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Synthesis and anti-hepatitis B virus evaluation of novel ethyl 6-hydroxyquinoline-3-carboxylates in vitro

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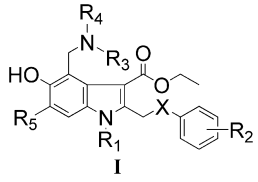
ABSTRACT

A series of non-nucleoside ethyl 6-hydroxyquinoline-3-carboxylate derivatives were prepared and evaluated in HepG2.2.15 cells. Most compounds inhibited the expression of viral antigens HBsAg or HBeAg at low concentration. Six compounds, **9f**$_3$, **12b**$_6$, **12f**$_6$, **13b**$_2$, **13b**$_6$, and **13f**$_6$, displayed excellent intracellular inhibitory activity and selectivity towards the replication of HBV DNA. Of these six initial hits, compound **13b**$_6$ was the most active.

1. Introduction

Hepatitis B virus (HBV) is a major cause of liver cirrhosis and hepatocellular carcinoma in humans. Until recently, it was estimated that 400 million people were chronically infected with HBV worldwide and that HBV was responsible for 750,000 deaths each year.[1] Rates of new HBV infections in developing countries continue to climb at an alarming rate. Currently, only two interferons and four nucleoside inhibitors have received FDA approval as single-agent treatments for HBV infection. Unfortunately, clinical response rates to interferon-α and peginterferon-α tend to be low (20–30%).[2] On the other hand, the required prolonged regimen of nucleoside analogues, such as lamivudine, adefovir dipivoxil, entecavir, and telbivudine, almost invariably leads to drug-resistance problems.[3–6] Drugs with novel structures and mechanisms of action are urgently needed.

Previously, we described a series of 5-hydroxy-1*H*-indole-3-carboxylate derivatives (**I**, Fig. 1) with anti-HBV activities in vitro.[7,8] In these studies, the structure–activity relationship (SAR) between the modification of substituents on the indole cycle and anti-HBV activity was assessed. In order to explore the SAR surrounding the indole region of this chemical series, we designed and prepared some novel ethyl 6-hydroxyquinoline-3-carboxylate derivatives in which the indole ring was expanded to a quinoline moiety. Other changes primarily targeted positions 2 and 5 of the quinoline ring. Aliphatic aminomethyl or *N*-heteroaromatic methyl functionality



I

Figure 1. Structure of 5-hydroxy-1*H*-indole-3-carboxylates.

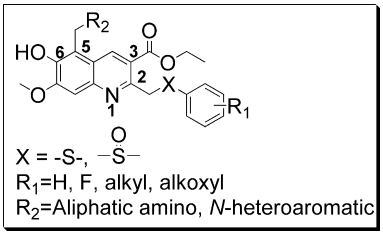


Figure 2. General structure of target compounds.

was introduced at the 5-position, and arylthiomethyl or arylsulfinylmethyl groups were incorporated into the 2-position in order to investigate the influence of these changes on anti-HBV activity (see Fig. 2).

2. Chemistry

The synthesis of target compounds **9a**–**9g** was achieved using a convenient seven-step procedure starting from 3-hydroxy-4-

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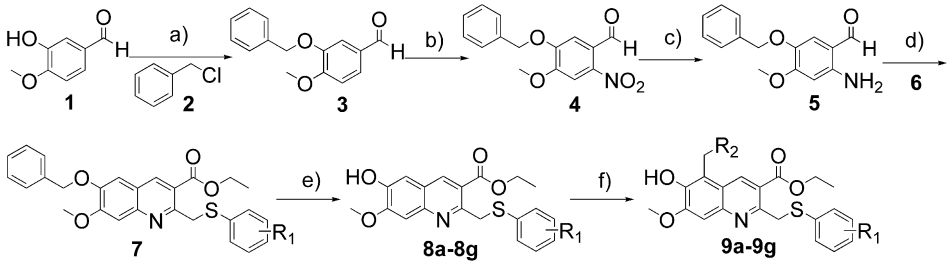
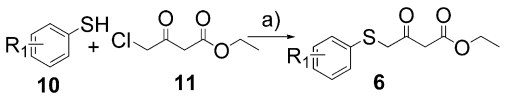


8a: R_1=H **8b**: R_1=4-Fluoro **8c**: R_1=2-Nitrogen **8d**: R_1=2-Methoxy
8e: R_1=3-Methoxy **8f**: R_1=3,4-Difluoro **8g**: R_1=3,4-Dimethoxy

$9a_3$: R_1=H, R_2=Piperidin-1-yl
$9b_1$: R_1=4-Fluoro, R_2=Dimethylamino
$9b_2$: R_1=4-Fluoro, R_2=Pyrrolidin-1-yl
$9c_2$: R_1=2-Nitrogen, R_2=Pyrrolidin-1-yl
$9d_1$: R_1=2-Methoxy, R_2=Dimethylamino
$9e_1$: R_1=3-Methoxy, R_2=Dimethylamino
$9f_1$: R_1=3,4-Difluoro, R_2=Dimethylamino
$9f_2$: R_1=3,4-Difluoro, R_2=Pyrrolidin-1-yl
$9f_3$: R_1=3,4-Difluoro, R_2=Piperidin-1-yl
$9f_4$: R_1=3,4-Difluoro, R_2=Morpholin-4-yl
$9f_5$: R_1=3,4-Difluoro, R_2=4-Methyl piperazin-1-yl
$9g_1$: R_1=3,4-Dimethoxy, R_2=Dimethylamino

Scheme 1. Synthesis of compounds **9a**–**9g**. Reagents and conditions: (a) K_2CO_3, DMF, 90 °C, 3 h, 90%; (b) fuming HNO_3, 40 °C, 5 h, 78%; (c) $HCONH_2$, Zn, absolute alcohol, reflux, 8 h, 50%; (d) piperidine, isopropanol, reflux, 12–15 h; (e) acetic acid/hydrochloric acid = 2/1 (volume ratio), 80 °C, 5–8 h, 44–59%; (f) aliphatic amine, 37% aqueous formaldehyde, methanol, HCl, 40 °C, 2–5 h, 68–76%. The overall yield from 10.5% to 15.7%.



