Full Paper

Synthesis and *in-vitro* Anti-hepatitis B Virus Activity of Ethyl 6-Bromo-8-hydroxyimidazo[1,2-*a*]pyridine-3-carboxylates

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A series of ethyl 6-bromo-8-hydroxyimidazo[1,2-*a*]pyridine-3-carboxylate derivatives were synthesized and evaluated for their anti-hepatitis B virus (HBV) activity and cytotoxicity in HepG2.2.15 cells. Nearly half of the tested compounds were proved to be highly effective in inhibiting the replication of HBV DNA with IC_{50} values ranging from 1.3 to 9.1 μM. Among them, **10o** and **10s** were identified as the most promising compounds.

Keywords: Anti-HBV activity / Ethyl 6-bromo-8-hydroxyimidazo[1,2-*a*]pyridine-3-carboxylates / Synthesis

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Introduction

Hepatitis B, a potentially life-threatening liver infection caused by the hepatitis B virus, is still a major health problem around the world, particularly in Asia. It can cause chronic liver disease and put people at high risk of death from cirrhosis and liver cancer [1, 2]. Of the approximately two billion people who have been infected with HBV worldwide, an estimated 400 million are chronically infected, with about one million deaths annually from HBV-related diseases [3]. Currently, the major therapeutic options for HBV carriers are interferon-α (IFN-α) and nucleoside analogues. The efficacy of IFN-α is limited and often associated with severe adverse effects [4]. Approved nucleoside analogues such as lamivudine, adefovir dipivoxil, and enticavir inhibit HBV replication by targeting the viral DNA polymerase, and after long-term treatment development of drug-resistance towards the virus becomes a problem [5–7]. Thus, there is a tremendous need for the development of novel classes of anti-HBV agents with novel structures and mechanisms of action for the chemotherapy of HBV infection.

Initially, we developed a series of ethyl 5-hydroxy-1*H*-indole-3-carboxylate analogues (**A**, Fig. 1), which exhibited significant anti-HBV activity [8, 9]. In view of the novel structural template, which differs from those of all reported anti-HBV agents, we were interested to further study the structure–activity relationship (SAR) of the related class of compounds. Thus, many efforts have been made to understand the SAR and we became interested in exploring new surrogates for the indole moiety of the series. In previous studies, we explored replacements for the indole with structures such as quinoline which maintains a key '$-CH_2-S-Ar$' linker and hydroxyl on the core heterocycle (**B** and **C**, Fig. 1). These quinoline-based series also displayed high potency against the replication of HBV DNA [10, 11]. This suggested to us that further modification of this region could provide more opportunities for the discovery of novel HBV inhibitors.

To further develop the SAR surrounding the core heterocycle region of this chemical series, we designed and synthesized a novel series of ethyl 6-bromo-8-hydroxyimidazo[1,2-*a*]pyridine-3-carboxylate derivatives (**D**, Fig. 2) and investigated their biological activity as potential HBV inhibitors.

Results and discussion

Chemistry

The title compounds were prepared as shown in Scheme 1. Treatment of commercially available 2-aminopyridin-3-ol **1** with benzyl chloride in the presence of NaOH in a mixture of dichloromethane and water led to the formation of compound **2** [12]. Bromination of **2** with bromine afforded the intermediate **3** [13], which was subsequently deprotected in a mixture of concentrated hydrochloric acid and acetic acid (1:2, v/v) to produce 2-amino-5-bromopyridin-3-ol **4**. We want to point out that this is a novel synthetic route for the

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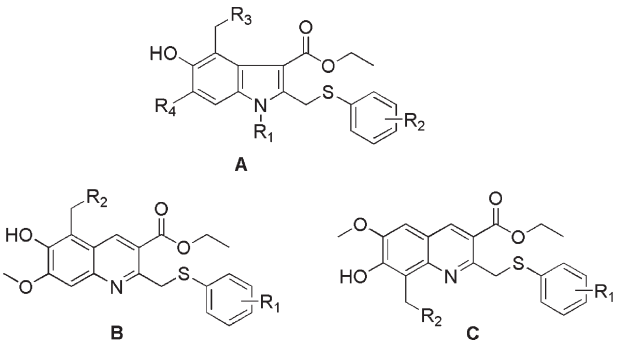


Figure 1. Structure of indole and quinoline derivatives.

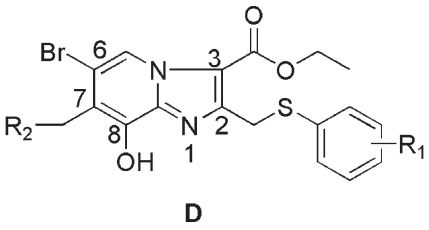


Figure 2. Structure of ethyl 6-bromo-8-hydroxyimidazo[1,2-*a*]pyridine-3-carboxylate derivatives.

