m/z): 445.1, ^{1}H-NMR (DMSO) δ: 8.5 (s, 1H, C^4-H), 7.6 (q, 2H, J = 5.2 Hz, Ph-H), 7.4 (t, 2H, J = 8.8 Hz, Ph-H), 7.3 (s, 1H, C^5-H), 4.7 (s, 2H, C*H*$_2$S), 4.2 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.9 (s, 2H, C*H*$_2$N), 3.8 (s, 3H, OC*H*$_3$), 2.1 (s, 6H, N(C*H*$_3$)$_2$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

10c: Yield 91%, m.p.: 153 – 155°C, MS [MH$^+$] (m/z): 457.2, ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.1 (d, 2H, J = 8.6 Hz, Ph-H), 6.9 (d, 2H, J = 8.8 Hz, Ph-H), 4.6 (s, 2H, C*H*$_2$S), 4.3 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 4.0 (s, 2H, C*H*$_2$N), 3.8 (s, 3H, OC*H*$_3$), 3.6 (s, 3H, OC*H*$_3$), 2.3 (s, 6H, N(C*H*$_3$)$_2$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

10d: Yield 79%, m.p.: 143 – 146°C, MS [MH$^+$] (m/z): 476.1, ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.4 (s, 1H, C^5-H), 7.1 (d, 2H, J = 8.5 Hz, Ph-H), 7.0 (d, 2H, J = 8.5 Hz, Ph-H), 4.3 (q, 2H, J = 6.9 Hz, C*H*$_2$CH$_3$), 4.2 (s, 2H, C*H*$_2$S), 3.9 (s, 2H, C*H*$_2$N), 3.7 (s, 3H, OC*H*$_3$), 3.5 (s, 2H, SC*H*$_2$), 2.6 (s, 6H, N(C*H*$_3$)$_2$), 1.3 (t, 3H, J = 6.9 Hz, CH$_2$C*H*$_3$).

10e: Yield 96%, m.p.: 147 – 149°C, MS [MH$^+$] (m/z): 441.1, ^{1}H-NMR (DMSO-d$_6$) δ: 8.4 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.2 – 7.1 (m, 3H, Ph-H), 6.9 (d, 2H, J = 7.1 Hz, Ph-H), 4.6 (s, 2H, CH$_2$S), 4.5 (s, 2H, C*H*$_2$N), 4.3 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.8 (s, 3H, OC*H*$_3$), 2.4 (s, 6H, N(C*H*$_3$)$_2$), 2.2 (s, 3H, CC*H*$_3$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$).

10f: Yield 89%, m.p.: 162 – 165°C, MS [MH$^+$] (m/z): 463.0, ^{1}H-NMR (DMSO-d$_6$) δ: 8.3 (s, 1H, C^4-H), 7.4 – 7.3 (m, 3H, Ph-H + C^5-H), 7.2 (s, 1H, Ph-H), 4.7 (s, 2H, C*H*$_2$S), 4.3 (q, 2H, J = 7.0 Hz, C*H*$_2$CH$_3$), 4.1 (s, 2H, C*H*$_2$N), 3.7 (s, 3H, OC*H*$_3$), 2.0 (s, 6H, N(C*H*$_3$)$_2$), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$C*H*$_3$).

General procedure for the synthesis of the target compounds ***11a – 11g***

To a solution of 1*H*-imidazole or 2-methyl-1*H*-imidazole (0.05 mol) and 36% HCl (4.3 mL, 0.05 mol) in 1,4-dioxane (30 mL) was added **10** (0.01 mol). The reaction mixture was further stirred at 60 – 80°C for 2 – 3 hours. After cooling, the resulting mixture was poured into water (100 mL), then the precipitate was collected by filtration, washed with water and acetone. The crude production was recrystallized from ethanol/water (3/1) to obtain the desired compounds **11a – g**.