Scheme 2. Synthesis of ethyl 3-oxo-4-((sub)phenylthio)butanoate **6**. Reagents and conditions: KOH, methanol, 25 °C, 8–14 h, 94–98%.

methoxylbenzaldehyde (**1**) depicted in Scheme 1. The commercially available compound **1** was treated with benzyl chloride **2** to give the compound **3**,[9] which was nitrated with a typical nitration reagent, fuming nitric acid, to provide 2-nitrobenzaldehyde **4**.[10] Activated zinc powder and catalytic formamide efficiently reduced the nitro group prior to the aldehyde group to get 2-aminobenzaldehyde **5**. The thioether **6** was prepared via etherification of ethyl 4-chloroacetoacetate **11** with the corresponding thiophenol **10** (Scheme 2).[8] Treatment of 2-aminobenzaldehyde **5** with **6** in the presence of piperidine afforded the key intermediate, ethyl quinoline-3-carboxylate **7**,[11] which was subsequently deprotected with a 2:1 mixture of acetic acid and hydrochloric acid to produce compound **8**. The target compounds, **9a**–**9g**, were then synthesized via a Mannich reaction as previously demonstrated.[7,8] When acetic acid was employed as the solvent, the reaction generated a mixture of products, including the desired mono-Mannich derivative and the bis-Mannich byproduct **A** (Scheme 3).[12] An alternative approach was explored, and the appropriate aliphatic amines were treated with compound **8** and 37% aqueous formaldehyde in methanol solvent to yield the expected compounds, **9a**–**9g**.

Imidazole or methylimidazole could not be introduced into the 5-position by a direct Mannich reaction with **8**. Alternatively, it can be realized through the following two steps: The corresponding dimethylamine derivatives of **9** were obtained via a Mannich reaction. And then treatment of **9** with imidazole or 2-methylimidazole in 1,4-dioxane generated five *N*-heteroaromatic derivatives **12b**–**12g** in good yields (Scheme 4).

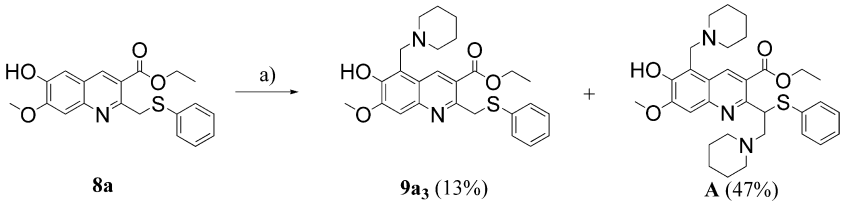
To assess whether 2-sulfinyl analogues could reduce cytotoxicity and increase potency, **$9b_1$**, **$9b_2$**, **$12f_6$**, etc. were oxidated using sodium perborate to provide seven target compounds **13b**–**13f** (Scheme 5).

3. Anti-HBV analysis

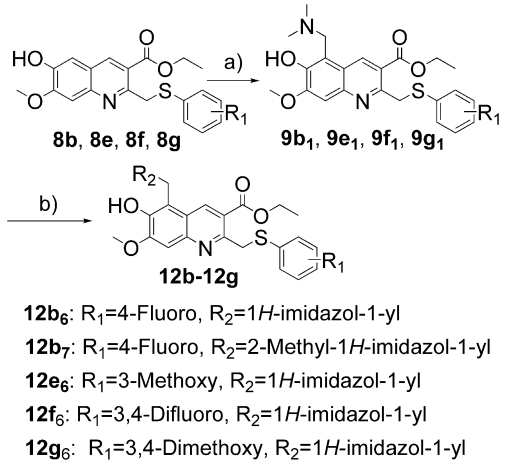
All of the target compounds were tested in vitro in HepG2.2.15 cells for anti-HBV activity and cytotoxicity. These compounds' properties are summarized in Table 1, in which they are compared to those of lamivudine. With the exception of **$9c_2$**, **$12g_6$**, and **$13c_2$**, all of the compounds exhibited an overall inhibition of virion including HBV antigen secretion and HBV DNA replication.

Although this series of compounds did not display any obvious SAR for anti-HBsAg and anti-HBeAg activity, six structural components, represented by 2-(fluoro-substituted phenyl)quinoline derivatives **$9b_1$**, **$9b_2$**, **$9f_4$**, **$9f_5$**, **$12b_7$**, and **$12f_6$**, were required for high potency (IC_{50} < 20 μM). Of these compounds, **$9f_4$** and **$9b_2$** were the most potent inhibitors of HBsAg production (IC_{50} = 5.8 μM, SI = 13.7) and HBeAg production (IC_{50} = 9.3 μM, SI = 5.1), respectively.

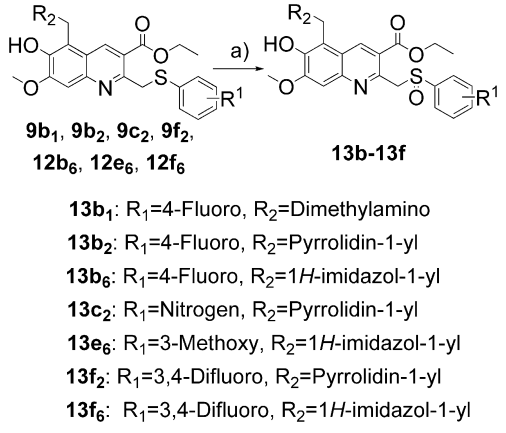
Twelve compounds (**$9a_3$**, **$9b_1$**,**$9b_2$**, **$9f_3$**, **$12b_6$**, **$12b_7$**, **$12f_6$**, **$13b_1$**, **$13b_2$**, **$13b_6$**, **$13f_2$**, and **$13f_6$**) exhibited more potent inhibition of HBV DNA replication (10- to 66-fold) than the control drug, lami-



Scheme 3. The Mannich reaction of **8a** in acetic acid.



Scheme 4. Synthesis of compounds **12b–12g**. Reagents and conditions: (a) 33% solution of dimethylamine, 37% aqueous formaldehyde, methanol, 40 °C, 2–5 h, 68–72%; (b) 1*H*-imidazole or 2-methyl-1*H*-imidazole, hydrochloric acid, 1,4-dioxane, 75–80 °C, 2–3 h, 60–73%.