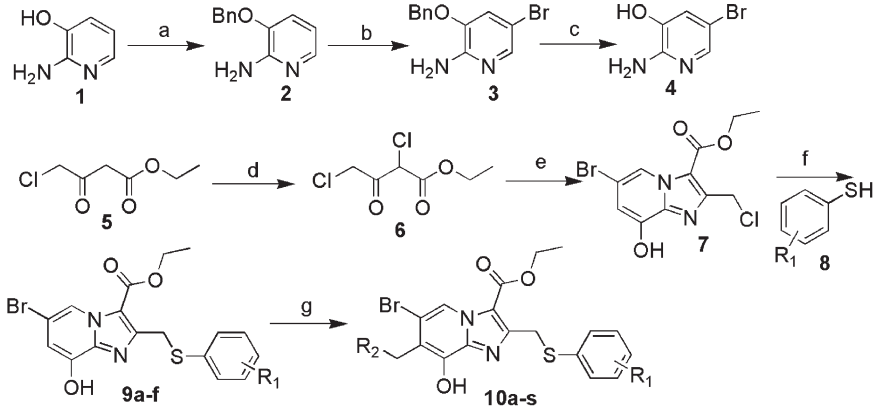
preparation of 2-amino-5-bromopyridin-3-ol. Condensation of **4** with **6** – resulting from the chlorination [14] of ethyl 4-chloroacetoacetate **5** – gave the key intermediate **7**, which, subsequently, was reacted with the corresponding thiophenol **8** to obtain compound **9**. The target compounds **10a–s** were synthesized by Mannich reactions of **9** with different secondary alkylamines.

Biological results and discussion

The synthesized compounds **10a–s** were evaluated for their cytotoxicity and anti-HBV activity, namely, the ability to inhibit the replication of HBV DNA and the production of HBsAg and HBeAg with lamivudine as positive control in HepG2.2.15 cells. The inhibition of the production of HBsAg and HBeAg by the tested compounds could not be demonstrated in this examination. The results of the inhibition of the replication of HBV DNA are summarized in Table 1.

As shown in Table 1, nine compounds **10c**, **10g**, **10i**, **10m**, **10n**, **10o**, **10q**, **10r**, and **10s** demonstrated good antiviral potency and were superior to the control with IC_{50} values ranging from 1.3 to 9.1 μM. Compound **10o**, the most potent one, was nearly 185 times more potent than lamivudine. The selective indices of **10m**, **10o**, and **10s** (SI = 8.5, 9.3, and 23.7, respectively) were comparable to or higher than that of lamivudine (SI = 9.1).

As we investigated the substituents on the phenyl ring of the 2-position, several substituents of different electronic, steric, and lipophilic properties were found to be acceptable. Among the *para*-position-substituted derivatives **10d–o**, the



9a: R_1=H **9b**: R_1=4-Fluoro

9c: R_1=4-Chloro **9d**: R_1=4-Bromo

9e: R_1=4-Methyl **9f**: R_1=3-Methoxy

Reagents and conditions: (a) Benzyl chloride, NaOH, TBAB, CH_2Cl_2, H_2O, r. t., 6 h; (b) Br_2, HOAc, H_2SO_4, 0°C, 2.5 h; (c) HOAc, HCl, reflux, 10–12 h; (d) SO_2Cl_2, CH_2Cl_2, r. t., 12 h; (e) **4**, C_2H_5OH, reflux, 5–8 h; (f) KOH, CH_3OH, r. t., 6 h; (g) alkyl secondary amine, 37% HCHO, CH_3OH, 30°C, overnight.

Scheme 1. Synthesis of target compounds.

Table 1. The substituents, anti-HBV activity, and cytotoxicity of target compounds *in vitro*.

Compound	R_1	R_2	CC_{50} (μM)[§]	HBV DNA replication	
				IC_{50} (μM)[#]	SI[&]
10a	H	Pyrrolidin-1-yl	27.8	–[$]	–
10b	H	Piperidin-1-yl	14.2	–	–
10c	H	4-Methylpiperazin-1-yl	31.5	7.8	4.0
10d	4-Fluoro	Dimethylamino	166.4	–	–
10e	4-Fluoro	Pyrrolidin-1-yl	35.3	–	–
10f	4-Fluoro	Piperidin-1-yl	27.8	–	–
10g	4-Fluoro	Morpholino	12.6	3.9	3.2
10h	4-Chloro	Pyrrolidin-1-yl	20.4	–	–
10i	4-Chloro	4-Methylpiperazin-1-yl	19.4	5.7	3.4
10j	4-Bromo	Dimethylamino	126.2	–	–
10k	4-Bromo	Morpholino	8.2	–	–
10l	4-Bromo	4-Methylpiperazin-1-yl	31.0	–	–
10m	4-Methyl	Dimethylamino	65.5	7.7	8.5
10n	4-Methyl	Pyrrolidin-1-yl	25.2	9.1	2.8
10o	4-Methyl	Morpholino	12.1	1.3	9.3
10p	3-Methoxy	Pyrrolidin-1-yl	51.4	–	–
10q	3-Methoxy	Piperidin-1-yl	20.5	3.2	6.4
10r	3-Methoxy	Morpholino	17.6	3.1	5.6
10s	3-Methoxy	4-Methylpiperazin-1-yl	61.7	2.6	23.7
Lamivudine			2183.1	240.0	9.1

[§] CC_{50} is 50% cytotoxic concentration in HepG2.2.15 cells;[#] IC_{50} is 50% inhibitory concentration;[&] selectivity index (SI: CC_{50}/IC_{50}); [$] – means no antiviral activity at the concentration lower than its CC_{50}.

methyl-substituted analogues **10m–o** showed potent antiviral activity (IC_{50} = 7.7, 9.1, and 1.3 μM, respectively), and were more active than the corresponding halide-substituted analogues. Enlarging the bulk of the substituent from F (**10g**, IC_{50} = 3.9 μM) or Cl (**10i**, IC_{50} = 5.7 μM) to Br resulted in a complete loss of inhibitory activity as seen in compounds **10k** and **10l**. These results suggest that the nature of the substituents at the *para*-position of the phenyl ring influences the anti-HBV activity remarkably. In addition, comparing compound **10c** with **10s**, compound **10s** (IC_{50} = 2.6 μM, CC_{50} = 61.7 μM) with a methoxy group at the *meta*-position exhibited a higher antiviral activity and much lower cytotoxicity than the unsubstituted derivative **10c** (IC_{50} = 7.8 μM, CC_{50} = 31.5 μM). This difference was also found when comparing **10b** and **10q**.