Ethyl 8-((1H-imidazole-1-yl)methyl)-7-hydroxy-6-methoxy-2-((phenylthio)methyl)quinoline-3-carboxylate ***11a***

Yield 83%, m.p.: 148-151°C, MS [MH$^+$] (m/z): 450.1, IR (KBr, cm^{-1}): 3415.8 (OH), 1703.1 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.5 (s, 1H, imidazolyl-H), 7.4 – 7.2 (m, 6H, Ph-H + C^5-H), 7.1 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.4 (s, 2H, C*H*$_2$N), 4.7 (s, 2H, C*H*$_2$S), 4.3 (q, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$), 3.8 (s, 3H, OC*H*$_3$), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$). Anal. calcd. for $C_{24}H_{23}N_3O_4S$: C 64.13, H 5.16, N 9.35. Found: C: 64.01, H 5.19, N 9.28.

Ethyl 7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazole-1-yl)methyl)-2-((phenylthio)methyl)quinoline-3-carboxylate ***11b***

Yield 76%, m.p.: 132 – 134°C, MS [MH$^+$] (m/z): 464.1, IR (KBr, cm^{-1}): 3430.0 (OH), 1705.8 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.3 (d, 2H, J = 7.1 Hz Ph-H), 7.2 (t, 2H, Ph-H), 7.1 (d, 1H, J = 7.1 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.5 (s, 1H, imida-

zolyl-H), 5.4 (s, 2H, CH_2N), 4.7 (s, 2H, CH_2S), 4.3 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.9 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{25}H_{25}N_3O_4S$: C 64.78, H 5.44, N 9.06. Found: C: 64.69, H 5.48, N 9.02.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((4-fluorophenylthio)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***11c***

Yield 81%, m.p.: 129–131°C, MS [MH$^+$] (m/z): 468.0, IR (KBr, cm^{-1}): 3424.7 (OH), 1700.5 (C=O); ^{1}H-NMR (DMSO) δ: 8.6 (s, 1H, C^4-H), 7.5 (s, 2H, imidazolyl-H + C^5-H), 7.4 (q, 2H, J = 5.2 Hz, Ph-H), 7.3 (t, 2H, J = 8.8 Hz, Ph-H), 7.0 (s, 1H, imidazolyl-H), 6.8 (s, 1H, imidazolyl-H), 5.3 (s, 2H, CH_2N), 4.7 (s, 2H, CH_2S), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{22}FN_3O_4S$: C 61.66, H 4.74, N 8.99. Found: C: 61.58, H 4.70, N 9.05.

Ethyl 2-((4-methoxyphenylthio)methyl)-7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)quinoline-3-carboxylate ***11d***

Yield 74%, m.p.: 144–147°C, MS [MH$^+$] (m/z): 494.1, IR (KBr, cm^{-1}): 3426.4 (OH), 1709.5 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.6 (s, 1H, C^5-H), 7.1 (d, 2H, J = 8.5 Hz, Ph-H), 6.9 (s, 2H, imidazolyl-H), 6.7 (d, 2H, J = 8.5 Hz, Ph-H), 5.4 (s, 2H, CH_2N), 4.6 (s, 2H, CH_2S), 4.3 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.9 (s, 3H, OCH_3), 3.6 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{26}H_{27}N_3O_5S$: C 63.27, H 5.51, N 8.51. Found: C: 63.21, H 5.47, N 8.56.

Ethyl 2-((4-chlorobenzylthio)methyl)-7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)quinoline-3-carboxylate ***11e***

Yield 79%, m.p.: 153–155°C, MS [MH$^+$] (m/z): 513.0, IR (KBr, cm^{-1}): 3423.7 (OH), 1715.2 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.6 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.2 (d, 2H, J = 8.5 Hz, Ph-H), 7.1 (d, 2H, J = 8.5 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.5 (s, 1H, imidazolyl-H), 5.5 (s, 2H, CH_2N), 4.3 (q, 2H, J = 7.0 Hz, CH_2CH$_3$), 4.2 (s, 2H, CH_2S), 3.9 (s, 3H, OCH_3), 3.6 (s, 2H, SCH_2), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). Anal. calcd. for $C_{26}H_{26}ClN_3O_4S$: C 60.99, H 5.12, N 8.21. Found: C: 60.87, H 5.14, N 8.17.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((3-methylphenylthio)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***11f***

