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Original article

Synthesis and antiviral activity of new acrylamide derivatives containing 1,2,3-thiadiazole as inhibitors of hepatitis B virus replication

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ABSTRACT

A series of new acrylamide derivatives containing 1,2,3-thiadiazole were synthesized, characterized, and evaluated for their anti-hepatitis B virus (HBV) activities *in vitro*. The IC_{50} of compounds **9b** (10.4 μg/mL), **9c** (3.59 μg/mL) and **17a** (9.00 μg/mL) of the inhibition on the replication of HBV DNA were higher than that of the positive control lamivudine (14.8 μg/mL). Compound **9d** exhibited significant activity against secretion of HBeAg (IC_{50} = 12.26 μg/mL).

1. Introduction

Hepatitis B virus (HBV) infection is one of the leading causes of death due to infectious diseases worldwide. Approximately 400 million people worldwide are chronically infected by HBV, with an annual global death toll of 1.2 million a year [1,2]. Although interferon-α (IFN-α) and several nucleoside analogues such as lamivudine (3-TC) and adefovir dipivoxil have been used to treat HBV infections, still the side effects of interferon and the viral resistance of nucleoside analogues make the current treatment regimens far from being satisfactory [3–5]. To address the toxicity and resistance problems of nucleoside inhibitors, a major goal in the field of medicinal chemistry is to discover and develop non-nucleoside inhibitors [6]. Currently some non-nucleoside inhibitors have also been reported to inhibit HBV replication [7–10].

A phenylacrylamide derivative, AT-130 (Fig. 1) has been discovered to possess potent anti-HBV activity through random screening [11–13]. It appears to act by inhibiting the packaging step in the life cycle of HBV [14]. A natural compound **2** extracted from *Dichondra repens forens* had a similar structure with compound **1**, exhibiting high anti-HBV activity [15]. To our knowledge, many compounds possessing 1,2,3-thiadiazole fragment exhibit useful biological properties, such as antiviral activities [16–18], antimicrobial [19] and antitumor activities [20]. Recently, we synthesized a series of 2-substituted-*N*-methyl-2-(1,2,3-thiadiazol-4-yl)-acetamides and disclosed their favorable anti-HBV activities first, such as compound **3** [21], which framework structure is also similar to that of acrylamide **1**. Prompted by the biological properties of 1,2,3-thiadiazole derivatives and the similar structure feature of antiviral compounds **1**, **2** and **3**, we reasoned that the bioisoteric replacement of the phenyl in phenylacrylamide with 1,2,3-thiadiazolyl would produce a new class of acrylamide analogues with improved anti-HBV activities. Herein, we designed and synthesized three series of novel acrylamide derivatives containing 1,2,3-thiadiazole in which the A or B ring of **1** was replaced by 4-methyl-1,2,3-thiadiazole (Fig. 2). The synthesized compounds were evaluated for their anti-HBV activities *in vitro* for the first time.

2. Chemistry

The Series **I** derivatives were prepared by replacing the B ring of **1** with 4-methyl-1,2,3-thidiazole, as shown in Scheme 1. The acid intermediate 5-carboxyl-4-methyl-1,2,3-thiadiazole **4**, which was previously synthesized [22], was treated with thionyl chloride at reflux to give 4-methyl-1,2,3-thiadiazole-5-carbonyl chloride **5**, which was then reacted with glycine to give 2-(4-methyl-1,2,3-thiadiazole-5-carboxamido) acetic acid **6**. Condensation of **6** with the appropriate substituted benzaldehydes in acetic anhydride under ultrasound irradiation afforded oxazolones **7a**–**c**, which was followed by ring-opening reaction with piperidine in $CHCl_3$ to give

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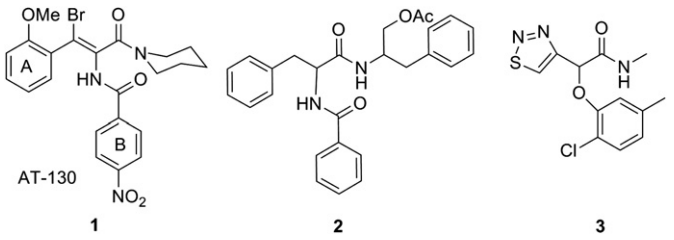


Fig. 1. Chemical structures of some of the reported compounds showing potent anti-HBV activity.

intermediates **8a–c** in high yields. Finally, halogenations of **8a–c** with the appropriate halogens afforded phenylacrylamide derivatives containing 1,2,3-thiadiazole **9a–d**.