Then, the effects of introducing different Mannich basic functionalities at the C-7 position of the imidazo[1,2-*a*]pyridine core were studied. In general, morpholino and 4-methyl-piperazinyl derivatives showed higher anti-HBV activity than pyrrolidinyl and dimethylamino derivatives. It is noteworthy that the morpholino derivatives **10g**, **10o**, and **10r** showed a remarkable inhibition of HBV DNA replication with IC_{50} values ranging between 1.3 and 3.9 μM, whereas an increase in cytotoxicity was also noted, resulting in a relatively small selectivity index. Besides, low cytotoxicity was observed with the introduction of a dimethylamino group (**10d**, **10j**, and **10m**) at the R_2 position.

Conclusion

In summary, a series of novel ethyl 6-bromo-8-hydroxyimidazo[1,2-*a*]pyridine-3-carboxylate derivatives were synthesized and assessed for their anti-HBV activity and cytotoxicity *in vitro*, using lamivudine as reference control. Nine of them displayed potential inhibition against the replication of HBV DNA with IC_{50} values of 1.3–9.1 μM. In particular, the most promising results were observed for compounds **10o** and **10s** which were more effective than lamivudine, with potent antiviral activities (IC_{50} = 1.3 and 2.6 μM, respectively) and extraordinarily high selectivity (SI = 9.3 and 23.7, respectively). The preliminary SARs showed that introduction of a methyl group at the *para*-position of the phenyl ring at the 2-position could enhance the inhibition of HBV-DNA replication. This finding was in agreement with the results of previous SAR studies in an ethyl 5-hydroxy-1*H*-indole-3-carboxylate series [9]. Different Mannich basic functionalities introduced at the 7-position had significant influence on antiviral activity and cytotoxicity. Generally, morpholino and 4-methyl-piperazinyl derivatives showed higher anti-HBV activity than pyrrolidinyl and dimethylamino derivatives. This trend seems to be not consistent with our previous observation on indole/quinoline analogues [9, 10]. Further studies of SARs and mechanisms of action of this new class of anti-HBV agents are currently performed in our laboratories.

Experimental

Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer. ^{1}H-NMR spectra were performed using Bruker 300 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). All chemicals were obtained from commercial suppliers and used without further purification. Concentration of the reaction solutions involved the use of a rotary evaporator at reduced pressure. Compounds **2** and **6** were synthesized in accordance with literature procedures [12, 14].

*2-Amino-5-bromopyridin-3-ol **4***

3-(Benzyloxy)pyridin-2-amine **2** (25.0 g, 125 mmol) was added to 10% H_2SO_4 (500 mL) at room temperature under stirring. At 0°C, to the mechanically stirred brown solution was added a mixture of bromine (24.0 g, 150 mmol) and AcOH (80 mL) dropwise over a period of 90 min. The resulting yellow suspension was stirred for 2.5 h at 0°C and then poured into ice water (500 mL). A pH value of 8 was adjusted by addition of 25% NH_4OH (220 mL). The aqueous phase was extracted with CH_2Cl_2 (4 × 300 mL). The combined organic phases were washed with water (300 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. A mixture of AcOH (200 mL) and concentrated HCl (100 mL) was added directly to the crude material, and the resulting solution was refluxed for 10–12 h under argon atmosphere. The solvent was removed under reduced pressure, the residue was poured into water (200 mL), and then filtered. The filtrate was adjusted to pH 7 with NH_4OH solution at 10°C, the precipitate was collected by filtration and dried to yield compound **4** (8.7 g) as a white solid. Yield: 36.8%; m.p.: 204°C (lit. [15]: 204–205); MS (ESI) *m*/*z*: 189.1, 187.0 $[M - H]^-$.

*Ethyl 6-bromo-2-(chloromethyl)-8-hydroxyimidazo[1,2-a] pyridine-3-carboxylate **7***

Compound **6** (0.11 mol) was added to a solution of 2-amino-5-bromopyridin-3-ol **4** (0.1 mol) in EtOH (100 mL). The reaction mixture was refluxed for 5–8 h and the precipitate formed. The solution was cooled and the precipitate was filtered, washed with EtOH, and dried to give **7** as a white solid. Yield: 78.0%; m.p.: 235–237°C; ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.38 (t, J = 7.1 Hz, 3H, $-CH_2CH_3$), 4.41 (q, J = 7.1 Hz, 2H, $-CH_2CH_3$), 5.02 (s, 2H, $-CH_2Cl$), 6.96 (d, J = 1.4 Hz, 1H, C^7-*H*), 8.87 (d, J = 1.4 Hz, 1H, C^5-*H*), 11.47 (s, 1H, −O*H*); MS (ESI) *m*/*z*: 333.0, 335.1 $[M + H]^+$.

*General procedure for the synthesis of ethyl 6-bromo-8-hydroxy-2-((arylthio)methyl)imidazo[1,2-a]pyridine-3-carboxylate derivatives **9a–f***

The appropriate arylthiol **8** (0.11 mol) was dropped into a solution of KOH (0.12 mol) in EtOH (100 mL) at 0°C and stirred for 0.5 h, then **7** (0.1 mol) was added and the mixture was stirred at room temperature for 6–8 h. The reaction mixture was adjusted to pH 5 with 2 M HCl solution, the precipitated white solid was collected, washed with water, and dried under vacuum to obtain compounds **9a–f** as white powder.