Scheme 5. Synthesis of compounds **13b**–**13f**. Reagents and conditions: (a) sodium perborate, sodium tungstate, acetic acid, 40 °C, 3–5 h, 56–74%.

vudine. Among these, $\mathbf{9f}_3$, $\mathbf{12b}_6$, $\mathbf{12f}_6$, $\mathbf{13b}_2$, $\mathbf{13b}_6$, and $\mathbf{13f}_6$ possessed selectivity indices from 5.6 ($\mathbf{9f}_3$) to 34.1 ($\mathbf{13b}_6$), which were comparable to or higher than that of lamivudine (7.1).

The anti-HBV activities of compounds $\mathbf{9b}_1$, $\mathbf{12b}_6$, $\mathbf{9f}_3$, $\mathbf{12f}_6$, and $\mathbf{13b}_6$, in which an electron-withdrawing fluoride group was introduced at the phenyl moiety of 2-position, were superior to those with electron-donating methoxy group or no substitution ($\mathbf{9d}_1$, $\mathbf{12e}_6$, $\mathbf{12g}_6$, $\mathbf{13e}_6$, and $\mathbf{9a}_3$). However, when the carbon was replaced with nitrogen at 2-position of phenyl ring, the anti-HBV activities were eliminated and the cytotoxicities were enhanced evidently (TC_{50}s of $\mathbf{9c}_2$ and $\mathbf{13c}_2$ were 17.5 μM and 9.4 μM, respectively). Interestingly, the IC_{50} values and SIs of 3,4-difluoro derivatives, such as $\mathbf{12f}_6$, $\mathbf{13f}_2$, and $\mathbf{13f}_6$, were not better than those of monofluorinated derivatives $\mathbf{12b}_6$, $\mathbf{13b}_2$, and $\mathbf{13b}_6$.

Different biological properties were observed when a variety of basic moieties were introduced via a Mannich reaction at the 5-position. Heterocyclic basic groups, such as pyrrolidinyl, piperidinyl, and imidazolyl groups, were preferred at this position. However, when morpholinyl and 4-methyl piperazinyl were introduced into 5-position of compounds, such as $\mathbf{9f}_4$ and $\mathbf{9f}_5$, the anti-HBV activities were eliminated.

While replacement of the sulfur group with a sulfinyl did not evidently alter the drugs' cytotoxicities, it either increased or maintained the drugs' anti-HBV activity (compounds $\mathbf{9b}_1$ vs. $\mathbf{13b}_1$, $\mathbf{12b}_6$ vs. $\mathbf{13b}_6$, $\mathbf{9b}_2$ vs. $\mathbf{13b}_2$, and $\mathbf{12f}_6$ vs. $\mathbf{13f}_6$). The most promising compound, $\mathbf{13b}_6$, which bore an imidazolyl group at the 5-position and a 4-fluorophenylsulifinyl at the 2-position, possessed exceptionally high-potency anti-HBV activity with an IC_{50} of 4.7 μM, a 66-fold improvement over lamivudine (IC_{50} = 311.2 μM).

4. Conclusion

In summary, a series of 6-hydroxyquinoline-3-carboxylate derivatives based on 5-hydroxy-1*H*-indole-3-carboxylate derivatives (**I**, Fig. 1) were synthesized in seven to nine steps and were assayed for their anti-HBV activity and cytotoxicity in vitro. As hoped, the reported anti-HBV properties of **I** also extended to this new series, and quinoline derivatives have shown higher

Table 1
Anti-HBV activity and cytotoxicity of target compounds in vitro

Compound	TC_{50} (μM)[a]	HBsAg		HBeAg		HBV DNA replication	
		IC_{50} (μM)[b]	SI[c]	IC_{50} (μM)	SI	IC_{50} (μM)	SI
$\mathbf{9a}_3$	60.7	–[d]	–	–	–	45.2	1.3
$\mathbf{9b}_1$	120.2	17.4	6.9	–	–	32.8	3.7
$\mathbf{9b}_2$	47.3	8.5	5.6	9.3	5.1	10.2	4.6
$\mathbf{9c}_2$	17.5	–	–	–	–	–	–
$\mathbf{9d}_1$	58.5	–	–	20.5	2.9	–	–
$\mathbf{9f}_3$	135.1	–	–	–	–	24.1	5.6
$\mathbf{9f}_4$	79.7	5.8	13.7	10.9	7.3	–	–
$\mathbf{9f}_5$	55.3	11.2	4.9	–	–	–	–
$\mathbf{12b}_6$	201.5	42.8	6.0	–	–	41.7	6.2
$\mathbf{12b}_7$	78.5	–	–	16.2	4.8	30.6	2.6
$\mathbf{12e}_6$	186.5	–	–	65.1	2.9	–	–
$\mathbf{12f}_6$	132.6	–	–	16.4	8.1	9.4	14.1
$\mathbf{12g}_6$	188.8	–	–	–	–	–	–
$\mathbf{13b}_1$	90.4	36.5	2.5	–	–	21.9	4.1
$\mathbf{13b}_2$	166.3	25.1	6.6	21.1	7.9	10.7	15.5
$\mathbf{13b}_6$	160.3	26.2	6.1	98.1	1.6	4.7	34.1
$\mathbf{13c}_2$	9.4	–	–	–	–	–	–
$\mathbf{13e}_6$	194.2	33.1	5.9	–	–	–	–
$\mathbf{13f}_2$	16.5	–	–	–	–	5.3	3.1
$\mathbf{13f}_6$	90.8	–	–	–	–	12.5	7.3
Lamivudine	2213.8	–	–	–	–	311.2	7.1

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

anti-HBeAg activities than indole derivatives. Most of these compounds demonstrated potential HBV inhibition, and compound **13b**$_6$ was the most potent, highly specific inhibitor of HBV DNA replication in cell culture.

Inhibition assay data reported in this study showed that the substituents on the phenyl ring of 2-position might play an important role in the tested drugs' activities. Fluoride could enhance the inhibition of HBV DNA replication, while 2-pyridyl derivatives could eliminate the anti-HBV activities completely. The different Mannich basic groups placed at the 5-position demonstrated significant distinctness of anti-HBV effects. Oxidation of the compounds' sulfide into a sulfinyl group had little influence on anti-HBV activity and cytotoxicity.