Yield 84%, m.p.: 131–133°C, MS [MH$^+$] (m/z): 464.1, IR (KBr, cm^{-1}): 3423.0 (OH), 1705.8 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.8 (s, 1H, imidazolyl-H), 7.5 (s, 1H, C^5-H), 7.2–7.1 (m, 3H, Ph-H), 7.1 (s, 1H, imidazolyl-H), 6.9 (d, 1H, J = 7.3 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 5.5 (s, 2H, CH_2N), 4.8 (s, 2H, CH_2S), 4.3 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.9 (s, 3H, OCH_3), 2.2 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{25}H_{25}N_3O_4S$: C 64.78, H 5.44, N 9.06. Found: C: 64.72, H 5.51, N 8.97.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((3,4-difluorophenylthio)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***11g***

Yield 64%, m.p.: 123–126°C, MS [MH$^+$] (m/z): 486.0, IR (KBr, cm^{-1}): 3417.5 (OH), 1703.6 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.6 (s, 1H, C^4-H), 7.8 (s, 1H, imidazolyl-H), 7.5–7.4 (m, 3H, Ph-H + C^5-H), 7.3 (s, 1H, Ph-H), 6.9 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 4.7 (s, 2H, CH_2S), 4.2 (q, 2H, J = 7.0 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{21}F_2N_3O_4S$: C 59.37, H 4.36, N 8.65. Found: C: 59.29, H 4.41, N 8.60.

General procedure for the synthesis of the target compounds ***12a–12g***

A solution of compound **11** (0.01 mol) and sodium perborate (1.7 g, 0.011 mol) in acetic acid (40 mL) was heated to 40°C for 3-5 hours. The solvent was evaporated *in vacuo,* and poured into water (100 mL). The precipitate was filtered, washed with acetone, dried, and recrystallized from ethanol to obtain the target compound **12a–g**.

Ethyl 8-((1H-imidazol-1-yl)methyl)-7-hydroxy-6-methoxy-2-((phenylsulfinyl)methyl)quinoline-3-carboxylate ***12a***

Yield 78%, m.p.: 135–137°C, MS [MH$^+$] (m/z): 466.1, IR (KBr, cm^{-1}): 3406.9 (OH), 1697.0 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.5 (s, 1H, C^4-H), 7.6 (s, 1H, imidazolyl-H), 7.5–7.4 (m, 6H, Ph-H + C^5-H), 7.1 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 4.9 (d, 1H, J = 12.7 Hz, CH_2SO), 4.7 (d, 1H, J = 12.7 Hz, CH_2SO) 4.2 (q, 2H, J = 7.0 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{23}N_3O_5S$: C 61.92, H 4.98, N 9.03. Found: C: 62.11, H 4.87, N 8.89.

Ethyl 7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)-2-((phenylsulfinyl)methyl)quinoline-3-carboxylate ***12b***

Yield 73%, m.p.: 154–156°C, MS [MH$^+$] (m/z): 480.0, IR (KBr, cm^{-1}): 3427.7 (OH), 1695.3 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.4 (s, 1H, C^4-H), 7.4 (s, 6H, Ph-H + C^5-H), 7.0 (s, 1H, imidazolyl-H), 6.4 (s, 1H, imidazolyl-H), 5.1 (s, 2H, CH_2N), 4.8 (d, 1H, J = 12.1 Hz, CH_2SO), 4.7 (d, 1H, J = 12.1 Hz, CH_2SO), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{25}H_{25}N_3O_5S$: C 62.61, H 5.25, N 8.76. Found: C: 62.57, H 5.28, N 8.71.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((4-fluorophenylsulfinyl)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***12c***

Yield 79%, m.p.: 131–134°C, MS [MH$^+$] (m/z): 484.1, IR (KBr, cm^{-1}): 3425.9 (OH), 1701.2 (C=O); ^{1}H-NMR (DMSO) δ: 8.4 (s, 1H, C^4-H), 7.5 (s, 2H, imidazolyl-H + C^5-H), 7.4 (q, 2H, J = 5.2 Hz, Ph-H), 7.3 (t, 2H, J = 8.8 Hz, Ph-H), 6.9 (s, 1H, imidazolyl-H), 6.8 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 4.8 (d, J = 12.6 Hz, 1H, CH_2SO), 4.6 (d, J = 12.6 Hz, 1H, CH_2SO), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{22}FN_3O_5S$: C 59.62, H 4.59, N 8.69. Found: C: 59.67, H 4.53, N 8.71.