*Ethyl 6-bromo-8-hydroxy-2-(phenylthiomethyl)imidazo [1,2-a]pyridine-3-carboxylate **9a***

Yield: 85.7%; m.p.: 190–192°C; ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.29 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 4.29 (q, 2H, J = 7.2 Hz, $-CH_2CH_3$), 4.53 (s, 2H, $-CH_2S-$), 6.89 (s, 1H, C^7-*H*), 7.20 (t, 1H, J = 7.2 Hz, −Ph*H*), 7.30 (t, 2H, J = 7.5 Hz, −Ph*H*), 7.41 (d, 2H, J = 7.5 Hz, −Ph*H*), 8.76 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 407.0, 409.1 $[M + H]^+$.

*Ethyl 6-bromo-2-((4-fluorophenylthio)methyl)-8-hydroxyimidazo[1,2-a]pyridine-3-carboxylate **9b***

Yield: 80.1%; m.p.: 211–213°C; ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.29 (t, 3H, J = 7.0 Hz, $-CH_2CH_3$), 4.27 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.53 (s, 2H, $-CH_2S-$), 6.86 (s, 1H, C^7-*H*), 7.05 (t, 2H, J = 8.5 Hz, −Ph*H*), 7.39 (dd, 2H, J = 8.4, 5.4 Hz, −Ph*H*), 8.69 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 425.0, 427.0 $[M + H]^+$.

*Ethyl 6-bromo-2-((4-chlorophenylthio)methyl)-8-hydroxyimidazo[1,2-a]pyridine-3-carboxylate **9c***

Yield: 85.0%; m.p.: 224°C (dec); ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.28 (t, 3H, J = 6.9 Hz, $-CH_2CH_3$), 4.26 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.48 (s, 2H, $-CH_2S-$), 6.82 (s, 1H, C^7-*H*), 7.25 (d, 2H, J = 8.1 Hz, −Ph*H*), 7.32 (d, J = 8.1 Hz, 2H, −Ph*H*), 8.66 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 441.5, 443.6 $[M + H]^+$.

*Ethyl 6-bromo-2-((4-bromophenylthio)methyl)-8-hydroxyimidazo[1,2-a]pyridine-3-carboxylate **9d***

Yield: 80.9%; m.p.: 236°C (dec); ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.27 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 4.24 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.47 (s, 2H, $-CH_2S-$), 6.78 (s, 1H, C^7-*H*), 7.31–7.40 (m, 4H, −Ph*H*), 8.67 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 485.0, 487.0 $[M + H]^+$.

*Ethyl 6-bromo-8-hydroxy-2-(p-tolylthiomethyl)imidazo-[1,2-a]pyridine-3-carboxylate **9e***

Yield: 82.8%; m.p.: 228°C (dec); ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.27 (t, 3H, J = 7.0 Hz, $-CH_2CH_3$), 2.25 (s, 3H, *p*-CH_3-phenyl), 4.25 (q, 2H, J = 6.8 Hz, $-CH_2CH_3$), 4.44 (s, 2H, $-CH_2S-$), 6.30 (s, 1H, C^7-*H*), 7.11 (d, 2H, J = 7.8 Hz, −Ph*H*), 7.29 (d, 2H, J = 7.9 Hz, −Ph*H*), 8.32 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 421.0, 423.0 $[M + H]^+$.

*Ethyl 6-bromo-8-hydroxy-2-((3-methoxyphenylthio)-methyl)imidazo[1,2-a]pyridine-3-carboxylate **9f***

Yield: 86.1%; m.p.: 240°C (dec); ^{1}H-NMR (DMSO-d_6, ppm) δ: 1.29 (t, 3H, J = 7.2 Hz, $-CH_2CH_3$), 3.76 (s, 3H, $-OCH_3$), 4.25 (q, 2H, J = 6.8 Hz, $-CH_2CH_3$), 4.50 (s, 2H, $-CH_2S-$), 6.72 (s, 1H, C^7-*H*), 6.78 (d, 1H, J = 7.8 Hz, −Ph*H*), 7.06–7.24 (m, 3H, −Ph*H*), 8.61 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 437.0, 439.0 $[M + H]^+$.

*General procedure for the synthesis of compounds **10a–s***

A solution of appropriate alkylamine (10 mmol) and 37% HCHO (4.4 mmol) in MeOH (20 mL) was stirred at room temperature for 30 min. Compound **9** (4 mmol) was added and the mixture was stirred overnight at 30°C. The MeOH was removed under vacuum and 50 mL of water were added in one portion. The resulting mixture was adjusted to pH 9 with 25% NH_4OH at 10°C and extracted with CH_2Cl_2. The organic phase was dried over

anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to yield the title compounds **10a–s**.

Ethyl 6-bromo-8-hydroxy-2-(phenylthiomethyl)-7-(pyrrolidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10a***

Light yellow solid, yield: 74.7%; m.p.: 126–128°C; IR (KBr, cm^{-1}): 3423.6 (ν_{OH}), 1692.5 ($\nu_{C=O}$), 2976.3, 1585.4, 1479.0, 1383.8, 1331.4, 1079.9; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.38 (t, 3H, J = 6.8 Hz, $-CH_2CH_3$), 1.94 (s, 4H, −pyrrolidinyl), 2.83 (s, 4H, −pyrrolidinyl), 4.15 (s, 2H, $-CH_2N-$), 4.35 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.58 (s, 2H, $-CH_2S-$), 7.19–7.25 (m, 3H, −Ph*H*), 7.48 (d, 2H, J = 7.5 Hz, −Ph*H*), 9.02 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 490.0, 492.1 $[M + H]^+$. Anal. calcd. for $C_{22}H_{24}BrN_3O_3S$ (%): C, 53.88; H, 4.93; N, 8.57. Found (%): C, 53.70; H, 5.01; N, 8.46.