Based on the study, the further structural alterations were focused on 5-position and the phenyl at 2-position of quinoline ring. The various halogens, such as chlorine, bromine, and iodine, would be introduced into phenyl which also could be replaced by *S*-heteroaromatic. Some *N*-heterocycles, such as pyrrolyl, pyrrolinyl, imidazolinyl, etc., would be incorporated into position 5.

5. Experimental

5.1. Chemistry

Melting points were measured with 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). ^{1}H NMR spectra were performed using Bruker 300 MHz and 600 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Compounds **3**, **4**, and thioether **6** were synthesized in accordance with literature procedures.[9,10]

5.1.1. 2-Amino-5-benzyloxy-4-methoxybenzaldehyde (5)

A mixture of **4** (28.7 g, 0.1 mol), formamide (2 mL) and absolute ethanol (60 mL) was heated with vigorous stirring until the solution boiled gently. Heating was discontinued, and 5 g portions of 32.5 g (0.5 mol) of activated zinc dust were added with sufficient frequency to sustain boiling while the reaction refluxed for 8 h. The hot mixture was filtered, and the filtrate was cooled to room temperature. The yellow crystals were filtered, washed with ethanol, and dried to yield **5** (12.8 g, 50%). Mp: 182–184 °C; MS [MH$^+$] (*m*/*z*): 258.1.

5.1.2. General procedure for the synthesis of ethyl 6-hydroxy-7-methoxy-2-((arylthio)methyl)quinoline-3-carboxylate derivatives (8a–8g)

A mixture of *o*-aminobenzaldehyde **5** (12.8 g, 0.05 mol), ethyl 3-oxo-4-(arylthio)butanoate **6** (0.05 mol), piperidine (0.5 mL, 0.005 mol) and isopropanol (10 mL) was stirred at reflux for 12–15 h and then cooled to room temperature. The solvent was removed under vacuum, and 20 mL of water was added. The aqueous solution was extracted with CH_2Cl_2, and the organic phase was washed with saturated sodium chloride solution and water. The organic phase was dried (Na_2SO_4) and evaporated to yield the crude compounds **7** as red oil. A 2:1 mixture of acetic acid and HCl acid (25 mL) was added directly to the red oil **7**, and the resulting solution was heated at 80 °C for 5–8 h. The reaction mixture was cooled to room temperature, and the isolated solids were filtered and dried to give compounds **8a**–**8g** as buff to yellow powder.

5.1.3. General procedure for the synthesis of compounds 9a–9g

A solution of aliphatic amine (0.025 mol), 37% HCHO (3 mL, 0.03 mol) and 36% HCl (0.1 mL) in MeOH was stirred at room temperature for 30 min. Compound **8** (0.01 mol) was added, and the mixture was heated to 40 °C for 2–5 h. The MeOH was removed under vacuum, and the residue was poured into water (100 mL). The resulting mixture was adjusted to pH 7 with 20% hydrochloric acid, and the solid product was collected by filtration and washed with water. The crude product was recrystallized from acetone to obtain compounds **9a**–**9g**.

5.1.3.1. Ethyl 6-hydroxy-7-methoxy-2-((phenylthio)methyl)-5-((piperidin-1-yl)methyl)quinoline-3-carboxylate (9a$_3$). White powder (3.5 g, 75%); Mp: 174–176 °C; MS [MH$^+$] (*m*/*z*): 467.2; ^{1}H NMR (DMSO-d_6): δ 1.38 (t, 3H, *J* = 7.1 Hz, –CH_2CH_3), 1.85 (m, 6H, piperidinyl), 2.67 (m, 4H, piperidinyl), 4.07 (s, 3H, –OC***H***$_3$), 4.35 (q, 2H, *J* = 7.1 Hz, –C***H***$_2$CH$_3$), 4.41 (s, 2H, –C***H***$_2$N–), 4.79 (s, 2H, –C***H***$_2$S–), 7.16 (m, 3H, –Ph***H***), 7.28 (m, 2H, –Ph***H***), 7.21 (s, 1H, C^8–**H**), 8.67 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3421.9 (**OH**), 1703.4 (**C═O**).

5.1.3.2. Ethyl 2-((4-fluorophenylthio)methyl)-5-((dimethylamino)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate (9b$_1$). White powder (3.2 g, 72%); Mp: 183–185 °C; MS [MH$^+$] (*m*/*z*): 445.1; ^{1}H NMR (DMSO-d_6): δ 1.37 (t, 3H, *J* = 7.2 Hz, –CH_2CH_3), 2.80 (d, 6H, *J* = 4.0 Hz, –N(C***H***$_3$)$_2$), 4.06 (s, 3H, –OC***H***$_3$), 4.36 (q, 2H, *J* = 7.2 Hz, –C***H***$_2$CH$_3$), 4.72 (s, 2H, –C***H***$_2$S–), 4.74 (d, 2H, *J* = 4.0 Hz, –C***H***$_2$N–), 7.13 (t, 2H, *J* = 8.7 Hz, –Ph***H***), 7.41 (q, 2H, *J* = 5.5 Hz, –Ph***H***), 7.53 (s, 1H, C^8–**H**), 8.87 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3431.2 (**OH**), 1701.4 (**C═O**).

5.1.3.3. Ethyl 2-((4-fluorophenylthio)methyl)-6-hydroxy-7-methoxy-5-((pyrrolidin-1-yl)methyl)quinoline-3-carboxylate (9b$_2$). White powder (3.5 g, 74%); Mp: 179–181 °C; MS [MH$^+$] (*m*/*z*): 471.0; ^{1}H NMR (DMSO-d_6): δ 1.43 (t, 3H, *J* = 6.9 Hz, –CH_2CH_3), 1.96 (s, 4H, –pyrrolidinyl), 2.90 (s, 4H, –pyrrolidinyl), 4.03 (s, 3H, –OC***H***$_3$), 4.39 (s, 2H, –C***H***$_2$N–), 4.41 (q, 2H, *J* = 6.9 Hz, –C***H***$_2$CH$_3$), 4.76 (s, 2H, –C***H***$_2$S–), 7.12 (t, 2H, *J* = 8.6 Hz, –Ph***H***), 7.39 (q, 2H, *J* = 5.4 Hz, –Ph***H***), 7.32 (s, 1H, C^8–**H**), 8.65 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3419.5 (**OH**), 1708.1 (**C═O**).