Ethyl 2-((4-methoxyphenylsulfinyl)methyl)-7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)quinoline-3-carboxylate ***12d***

Yield 80%, m.p.: 138–141°C, MS [MH$^+$] (m/z): 510.0, IR (KBr, cm^{-1}): 3449.5 (OH), 1699.5 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.3 (d, 2H, J = 8.8 Hz, Ph-H), 7.0 (d, 2H, J = 8.8 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.5 (s, 1H, imidazolyl-H), 5.4 (s,

2H, CH_2N), 4.9 (d, 1H, J = 12.1 Hz, CH_2SO), 4.7 (d, 1H, J = 12.1 Hz, CH_2SO), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 3.6 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{26}H_{27}N_3O_6S$: C 61.28, H 5.34, N 8.25. Found: C: 61.25, H 5.27, N 8.31.

Ethyl 2-((4-chlorobenzylsulfinyl)methyl)-7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)quinoline-3-carboxylate ***12e***

Yield 65%, m.p.: 168 – 171°C, MS [MH$^+$] (m/z): 529.1, IR (KBr, cm^{-1}): 3424.5 (OH), 1712.3 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.6 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.2 (t, 4H, J = 8.5 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.5 (s, 1H, imidazolyl-H), 5.5 (s, 2H, CH_2N), 4.8 (d, 1H, J = 12.3 Hz, CH_2SO), 4.5 (d, 1H, J = 12.3 Hz, CH_2SO), 4.3 (q, 2H, J = 7.0 Hz, CH_2CH$_3$), 3.9 (s, 2H, SCH_2), 3.8 (s, 3H, OCH_3), 2.5 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). Anal. calcd. for $C_{26}H_{26}ClN_3O_5S$: C 59.14, H 4.96, N 7.96. Found: C: 58.93, H 5.03, N 7.89.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((3-methylphenylsulfinyl)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***12f***

Yield 77%, m.p.: 144 – 146°C, MS [MH$^+$] (m/z): 480.1, IR (KBr, cm^{-1}): 3426.1 (OH), 1708.5 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.5 (s, 2H, imidazolyl-H + C^5-H), 7.3 – 7.2 (m, 3H, Ph-H), 7.1 (d, 1H, J = 5.2 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 4.8 (d, 1H, J = 12.1 Hz, CH_2SO), 4.6 (d, 1H, J = 12.1 Hz, CH_2SO), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.9 (s, 3H, OCH_3), 2.2 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{25}H_{25}N_3O_5S$: C 62.61, H 5.25, N 8.76. Found: C: 62.55, H 5.20, N 8.71.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((3,4-difluorophenylsulfinyl)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***12g***

Yield 72%, m.p.: 129 – 131°C, MS [MH$^+$] (m/z): 502.1, IR (KBr, cm^{-1}): 3408.3 (OH), 1710.9 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.7 (s, 1H, imidazolyl-H), 7.6 – 7.5 (m, 3H, Ph-H + C^5-H), 7.3 (s, 1H, Ph-H), 7.0 (s, 1H, imidazolyl-H), 6.7 (s, 1H, imidazolyl-H), 5.5 (s, 2H, CH_2N), 5.0 (d, 1H, J = 12.7 Hz, CH_2SO), 4.7 (d, 1H, J = 12.7 Hz, CH_2SO), 4.3 (q, J = 7.1 Hz, 2H, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{21}F_2N_3O_5S$: C 57.48, H 4.22, N 8.38. Found: C: 57.41, H 4.27, N 8.35.

General procedure for the synthesis of the target compounds ***13a – 13g***

Compound **11** (0.01 mol) was first dissolved in methanol (40 mL). Then, sodium wolframate dihydrate (0.3 g, 0.001 mol) and 30% hydrogen peroxide (3.1 mL, 0.03 mol) were successively dropped into the solution and stirred at 20°C for 3 – 7 hours. The reaction mixture was poured into 100 mL water, and the precipitate was filtered, washed with acetone, dried, to give compound **13a – g**.