Ethyl 6-bromo-8-hydroxy-2-(phenylthiomethyl)-7-(piperidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10b***

White solid, yield: 70.8%; m.p.: 159–161°C; IR (KBr, cm^{-1}): 3424.0 (ν_{OH}), 1690.5 ($\nu_{C=O}$), 2935.2, 1585.3, 1478.6, 1399.6, 1336.1, 1079.5; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.37 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 1.54 (s, 2H, −piperidinyl), 1,71 (s, 4H, −piperidinyl), 2.64 (s, 4H, −piperidinyl), 3.95 (s, 2H, $-CH_2N-$), 4.34 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.57 (s, 2H, $-CH_2S-$), 7.16 (d, 1H, J = 7.1 Hz, −Ph*H*), 7.23–7.28 (m, 2H, −Ph*H*), 7.46 (d, 2H, J = 7.5 Hz, −Ph*H*), 9.02 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 504.0, 506.0 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}BrN_3O_3S$ (%): C, 54.76; H, 5.20; N, 8.33. Found (%): C, 54.84; H, 5.32; N, 8.05.

Ethyl 6-bromo-8-hydroxy-7-((4-methylpiperazin-1-yl)-methyl)-2-(phenylthiomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10c***

Light yellow solid, yield: 65.1%; m.p.: 168–170°C; IR (KBr, cm^{-1}): 3422.2 (ν_{OH}), 1686.3 ($\nu_{C=O}$), 2934.4, 1585.5, 1469.8, 1397.8, 1332.6, 1074.0; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.37 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.32 (s, 3H, $-NCH_3$), 2.73 (m, 8H, −piperazinyl), 3.99 (s, 2H, $-CH_2N-$), 4.34 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.57 (s, 2H, $-CH_2S-$), 7.16 (t, 1H, J = 7.3 Hz, −Ph*H*), 7.24–7.29 (m, 2H, −Ph*H*), 7.46 (d, 2H, J = 7.6 Hz, −Ph*H*), 9.05 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 519.1, 521.0 $[M + H]^+$. Anal. calcd. for $C_{23}H_{27}BrN_4O_3S$ (%): C, 53.18; H, 5.24; N, 10.79. Found (%): C, 53.40; H, 5.35; N, 10.72.

Ethyl 6-bromo-7-((dimethylamino)methyl)-2-((4-fluorophenylthio)methyl)-8-hydroxyimidazo[1,2-a]pyridine-3-carboxylate ***10d***

White solid, yield: 79.6%; m.p.: 125–127°C; IR (KBr, cm^{-1}): 3420.4 (ν_{OH}), 1692.8 ($\nu_{C=O}$), 2927.0, 1589.8, 1491.2, 1383.7, 1330.7, 1225.3, 1076.4; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.37 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.54 (s, 6H, $-N(CH_3)_2$), 4.04 (s, 2H, $-CH_2N-$), 4.33 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.50 (s, 2H, $-CH_2S-$), 6.95 (t, 2H, J = 8.7 Hz, −Ph*H*), 7.41 (dd, 2H, J = 8.4, 5.4 Hz, −Ph*H*), 8.98 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 482.0, 484.1 $[M + H]^+$. Anal. calcd. for $C_{20}H_{21}BrFN_3O_3S$ (%): C, 49.80; H, 4.39; N, 8.71. Found (%): C, 50.06; H, 4.51; N, 8.63.

Ethyl 6-bromo-2-((4-fluorophenylthio)methyl)-8-hydroxy-7-(pyrrolidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10e***

Light yellow solid, yield: 82.2%; m.p.: 131–133°C; IR (KBr, cm^{-1}): 3418.2 (ν_{OH}), 1691.9 ($\nu_{C=O}$), 2977.4, 1589.5, 1490.7, 1399.4, 1332.1, 1225.1, 1078.7; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.37 (t, 3H, J = 7.0 Hz, $-CH_2CH_3$), 1.98 (s, 4H, −pyrrolidinyl), 2.92 (s, 4H, −pyrrolidinyl), 4.20 (s, 2H, $-CH_2N-$), 4.33 (q, 2H, J = 7.2 Hz, $-CH_2CH_3$), 4.48 (s, 2H, $-CH_2S-$), 6.94 (t, 2H, J = 8.6 Hz, −Ph*H*), 7.39 (dd, 2H, J = 8.4, 5.6 Hz, −Ph*H*), 8.93 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 508.0, 510.0 $[M + H]^+$. Anal. calcd. for $C_{22}H_{23}BrFN_3O_3S$ (%): C, 51.97; H, 4.56; N, 8.27. Found (%): C, 51.79; H, 4.58; N, 8.15.

Ethyl 6-bromo-2-((4-fluorophenylthio)methyl)-8-hydroxy-7-(piperidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10f***

White solid, yield: 70.8%; m.p.: 186–188°C; IR (KBr, cm^{-1}): 3424.1 (ν_{OH}), 1694.9 ($\nu_{C=O}$), 2934.8, 1589.2, 1490.6, 1400.5, 1338.3, 1225.5, 1079.8; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.38 (t, 3H, J = 7.2 Hz, $-CH_2CH_3$), 1.55 (s, 2H, −piperidinyl), 1,71 (s, 4H, −piperidinyl), 2.66 (s, 4H, −piperidinyl), 3.96 (s, 2H, $-CH_2N-$), 4.34 (q, 2H, J = 7.2 Hz, $-CH_2CH_3$), 4.50 (s, 2H, $-CH_2S-$), 6.95 (t, 2H, J = 8.5 Hz, −Ph*H*), 7.43 (dd, 2H, J = 8.4, 5.6 Hz, −Ph*H*), 9.01 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 522.1, 524.1 $[M + H]^+$. Anal. calcd. for $C_{23}H_{25}BrFN_3O_3S$ (%): C, 52.88; H, 4.82; N, 8.04. Found (%): C, 53.08; H, 4.96; N, 7.98.