5.1.3.4. Ethyl 6-hydroxy-7-methoxy-2-((pyridin-2-ylthio)methyl)-5-((pyrrolidin-1-yl)methyl)quinoline-3-carboxylate (9c$_2$). White powder (3.2 g, 70%); Mp: 142–143 °C; MS [MH$^+$] (*m*/*z*): 454.1; ^{1}H NMR (DMSO-d_6): δ 1.43 (t, 3H, *J* = 7.1 Hz, –CH_2CH_3), 1.89 (s, 4H, –pyrrolidinyl), 2.81 (s, 4H, –pyrrolidinyl), 3.92 (s, 3H, –OC***H***$_3$), 4.11 (s, 2H, –C***H***$_2$N–), 4.40 (q, 2H, *J* = 7.1 Hz, –C***H***$_2$CH$_3$), 4.72 (s, 2H, –C***H***$_2$S–), 7.51 (m, 3H, –pyridyl+C^8–**H**), 7.65 (m, 2H, –pyridyl), 8.66 (s, 1H, C^4-**H**); IR (KBr, cm^{-1}): 3430.5 (**OH**), 1704.6 (**C═O**).

5.1.3.5. Ethyl 2-((2-methoxyphenylthio)methyl)-5-((dimethylamino)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate (9d$_1$). White powder (3.1 g, 68%); Mp: 171–172 °C; MS [MH$^+$] (*m*/*z*): 457.2; ^{1}H NMR (DMSO-d_6): δ 1.42 (t, 3H, *J* = 7.1 Hz, –CH_2CH_3), 2.91 (s, 6H, –N(C***H***$_3$)$_2$), 3.80 (s, 3H, –OC***H***$_3$), 4.03 (s, 3H, –OC***H***$_3$), 4.31 (s, 2H, –C***H***$_2$N–), 4.42 (q, 2H, *J* = 7.1 Hz, –C***H***$_2$CH$_3$), 4.77 (s, 2H, –C***H***$_2$S–), 6.81 (m, 2H, –Ph***H***), 7.15 (m, 1H, –Ph***H***), 7.27 (s, 1H, C^8–**H**), 7.46 (m, 1H, –Ph***H***), 8.62 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3427.1 (**OH**), 1700.8 (**C═O**).

5.1.3.6. Ethyl 2-((3,4-difluorophenylthio)methyl)-6-hydroxy-7-methoxy-5-((piperidin-1-yl)methyl)quinoline-3-carboxylate (9f$_3$). Yellow powder (3.6 g, 72%); Mp: 168–170 °C; MS [MH]$^+$ (*m*/*z*): 503.2; ^{1}H NMR (DMSO-d_6): δ 1.35 (t, 3H, *J* = 7.1 Hz, –CH_2CH_3), 1.53 (s, 6H, –piperidinyl), 2.64 (s, 4H, –piperidinyl), 3.95 (s, 3H, –OC***H***$_3$), 4.06 (s, 2H, –C***H***$_2$N–), 4.32 (q, 2H, *J* = 7.1 Hz, –C***H***$_2$CH$_3$), 4.70 (s, 2H, –C***H***$_2$S–), 7.14–7.18 (m, 2H, –Ph***H***), 7.40–7.45 (m, 2H, –Ph***H***+C^8–**H**) 8.67 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3415.0 (**OH**), 1708.1 (**C=O**).

5.1.3.7. Ethyl 2-((3,4-difluorophenylthio)methyl)-6-hydroxy-7-methoxy-5-(morpholinomethyl)quinoline-3-carboxylate ($9f_4$). White powder (4.1 g, 81%); Mp: 191–193 °C; MS [MH^+] (m/z): 505.2; ^{1}H NMR (DMSO-d_6): δ 1.35 (t, 3H, J = 7.1 Hz, –CH_2CH_3), 3.35 (s, 4H, –morpholino), 3.54 (s, 4H, –morpholino), 3.95 (s, 3H, –OCH_3), 4.38 (s, 2H, –CH_2N–), 4.41 (q, 2H, J = 7.1 Hz, –CH_2CH_3), 4.65 (s, 2H, –CH_2S–), 7.12–7.35 (m, 3H, –Ph***H***), 7.29 (s, 1H, C^8–**H**), 8.75 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3420.1 (**OH**), 1708.4 (**C=O**).

5.1.3.8. Ethyl 2-((3,4-difluorophenylthio)methyl)-6-hydroxy-7-methoxy-5-((4-methylpiperazin-1-yl)methyl)quinoline-3-carboxylate ($9f_5$). Pale yellow powder (3.7 g, 71%); Mp: 186–188°C; MS [MH^+] (m/z): 518.2; ^{1}H NMR (DMSO-d_6): δ 1.41 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 2.36 (s, 3H, –NCH_3), 2.78 (m, 8H, –piperazinyl), 4.01 (s, 3H, –OCH_3), 4.35 (s, 2H, –CH_2N–), 4.46 (q, 2H, J = 7.0 Hz, –CH_2CH_3), 4.68 (s, 2H, –CH_2S–), 7.15–7.36 (m, 3H, –Ph***H***), 7.33 (s, 1H, C^8–**H**), 8.71 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3427.5 (**OH**), 1702.3 (**C=O**).

5.1.4. General procedure for the synthesis of compounds12b–12g

Either 1*H*-imidazole or 2-methyl-1*H*-imidazole (0.05 mol), 36% HCl (4.3 mL, 0.05 mol) and dimethylamino analogues **9** (0.01 mol) were dissolved in 1,4-dioxane (30 mL) and stirred at 75–80 °C for 2–3 h. After cooling to room temperature, the resulting mixture was poured into water (100 mL), and the precipitate was collected by filtration and washed with water and acetone. The crude product was recrystallized from ethanol/water to obtain the desired compounds **12b**–**12g**.