Ethyl 8-((1H-imidazol-1-yl)methyl)-7-hydroxy-6-methoxy-2-((phenylsulfonyl)methyl)quinoline-3-carboxylate ***13a***

Yield 85%, m.p.: 143 – 145°C, MS [MH$^+$] (m/z): 482.0, IR (KBr, cm^{-1}): 3422.6 (OH), 1714.9 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.6 – 7.5 (m, 3H, imidazolyl-H + Ph-H), 7.5 – 7.4 (m, 4H, Ph-H + C^5-H), 6.9 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 5.1 (s, 2H, CH_2SO$_2$), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.9 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{23}N_3O_6S$: C 59.86, H 4.81, N 8.73. Found: C: 59.72, H 4.89, N 8.64.

Ethyl 7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)-2-((phenylsulfonyl)methyl)quinoline-3-carboxylate ***13b***

Yield 87%, m.p.: 146 – 149°C, MS [MH$^+$] (m/z): 496.1, IR (KBr, cm^{-1}): 3427.6 (OH), 1707.8 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.6 (s, 1H, C^4-H), 7.6 (s, 1H, C^5-H), 7.5 – 7.4 (m, 4H, Ph-H), 7.2 (d, 1H, J = 7.3 Hz, Ph-H), 6.9 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 5.2 (s, 2H, CH_2SO$_2$), 4.3 (q, J = 7.0 Hz, 2H, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). Anal. calcd. for $C_{25}H_{25}N_3O_6S$: C 60.59, H 5.08, N 8.48. Found: C: 60.47, H 5.12, N 8.41.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((4-fluorophenylsulfonyl)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***13c***

Yield 83%, m.p.: 137 – 139°C, MS [MH$^+$] (m/z): 500.1, IR (KBr, cm^{-1}): 3424.5 (OH), 1712.6 (C=O); ^{1}H-NMR (DMSO) δ: 8.5 (s, 1H, C^4-H), 7.6 (s, 1H, imidazolyl-H), 7.5 (s, 1H, C^5-H), 7.4 (q, 2H, J = 5.3 Hz, Ph-H), 7.3 (t, 2H, J = 8.9 Hz, Ph-H), 7.0 (s, 1H, imidazolyl-H), 6.9 (s, 1H, imidazolyl-H), 5.3 (s, 2H, CH_2N), 5.1 (s, 2H, CH_2SO$_2$), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{24}H_{22}FN_3O_6S$: C 57.71, H 4.44, N 8.41. Found: C: 57.63, H 4.49, N 8.47.

Ethyl 2-((4-methoxyphenylsulfonyl)methyl)-7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)quinoline-3-carboxylate ***13d***

Yield 79%, m.p.: 136 – 139°C, MS [MH$^+$] (m/z): 526.2, IR (KBr, cm^{-1}): 3422.6 (OH), 1716.8 (C=O); ^{1}HNMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.5 (s, 1H, C^5-H), 7.4 (d, 2H, J = 8.8 Hz, Ph-H), 7.0 (d, 2H, J = 8.8 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.5 (s, 1H, imidazolyl-H), 5.3 (s, 2H, CH$_2$N), 5.1 (s, 2H, CH$_2$SO$_2$), 4.3 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.9 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{26}H_{27}N_3O_7S$: C 59.42, H 5.18, N 8.00. Found: C: 59.38, H 5.23, N 7.96.

Ethyl 2-((4-chlorobenzylsulfonyl)methyl)-7-hydroxy-6-methoxy-8-((2-methyl-1H-imidazol-1-yl)methyl)quinoline-3-carboxylate ***13e***

Yield 82%, m.p.: 147 – 149°C, MS [MH$^+$] (m/z): 545.1, IR (KBr, cm^{-1}): 3422.8 (OH), 1705.1 (C=O); ^{1}H-NMR (DMSO-d$_6$) δ: 8.7 (s, 1H, C^4-H), 7.6 (s, 1H, C^5-H), 7.4 (d, 2H, J = 8.5 Hz, Ph-H), 7.3 (d, 2H, J = 8.5 Hz, Ph-H), 6.8 (s, 1H, imidazolyl-H), 6.5 (s, 1H, imidazolyl-H), 5.5 (s, 2H, CH_2N), 5.2 (s, 2H, CH_2SO$_2$), 4.4 (s, 2H, SCH_2), 4.2 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 3.8 (s, 3H, OCH_3), 2.4 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd. for $C_{26}H_{26}ClN_3O_6S$: C 57.40, H 4.82, N 7.72. Found: C: 57.52, H 4.88, N 7.63.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((3-methylphenylsulfonyl)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***13f***