Ethyl 6-bromo-2-((4-fluorophenylthio)methyl)-8-hydroxy-7-(morpholinomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10g***

Light yellow solid, yield: 77.6%; m.p.: 179–181°C; IR (KBr, cm^{-1}): 3422.1 (ν_{OH}), 1694.8 ($\nu_{C=O}$), 2930.4, 1589.3, 1490.9, 1400.0, 1333.3, 1225.2, 1076.3; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.38 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.71 (s, 4H, −morpholino), 3.81 (s, 4H, −morpholino), 4.01 (s, 2H, $-CH_2N-$), 4.34 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.50 (s, 2H, $-CH_2S-$), 6.95 (t, 2H, J = 8.5 Hz, −Ph*H*), 7.42 (dd, 2H, J = 8.4, 5.4 Hz, −Ph*H*), 9.07 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 524.0, 526.1 $[M + H]^+$. Anal. calcd. for $C_{22}H_{23}BrFN_3O_4S$ (%): C, 50.39; H, 4.42; N, 8.01. Found (%): C, 50.16; H, 4.51; N, 8.26.

Ethyl 6-bromo-2-((4-chlorophenylthio)methyl)-8-hydroxy-7-(pyrrolidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10h***

White solid, yield: 82.2%; m.p.: 125–126°C; IR (KBr, cm^{-1}): 3428.9 (ν_{OH}), 1693.6 ($\nu_{C=O}$), 2976.2, 1598.1, 1476.2, 1402.3, 1332.2, 1095.2, 1080.1 ($\nu_{C\text{-}Br}$); ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.39 (t, 3H, J = 6.9 Hz, $-CH_2CH_3$), 2.02 (s, 4H, −pyrrolidinyl), 3.05 (s, 4H, −pyrrolidinyl), 4.17 (s, 2H, $-CH_2N-$), 4.36 (q, 2H, J = 7.2 Hz, $-CH_2CH_3$), 4.50 (s, 2H, $-CH_2S-$), 7.20 (d, 2H, J = 7.6 Hz, −Ph*H*), 7.34 (d, 2H, J = 8.0 Hz, −Ph*H*), 8.94 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 524.5, 525.5 $[M + H]^+$. Anal. calcd. for $C_{22}H_{23}BrClN_3O_3S$ (%): C, 50.34; H, 4.42; N, 8.01. Found (%): C, 50.55; H, 4.60; N, 7.78.

Ethyl 6-bromo-2-((4-chlorophenylthio)methyl)-8-hydroxy-7-((4-methylpiperazin-1-yl)methyl)imidazo[1,2-a]pyridine-3-carboxylate ***10i***

Light yellow solid, yield: 65.1%; m.p.: 178–180°C; IR (KBr, cm^{-1}): 3429.4 (ν_{OH}), 1693.2 ($\nu_{C=O}$), 2937.1, 1476.1, 1397.6, 1334.4,

1094.9, 1076.4; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.40 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.33 (s, 3H, $-NCH_3$), 2.65 (m, 8H, −piperazinyl), 3.99 (s, 2H, $-CH_2N-$), 4.38 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.53 (s, 2H, $-CH_2S-$), 7.22 (d, 2H, J = 7.9 Hz, −Ph*H*), 7.40 (d, J = 8.1 Hz, 2H, −Ph*H*), 9.04 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 553.7, 555.6 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}BrClN_4O_3S$ (%): C, 49.87; H, 4.73; N, 10.11. Found (%): C, 50.13; H, 4.81; N, 9.98.

Ethyl 6-bromo-2-((4-bromophenylthio)methyl)-7-((dimethylamino)methyl)-8-hydroxyimidazo[1,2-a]pyridine-3-carboxylate ***10j***

Light yellow solid, yield: 57.6%; m.p.: 133–135°C; IR (KBr, cm^{-1}): 3424.3 (ν_{OH}), 1695.6 ($\nu_{C=O}$), 2978.5, 1473.7, 1401.0, 1332.7, 1067.5; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.39 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.55 (s, 6H, $-N(CH_3)_2$), 4.05 (s, 2H, $-CH_2N-$), 4.39 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.53 (s, 2H, $-CH_2S-$), 7.29–7.36 (m, 4H, −Ph*H*), 8.98 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 542.3, 544.1 $[M + H]^+$. Anal. calcd. for $C_{20}H_{21}Br_2N_3O_3S$ (%): C, 44.22; H, 3.90; N, 7.73. Found (%): C, 44.53; H, 4.01; N, 7.66.