5.1.4.1. Ethyl 5-((1*H*-imidazole-1-yl)methyl)-2-((4-fluorophenylthio)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate ($12b_6$). Pale yellow powder (3.1 g, 66%); Mp: 153–155 °C; MS [MH^+] (m/z): 468.1; ^{1}H NMR (DMSO-d_6): δ 1.34 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 4.01 (s, 3H, –OCH_3), 4.30 (q, 2H, J = 7.0 Hz, –CH_2CH_3), 4.66 (s, 2H, –CH_2S–), 5.60 (s, 2H, –CH_2N–), 6.81 (s, 1H, –imidazolyl-***H***), 7.02 (s, 1H, –imidazolyl-***H***), 7.09 (t, 2H, J = 8.7 Hz, –Ph***H***), 7.38 (m, 3H, –Ph***H***+C^8–***H***), 7.68 (s, 1H, –imidazolyl-***H***), 8.79 (s, 1H, C^4-H); IR (KBr, cm^{-1}): 3416.8 (**OH**), 1704.1 (**C=O**).

5.1.4.2. Ethyl 2-((4-fluorophenylthio)methyl)-6-hydroxy-7-methoxy-5-(2-methyl-1*H*-imidazol-1-yl)quinoline-3-carboxylate ($12b_7$). Yellow powder (3.4 g, 71%); Mp: 167–169 °C; MS [MH^+] (m/z): 482.0; ^{1}H NMR (DMSO-d_6): δ 1.34 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 2.44 (s, 3H, –CCH_3), 4.04 (s, 3H, –OCH_3), 4.31 (q, 2H, J = 7.0 Hz, –CH_2CH_3), 4.69 (s, 2H, –CH_2S–), 5.47 (s, 2H, –CH_2N–), 6.64 (s, 1H, –imidazolyl-***H***), 6.70 (s, 1H, –imidazolyl-***H***), 7.11 (t, 2H, J = 8.7 Hz, –Ph***H***), 7.28 (s, 1H, C^8–***H***), 7.40 (q, 2H, J = 5.4 Hz, –Ph***H***), 8.68 (s, 1H, C^4–H); IR (KBr, cm^{-1}): 3417.5 (**OH**), 1703.7 (**C=O**).

5.1.4.3. Ethyl 5-((1*H*-imidazole-1-yl)methyl)-2-((3-methoxyphenylthio)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate ($12e_6$). White powder (3.5 g, 73%); Mp: 173–175 °C; MS [MH^+] (m/z): 480.1; ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 3.65 (s, 3H, –OCH_3), 3.86 (s, 3H, –OCH_3), 4.23 (q, 2H, J = 7.0 Hz, –CH_2CH_3), 4.61 (s, 2H, –CH_2S–), 5.43 (s, 2H, –CH_2N–), 6.74 (s, 1H, –imidazolyl-***H***), 6.95 (s, 1H, –imidazolyl-***H***), 7.10 (m, 4H, –Ph***H***+C^8–***H***), 7.38 (t, 1H, J = 7.5 Hz, –Ph***H***), 7.60 (s, 1H, –imidazolyl-***H***), 8.40 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3418.5 (**OH**), 1710.2 (**C=O**).

5.1.4.4. Ethyl 5-((1*H*-imidazole-1-yl)methyl)-2-((3,4-difluorophenylthio)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate ($12f_6$). White powder (2.9 g, 60%); Mp: 158–160 °C; MS [MH^+] (m/z): 486.3; ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 3.89 (s, 3H, –OCH_3), 4.25 (q, 2H, J = 7.0 Hz, –CH_2CH_3), 4.67 (s, 2H, –CH_2S–), 5.57 (s, 2H, –CH_2N–), 6.75 (s, 1H, –imidazolyl-***H***), 7.04 (s, 1H, –imidazolyl-***H***), 7.33 (s, 1H, C^8–***H***), 7.58 (m, 3H, –Ph***H***), 7.67 (s, 1H, –imidazolyl-***H***), 8.52 (s, 1H, C^4–**H**); IR (KBr, cm^{-1}): 3427.4 (**OH**), 1703.5 (**C=O**).

5.1.4.5. Ethyl 5-((1*H*-imidazole-1-yl)methyl)-2-((3,4-dimethoxyphenylthio)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate ($12g_6$). White powder (3.1 g, 61%); Mp: 179–181 °C; MS [MH^+] (m/z): 510.2; ^{1}H NMR (DMSO-d_6): δ 1.35 (t, 3H, J = 7.1 Hz, –CH_2CH_3), 3.55 (s, 3H, –OCH_3), 3.68 (s, 3H, –OCH_3), 4.01 (s, 3H, –OCH_3), 4.29 (q, 2H, J = 7.1 Hz, –CH_2CH_3), 4.60 (s, 2H, –CH_2S–), 5.62 (s, 2H, –CH_2N–), 6.82 (m, 3H, –Ph***H***), 7.05 (s, 1H, –imidazolyl-***H***), 7.16 (s, 1H, –imidazolyl-***H***), 7.37 (m, 1H, C^8-***H***), 7.76 (s, 1H, –imidazolyl-***H***), 8.80 (s, 1H, C^4-**H**); IR (KBr, cm^{-1}): 3409.1 (**OH**), 1698.3 (**C=O**).

5.1.5. General procedure for the synthesis of the target compounds 13b–13f

A solution of compound **9** or **12** (0.01 mol) and sodium perborate (1.7 g, 0.011 mol) in acetic acid (40 mL) was heated at 40 °C for 3–5 h. The solvent was evaporated under vacuum, and the residue was poured into water (100 mL). The resulting mixture was adjusted to pH 8 with 20% NaOH, and the precipitate was filtered, washed with acetone, dried, and recrystallized from ethanol to obtain the target compounds **13b**–**13f**.

5.1.5.1. Ethyl 2-((4-fluorophenylsulfinyl)methyl)-5-((dimethylamino)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate ($13b_1$). Yellow powder (3.4 g, 74%); Mp: 171–173 °C; MS [MH^+] (m/z): 461.3; ^{1}H NMR (DMSO-d_6): δ 1.36 (t, 3H, J = 7.0 Hz, –CH_2CH_3), 2.79 (s, 6H, –$N(CH_3)_2$), 3.97 (s, 3H, –OCH_3), 4.32 (q, 2H, J = 7.0 Hz, –CH_2CH_3), 4.69 (d, 1H, J = 12.7 Hz, –CH_2SO–), 4.75 (s, 2H, –CH_2N–), 4.76 (d, 1H, J = 12.7 Hz, –CH_2SO–), 7.21 (t, 2H, J = 8.8 Hz, –Ph***H***), 7.40 (q, 2H, J = 5.5 Hz, –Ph***H***), 7.49 (s, 1H, C^8–***H***), 8.82 (s, 1H, C^4–***H***); IR (KBr, cm^{-1}): 3429.6 (**OH**), 1712.5 (**C=O**).