Yield 85%, m.p.: 137–139°C, MS [MH^+] (m/z): 496.1, IR (KBr, cm^{-1}): 3417.0 (OH), 1710.8 (C=O); ^{1}H-NMR (DMSO-d_6) δ 8.7 (s, 1H, C^4-H), 7.5 (s, 2H, imidazolyl-H + C^5-H), 7.5–7.4 (m, 2H, Ph-H), 7.3 (d, 2H, J = 4.9 Hz, Ph-H), 6.9 (s, 1H, imidazolyl-H), 6.7 (s, 1H, imidazolyl-H), 5.4 (s, 2H, CH_2N), 5.2 (s, 2H, CH_2SO_2) 4.3 (q, 2H, J = 7.1 Hz, CH_2CH_3), 3.9 (s, 3H, OCH_3), 2.2 (s, 3H, CCH_3), 1.3 (t, 3H, J = 7.1 Hz, CH_2CH_3). Anal. calcd. for $C_{25}H_{25}N_3O_6S$: C 60.59, H 5.08, N 8.48. Found: C: 60.51, H 5.14, N 8.42.

Ethyl 8-((1H-imidazol-1-yl)methyl)-2-((3,4-difluorophenylsulfonyl)methyl)-7-hydroxy-6-methoxyquinoline-3-carboxylate ***13g***

Yield 80%, m.p.: 146–149°C, MS [MH^+] (m/z): 518.1, IR (KBr, cm^{-1}): 3427.7 (OH), 1710.1 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 8.6 (s, 1H, C^4-H), 7.8 (s, 1H, imidazolyl-H), 7.5–7.4 (m, 3H, Ph-H + C^5-H), 7.4 (s, 1H, Ph-H), 7.0 (s, 1H, imidazolyl-H), 6.6 (s, 1H, imidazolyl-H), 5.5 (s, 2H, CH_2N), 5.2 (s, 2H, CH_2SO_2), 4.3 (q, 2H, J = 7.0 Hz, CH_2CH_3), 3.9 (s, 3H, OCH_3), 1.3 (t, 3H, J = 7.0 Hz, CH_2CH_3). Anal. calcd. for $C_{24}H_{21}F_2N_3O_6S$: C 55.70, H 4.09, N 8.12. Found: C: 55.63, H 4.14, N 8.07.

Pharmacology

In-vitro anti-HBV activity assay. The antiviral activities of compounds **11a–13g** against HBV in 2.2.15 cells were evaluated by methods reported [14, 15], including the ability to inhibit the production of HBsAg and HBeAg and the replication of HBV DNA in HBV-infected 2.2.15 cells. Briefly, confluent cultures of 2.2.15 cells were maintained on 96-well flat-bottomed tissue culture plates in RPMI 1640 medium with 2% fetal bovine serum. Cultures were treated with eight consecutive daily doses of the test compounds and lamivudine (purchased by Glaxo & Welcome Co., UK). Medium was changed daily with fresh test compounds and positive control for eight days. Extracellular HBV surface and e antigen levels produced from 2.2.15 cells were evaluated by semiquantitative enzyme immunoassay (EIA) methods using commercial kits (HBsAg, Abbott Laboratories, Shanghai, China; HBeAg, Diasorin, Inc., Shanghai, China) as previously described [16]. Intracellular HBV DNA levels were measured by quantitative Southern blot hybridization. The IC_{50} and selected index of the evaluated compounds and lamivudine were calculated, respectively.

Cytotoxicity assay

Cytotoxicity of compounds **11a–13g** was assessed in HepG2.2.15 cells. Briefly, 2.2.15 cells were cultured in 96-well flat-bottomed tissue culture plates and treated with test compound (in 0.2 mL culture medium/well) as described above. Untreated control cultures were maintained on each 96-well plate. Toxicity was determined by measuring neutral red dye uptake, as determined from the absorbance at 510 nm relative to untreated cells, at 24 h following day nine of treatment.

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