Ethyl 6-bromo-2-((4-bromophenylthio)methyl)-8-hydroxy-7-(morpholinomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10k***

Light pink solid, yield: 72.0%; m.p.: 158–160°C; IR (KBr, cm^{-1}): 3421.3 (ν_{OH}), 1699.8 ($\nu_{C=O}$), 2924.0, 1473.9, 1400.9, 1332.9, 1077.3; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.40 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.81 (s, 4H, −morpholino), 3.85 (s, 4H, −morpholino), 4.01 (s, 2H, $-CH_2N-$), 4.38 (q, 2H, J = 7.2 Hz, $-CH_2CH_3$), 4.50 (s, 2H, $-CH_2S-$), 7.34 (m, 4H, −Ph*H*), 9.02 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 584.1, 586.1 $[M + H]^+$. Anal. calcd. for $C_{22}H_{23}Br_2N_3O_4S$ (%): C, 45.14; H, 3.96; N, 7.18. Found (%): C, 45.42; H, 4.06; N, 7.11.

Ethyl 6-bromo-2-((4-bromophenylthio)methyl)-8-hydroxy-7-((4-methylpiperazin-1-yl)methyl)imidazo[1,2-a]pyridine-3-carboxylate ***10l***

Light yellow solid, yield: 65.9%; m.p.: 146–147°C; IR (KBr, cm^{-1}): 3422.5 (ν_{OH}), 1683.2 ($\nu_{C=O}$), 2924.2, 1471.6, 1396.7, 1330.3, 1071.3; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.40 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.35 (s, 3H, $-NCH_3$), 2.76 (m, 8H, −piperazinyl), 4.00 (s, 2H, $-CH_2N-$), 4.38 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.53 (s, 2H, $-CH_2S-$), 7.31–7.40 (m, 4H, −Ph*H*), 9.05 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 597.0, 599.0 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}Br_2N_4O_3S$ (%): C, 46.17; H, 4.38; N, 9.36. Found (%): C, 46.37; H, 4.48; N, 9.24.

Ethyl 6-bromo-7-((dimethylamino)methyl)-8-hydroxy-2-(p-tolylthiomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10m***

White solid, yield: 69.6%; m.p.: 191–193°C; IR (KBr, cm^{-1}): 3408.4 (ν_{OH}), 1694.4 ($\nu_{C=O}$), 2976.9, 1597.6, 1494.0, 1402.6, 1326.5, 1080.4; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.36 (t, 3H, J = 6.9 Hz, $-CH_2CH_3$), 2.29 (s, 3H, *p*-CH_3-phenyl), 2.46 (s, 6H, $-N(CH_3)_2$), 3.96 (s, 2H, $-CH_2N-$), 4.32 (q, 2H, J = 6.8 Hz, $-CH_2CH_3$), 4.53 (s, 2H, $-CH_2S-$), 7.06 (d, 2H, J = 7.1 Hz, −Ph*H*), 7.34 (d, 2H, J = 6.7 Hz, −Ph*H*), 9.04 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 478.1, 480.0 $[M + H]^+$. Anal. calcd. for $C_{21}H_{24}BrN_3O_3S$ (%): C, 52.72; H, 5.06; N, 8.78. Found (%): C, 52.80; H, 5.15; N, 8.65.

Ethyl 6-bromo-8-hydroxy-7-(pyrrolidin-1-ylmethyl)-2-(p-tolylthiomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10n***

White solid, yield: 71.5%; m.p.: 190–192°C; IR (KBr, cm^{-1}): 3423.5 (ν_{OH}), 1693.0 ($\nu_{C=O}$), 2962.9, 1494.0, 1401.1, 1330.2, 1079.5 (ν_{C-Br}); ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.36 (t, 3H, J = 6.9 Hz, $-CH_2CH_3$), 1.92 (s, 4H, −pyrrolidinyl), 2.29 (s, 3H, *p*-CH_3-phenyl), 2.80 (s, 4H, −pyrrolidinyl), 4.12 (s, 2H, $-CH_2N-$), 4.31 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.52 (s, 2H, $-CH_2S-$), 7.06 (d, 2H, J = 7.2 Hz, −Ph*H*), 7.35 (d, 2H, J = 6.7 Hz, −Ph*H*), 9.01 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 504.0, 505.9 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}BrN_3O_3S$ (%): C, 54.76; H, 5.20; N, 8.33. Found (%): C, 54.83; H, 5.29; N, 8.21.

Ethyl 6-bromo-8-hydroxy-7-(morpholinomethyl)-2-(p-tolylthiomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10o***

Light pink solid, yield: 78.2%; m.p.: 202–204°C; IR (KBr, cm^{-1}): 3430.1 (ν_{OH}), 1699.0 ($\nu_{C=O}$), 2923.3, 1491.4, 1399.9, 1332.8, 1077.3; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.39 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 2.30 (s, 3H, *p*-CH_3-phenyl), 2.81 (s, 4H, −morpholino), 3.84 (s, 4H, −morpholino), 4.04 (s, 2H, $-CH_2N-$), 4.34 (q, 2H, J = 7.1 Hz, $-CH_2CH_3$), 4.49 (s, 2H, $-CH_2S-$), 7.09 (d, 2H, J = 7.1 Hz, −Ph*H*), 7.34 (d, 2H, J = 6.8 Hz, −Ph*H*), 9.08 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 520.1, 522.0 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}BrN_3O_4S$ (%): C, 53.08; H, 5.04; N, 8.07. Found (%): C, 52.89; H, 5.07; N, 8.11.