5.1.5.2. Ethyl 2-((4-fluorophenylsulfinyl)methyl)-6-hydroxy-7-methoxy-5-((pyrrolidin-1-yl)methyl)-quinoline-3-carboxylate ($13b_2$). Pale yellow powder (2.7 g, 56%); Mp: 163–165 °C; MS [MH^+] (m/z): 487.1; ^{1}H NMR (DMSO-d_6): δ 1.34 (t, 3H, J = 7.1 Hz, –CH_2CH_3), 1.93 (s, 4H, –pyrrolidinyl), 2.77 (s, 4H, –pyrrolidinyl), 4.01 (s, 3H, –OCH_3), 4.37 (q, 2H, J = 7.1 Hz, –CH_2CH_3), 4.72 (d, 1H, J = 12.6 Hz, –CH_2SO–), 4.77 (s, 2H, –CH_2N–), 4.93 (d, 1H, J = 12.6 Hz, –CH_2SO–), 7.31 (t, 2H, J = 8.7 Hz, –Ph***H***), 7.51 (q, 2H, J = 5.5 Hz, –Ph***H***), 7.35 (s, 1H, C^8–***H***), 8.69 (s, 1H, C^4–***H***); IR (KBr, cm^{-1}): 3422.1 (**OH**), 1708.6 (**C=O**).

5.1.5.3. Ethyl 5-((1*H*-imidazol-1-yl)methyl)-2-((4-fluorophenylsulfinyl)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate ($13b_6$). Yellow powder (3.4 g, 70%); Mp: 193–195 °C; MS [MH^+] (m/z): 484.1; ^{1}H NMR (DMSO-d_6): δ 1.35 (t, 3H, J = 7.1 Hz, –CH_2CH_3), 4.06 (s, 3H, –OCH_3), 4.32 (q, 2H, J = 7.1 Hz, –CH_2CH_3), 4.73 (d, 1H, J = 12.4 Hz, –CH_2SO–), 4.92 (d, 1H, J = 12.4 Hz, –CH_2SO–), 5.88 (s, 2H, –CH_2N–), 7.37 (t, 2H, J = 8.7 Hz, –Ph***H***), 7.48 (s, 1H, –imidazolyl), 7.57 (m, 3H, –imidazolyl+–Ph***H***), 7.64 (s, 1H, C^8–**H**), 8.83 (s, 1H, –imidazolyl), 9.13 (s, 1H, C^4–H); IR (KBr, cm^{-1}): 3438.2 (**OH**), 1721.5 (**C=O**).

5.1.5.4. Ethyl 6-hydroxy-7-methoxy-2-((pyridin-2-ylsulfinyl)-methyl)-5-((pyrrolidin-1-yl)methyl)quinoline-3-carboxylate ($13c_2$). Yellow powder (3.0 g, 64%); Mp: 146–148 °C; MS [MH^+] (m/z): 470.2; ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 3H, J = 7.1 Hz, –CH_2CH_3), 1.91 (s, 4H, –pyrrolidinyl), 2.83 (s, 4H, –pyrrolidinyl), 4.01 (s, 3H, –OCH_3), 4.28 (s, 2H, –CH_2N–), 4.37 (q, 2H, J = 7.1 Hz, –CH_2CH_3), 4.74 (d, 1H, J = 12.7 Hz, –CH_2SO–), 4.82 (d, 1H, J = 12.7 Hz, –CH_2SO–), 7.29 (s, 1H, C^8–***H***), 7.52 (m, 2H, –pyridyl), 7.74 (m, 2H, –pyridyl), 8.58 (s, 1H, C^4–***H***); IR (KBr, cm^{-1}): 3429.2 (**OH**), 1713.2 (**C=O**).

5.1.5.5. Ethyl 5-((1*H*-imidazol-1-yl)methyl)-2-((3-methoxyphenylsulfinyl)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate (13e$_6$). Yellow powder (3.5 g, 67%); Mp: 176–178 °C; MS [MH$^+$] (*m*/*z*): 496.2; ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 3H, *J* = 7.0 Hz, –CH_2CH_3), 3.65 (s, 3H, –OCH_3), 3.86 (s, 3H, –OCH_3), 4.25 (q, 2H, *J* = 7.0 Hz, –CH_2CH_3), 4.61 (d, 1H, *J* = 12.2 Hz, –CH_2SO–), 4.73 (d, 1H, *J* = 12.2 Hz, –CH_2SO–), 5.42 (s, 2H, –CH_2N–), 6.75 (s, 1H, –imidazolyl), 6.95 (s, 1H, –imidazolyl), 7.05 (m, 4H, –Ph*H*+C^8–*H*), 7.41 (t, 1H, *J* = 7.5 Hz, –Ph*H*), 7.60 (s, 1H, –imidazolyl), 8.39 (s, 1H, C^4–*H*); IR (KBr, cm^{-1}): 3420.1 (**OH**), 1718.4 (**C=O**).

5.1.5.6. Ethyl 2-((3,4-difluorophenylsulfinyl)methyl)-6-hydroxy-7-methoxy-5-((pyrrolidin-1-yl)methyl)quinoline-3-carboxylate (13f$_2$). Yellow powder (3.2 g, 63%); Mp: 181–183°C; MS [MH$^+$] (*m*/*z*): 505.2; ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, *J* = 7.1 Hz, –CH_2CH_3), 1.93 (s, 4H, –pyrrolidinyl), 2.77 (s, 4H, –pyrrolidinyl), 3.94 (s, 3H, –OCH_3), 4.27 (q, 2H, *J* = 7.1 Hz, –CH_2CH_3), 4.65 (d, 1H, *J* = 12.7 Hz, –CH_2SO–), 4.76 (s, 2H, –CH_2N–), 4.79 (d, 1H, *J* = 12.7 Hz, –CH_2SO–), 7.35 (s, 1H, C^8–*H*), 7.56 (m, 3H, –Ph*H*), 8.42 (s, 1H, C^4-*H*); IR (KBr, cm^{-1}): 3417.1 (**OH**), 1709.3 (**C=O**).

5.1.5.7. Ethyl 5-((1*H*-imidazol-1-yl)methyl)-2-((3,4-difluorophenylsulfinyl)methyl)-6-hydroxy-7-methoxyquinoline-3-carboxylate (13f$_6$). Pale yellow powder (3.1 g, 62%); Mp: 183–185 °C; MS [MH$^+$] (*m*/*z*): 502.1; ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, *J* = 7.0 Hz, –CH_2CH_3), 3.87 (s, 3H, –OCH_3), 4.25 (q, 2H, *J* = 7.0 Hz, –CH_2CH_3), 4.65 (d, 1H, *J* = 12.3 Hz, –CH_2SO–), 4.83 (d, 1H, *J* = 12.3 Hz, –CH_2SO–), 5.45 (s, 2H, –CH_2N–), 6.75 (s, 1H, –imidazolyl), 7.04 (s, 1H, –imidazolyl), 7.33 (s, 1H, C^8–*H*), 7.55 (m, 4H, –Ph*H*+–imidazolyl), 8.44 (s, 1H, C^4–*H*); IR (KBr, cm^{-1}): 3421.6 (**OH**), 1709.3 (**C=O**).

5.2. Pharmacology

5.2.1. In vitro anti-HBV activity assay

The anti-HBV activities of compounds **9a–13f** were evaluated in HepG2.2.15 cells by previously reported methods.[13,14] This assay included measurement of the drugs' abilities to inhibit the production of both HBsAg and HBeAg and to inhibit HBV DNA replication. Briefly, confluent cell cultures in 96-well flat-bottomed tissue culture plates were treated with various doses of the test compounds or lamivudine (purchased by Glaxo & Welcome Co.) in RPMI 1640 medium supplemented with 2% fetal bovine serum. Medium was changed daily with fresh test compounds and positive control for 8 days. Extracellular HBV surface- and e-antigen secretion levels were evaluated for HepG2.2.15 cells by semiquantitative enzyme immunoassay (EIA) methods using commercial kits (HBsAg, Abbott Laboratories; HBeAg, Diasorin, Inc.) as previously described.[8] Intracellular HBV DNA levels were measured by quantitative Southern blot hybridization.

5.2.2. Cytotoxicity assay

The cytotoxicities of compounds **9a–13f** to HepG2.2.15 cells were assessed by MTT assay.[15] Briefly, HepG2.2.15 cells were treated as described above. Untreated control cultures were also maintained on each 96-well plate. Toxicity was determined by measuring neutral red dye uptake, as determined from the cells' 510 nm absorbance relative to untreated cells, 9 days of treatment.

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References and notes

1. Custer, B.; Sullivan, S. D.; Hazlet, T. K.; Iloeje, U.; Veenstra, D. L.; Kowdley, K. V. *J. Clin. Gastroenterol.* **2004**, *38*, S158.
2. Fattovich, G.; Brollo, L.; Alberti, A.; Pontisso, P.; Giustina, G.; Realdi, G. *Hepatology* **1988**, *8*, 1651.
3. Fischer, K. P.; Gutfreund, K. S.; Tyrrell, D. L. *Drug Resist. Updat.* **2001**, *4*, 98.
4. Danta, M.; Dusheiko, G. *Int. J. Clin. Practice* **2004**, *58*, 877.
5. Colonno, R. J.; Rose, R.; Baldick, C. J.; Levine, S.; Podornowski, K.; Yu, C. F.; Walsh, A.; Fang, J.; Hsu, M.; Mazzucco, C.; Eggers, B.; Zhang, S.; Plym, M.; Klesczewski, K.; Tenney, D. J. *Hepatology* **2006**, *44*, 1656.
6. Sims, K. A.; Woodland, A. M. *Pharmacotherapy* **2006**, *26*, 1745.
7. Chai, H. F.; Zhao, Y. F.; Zhao, C. S.; Gong, P. *Bioorg. Med. Chem.* **2006**, *14*, 911.
8. Zhao, C. S.; Zhao, Y. F.; Chai, H. F.; Gong, P. *Bioorg. Med. Chem.* **2006**, *14*, 2552.
9. Sumiaki, K.; Hirofumi, T.; Tetsuya, K.; Kiyoharu, N.; Manabu, N. *Tetrahedron* **2004**, *60*, 4901.
10. Patteux, C.; Foucout, L.; Bohn, P.; Dupas, G.; Leprince, J.; Tonon, M. C.; Dehouck, B.; Marsais, F.; Papamicael, C.; Levacher, V. *Org. Biomol. Chem.* **2006**, *4*, 817.
11. Friedländer, P.; Gohring, C. F. *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 1833.
12. The spectral data for compound **A**: White powder; Mp: 184–186; MS [MH$^+$] (*m*/*z*): 564.0; ^{1}H NMR (DMSO-d_6): δ 1.28–1.46 (m, 10H, –CH_2CH_3+–piperidinyl), 1.54–2.14 (m, 6H, –piperidinyl), 2.16 (t, 2H, –piperidinyl), 2.36 (d, 2H, –piperidinyl), 2.50–2.55 (m, 3H, –piperidinyl), 2.82 (dd, *J* = 5.3, 12.7 Hz, 1H, –CHCH_2–), 3.26 (dd, *J* = 8.7, 12.7 Hz, 1H, –CHCH_2–), 3.98 (s, 3H, –OCH_3), 4.07 (s, 2H, –CH_2N–), 4.30 (q, *J* = 7.1 Hz, 2H, –CH_2CH_3), 5.78 (dd, *J* = 5.3, 8.7 Hz, 1H, –CHS–), 7.23–7.26 (m, 2H, C^8-*H*+–Ph*H*), 7.29–7.40 (m, 2H, –Ph*H*), 7.42 (d, 2H, –Ph*H*), 8.71 (s, 1H, C^4–*H*). IR (KBr, cm^{-1}) 3437 (**OH**), 1710 (**C=O**).
13. Korba, B. E.; Milman, G. *Antiviral Res.* **1991**, *15*, 217.
14. Sells, M. A.; Zelent, A. Z.; Shvartsan, M.; Acs, G. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1144.
15. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.