Ethyl 6-bromo-8-hydroxy-2-((3-methoxyphenylthio)methyl)-7-(pyrrolidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10p***

Light yellow solid, yield: 76.5%; m.p.: 122–124°C; IR (KBr, cm^{-1}): 3425.8 (ν_{OH}), 1692.1 ($\nu_{C=O}$), 2959.2, 1589.7, 1477.4, 1401.1, 1329.8, 1078.4; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.38 (t, 3H, J = 7.1 Hz, $-CH_2CH_3$), 1.91 (s, 4H, −pyrrolidinyl), 2.81 (s, 4H, −pyrrolidinyl), 3.79 (s, 3H, $-OCH_3$), 4.12 (s, 2H, $-CH_2N-$), 4.36 (q, 2H, J = 6.8 Hz, $-CH_2CH_3$), 4.56 (s, 2H, $-CH_2S-$), 6.68 (d, 1H, J = 7.8 Hz, −Ph*H*), 7.10 (m, 3H, −Ph*H*), 8.89 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 520.0, 522.1 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}BrN_3O_4S$ (%): C, 53.08; H, 5.04; N, 8.07. Found (%): C, 53.14; H, 5.13; N, 7.96.

Ethyl 6-bromo-8-hydroxy-2-((3-methoxyphenylthio)methyl)-7-(piperidin-1-ylmethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10q***

White solid, yield: 80.6%; m.p.: 150–152°C; IR (KBr, cm^{-1}): 3422.0 (ν_{OH}), 1690.9 ($\nu_{C=O}$), 2934.7, 1589.7, 1476.2, 1399.6, 1336.3, 1078.0; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.39 (t, 3H, J = 7.0 Hz, $-CH_2CH_3$), 1.55 (s, 2H, −piperidinyl), 1,70 (s, 4H, −piperidinyl), 2.67 (s, 4H, −piperidinyl), 3.80 (s, 3H, $-OCH_3$), 3.95 (s, 2H, $-CH_2N-$), 4.36 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.57 (s, 2H, $-CH_2S-$), 6.70 (d, 1H, J = 7.8 Hz, −Ph*H*), 7.11 (m, 3H, −Ph*H*), 9.02 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 534.0, 536.0 $[M + H]^+$. Anal. calcd. for $C_{24}H_{28}BrN_3O_4S$ (%): C, 53.93; H, 5.28; N, 7.86. Found (%): C, 54.02; H, 5.38; N, 7.78.

Ethyl 6-bromo-8-hydroxy-2-((3-methoxyphenylthio)- methyl)-7-(morpholinomethyl)imidazo[1,2-a]pyridine-3-carboxylate ***10r***

Light yellow solid, yield: 76.0%; m.p.: 167–169°C; IR (KBr, cm^{-1}): 3424.1 (ν_{OH}), 1692.6 ($\nu_{C=O}$), 2933.1, 1589.5, 1476.2, 1400.1, 1332.0, 1076.2; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.39 (t, 3H, J = 7.0 Hz, $-CH_2CH_3$), 2.70 (s, 4H, −morpholino), 3.77–3.81 (m, 7H, −morpholino, $-OCH_3$), 4.00 (s, 2H, $-CH_2N-$), 4.37 (q, 2H, J = 7.0 Hz, $-CH_2CH_3$), 4.57 (s, 2H, $-CH_2S-$), 6.70 (d, 1H, J = 8.1 Hz, −Ph*H*), 7.11 (m, 3H, −Ph*H*), 9.08 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 536.0, 538.1 $[M + H]^+$. Anal. calcd. for $C_{23}H_{26}BrN_3O_5S$ (%): C, 51.50; H, 4.89; N, 7.83. Found (%): C, 51.67; H, 5.02; N, 7.71.

Ethyl 6-bromo-8-hydroxy-2-((3-methoxyphenylthio)-methyl)-7-((4-methylpiperazin-1-yl)methyl)imidazo[1,2-a]pyridine-3-carboxylate ***10s***

Light yellow solid, yield: 58.1%; m.p.: 143–145°C; IR (KBr, cm^{-1}): 3431.0 (ν_{OH}), 1688.5 ($\nu_{C=O}$), 2935.7, 1589.8, 1476.3, 1398.9, 1333.2, 1077.0; ^{1}H-NMR ($CDCl_3$, ppm) δ: 1.39 (t, 3H, $J = 7.1$ Hz, $-CH_2CH_3$), 2.33 (s, 3H, $-NCH_3$), 2.69 (s, 8H, −piperazinyl), 3.80 (s, 3H, $-OCH_3$), 3.99 (s, 2H, $-CH_2N-$), 4.37 (q, 2H, $J = 7.1$ Hz, $-CH_2CH_3$), 4.57 (s, 2H, $-CH_2S-$), 6.71 (d, 1H, $J = 8.1$ Hz, −Ph*H*), 7.11 (m, 3H, −Ph*H*), 9.06 (s, 1H, C^5-*H*); MS (ESI) *m*/*z*: 549.1, 551.2 $[M + H]^+$. Anal. calcd. for $C_{24}H_{29}BrN_4O_4S$ (%): C, 52.46; H, 5.32; N, 10.20. Found (%): C, 52.67; H, 5.43; N, 10.14.

Pharmacology

In-vitro anti-HBV activity assay

Details of the design of the antiviral procedure and the growth conditions for HepG2.2.15 cells have been described previously [16, 17]. The *in-vitro* anti-HBV activity included the ability to inhibit the production of HBsAg and HBeAg and the replication of HBV DNA in HBV-infected 2.2.15 cells. For the antiviral analyses, 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 eight days. Extracellular HBsAg and HBeAg were analyzed in culture medium by semiquantitative enzyme immunoassay (EIA). Intracellular HBV DNA levels were measured by quantitative Southern blot hybridization.

Cytotoxicity assay

Cytotoxicity of compounds was assessed by the MTT assay as previously described [18]. Briefly, HepG2.2.15 cells were cultured in 96-well tissue culture plates with various doses of test compounds (in 0.2 mL culture medium/well) as described above. The cells with media alone were used as controls. 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 9 of treatment.

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