For the aromatic aldehyde to react with compound **6**, the conventional heating method (Erlenmeyer's method [23]) was unsuitable, as two compounds (compounds **7** and **10**) would appear at a higher temperature (100 °C). In the reaction procedure, only compound **10** would be obtained at 140 °C, similar to the reference [23]. At 140 °C in the presence of sodium acetate and acetic anhydride, compound **7** was completely converted to the rearranged product **10**. To optimize the synthetic reaction conditions for the key intermediate **7** and to avoid the formation of the side product **10** at high temperatures, we used the ultrasound irradiation method for this transformation. Without much difficulty, the desired **7** from compound **6** with aromatic aldehyde was successfully obtained by the ultrasound irradiation (70 °C, 2 h). The new compound **11** was obtained after aminated ring-opening and halogenation of **10**, and the structure of the rearranged product **10** was confirmed by an X-ray diffraction analysis of compound **11** (Fig. 3). The mechanism of this transformation is postulated as follows (Fig. 4). The acetate ion attacks the carbonyl of substrate compound **7** to generate intermediate **A**. It is followed by the subsequent nucleophilic attack of the nitrogen negative ion of **A** on a carbonyl moiety of acetic anhydride to provide intermediate **B**. A stable compound **10** is then formed through intramolecular cyclic reaction.

The Series **II** derivatives were prepared by replacing the A ring of **1** with 4-methyl-1,2,3-thidiazole, as shown in Scheme 2. 5-Ethoxycarbonyl-4-methyl-1,2,3-thiadiazole **12**, which was previously synthesized [22], was reduced with KBH_4 in ethanol to give 5-hydroxymethyl-4-methyl-1,2,3-thiadiazole **13**. Treatment of **13** with pyridinium chlorochromate (PCC) in methylene chloride afforded 4-methyl-1,2,3-thiadiazole-5-carboxaldehyde **14**, which was subsequently condensed with hippuric acid in acetic anhydride at 100 °C to provide oxazolone **15**. Finally, the 1,2,3-thiadiazolylacrylamide derivatives **17a–d** were synthesized with a similar procedure to that described for the synthesis compounds **9a–d**.

The Series **III** derivative **20** was prepared by simultaneously replacing the A and B rings of **1** with 4-methyl-1,2,3-thidiazole, as shown in Scheme 3. The intermediate oxazolone **18** was obtained by condensation of **6** and **14** in acetic anhydride under ultrasound irradiation (70 °C, 2 h). The bis-1,2,3-thiadiazole substituted compound **20** was synthesized in a similar method as described for the synthesis compounds **9a–d**.

3. Results and discussion

The synthesized acrylamide derivatives containing 1,2,3-thiadiazole were evaluated for their cytotoxicities and anti-HBV activities using the antiviral drug lamivudine as the reference drug in 2.2.15 cells. The results are summarized in Table 1.

Initial studies were conducted on the Series **I** derivatives **9a–d**, which has different patterns of substitution on the phenyl ring of acrylamide derivatives. Among derivatives **9a–c**, the chlorine substituted analogues (**9b** and **9c**) demonstrated good antiviral activity, and the para-substituent analogue (**9c**) displayed more potent antiviral activity (IC_{50} = 3.59 μg/mL) than the ortho-substituent analogue **9b** (IC_{50} = 10.4 μg/mL). However, compounds (**9a** and **9d**), which bear no substituent on the phenyl, proved to be virtually inactive. This result suggests that the substituent on the phenyl ring is an important feature in conferring relatively potent inhibitory activity. Surprisingly, compound **9d**, replacing the bromine atom at the double bond of **9a** with a chlorine atom, exhibited excellent inhibitory activity against secretion of HBeAg (IC_{50} = 12.26 μg/mL). The most potent compound in this subseries was compound **9c** (IC_{50} = 3.59 μg/mL), which was nearly four times more potent than the reference drug lamivudine (IC_{50} = 14.80 μg/mL).

Replacement of the ring A of **1** with 1,2,3-thiadiazole produced the Series **II** derivatives (Table 1, **16a** and **17a–d**). Among derivatives **17a–c**, the compound containing piperidine group (**17a**) was more effective than morpholine derivative (**17b**), but the anti-HBV activity was eliminated when the amine position was substituted with the none-ring structural diethylamine group (**17c**). Besides, when the bromine atom at the double bond of **17a** was replaced with the hydrogen atom (**17a**) or chlorine atom (**17d**), anti-HBV activities were also eliminated. This result suggests that both the substituent on the amine and the substituent on the double bond have significant influence on anti-HBV DNA activity. Of these compounds, the most active compound **17a** exhibited high activity against HBV (IC_{50} = 9.00 μg/mL), which was more potent than the reference drug lamivudine (IC_{50} = 14.80 μg/mL).

The selective indexes of **9b**, **9c**, and **17a** were 8.29, 4.96, and 21.38, respectively. Although their SIs were lower than that of lamivudine, their anti-HBV potencies were higher than that of lamivudine.

When rings A and B were simultaneously replaced by 1,2,3-thiadiazole (Series **III**, **20**), the anti-HBV activity was eliminated, and the cytotoxicity of the disubstituted 1,2,3-thiadiazole derivative was higher than that of the corresponding monosubstituted analogues.

4. Conclusions

In summary, a series of acrylamide derivatives containing 1,2,3-thiadiazole based on compound **1** were synthesized and

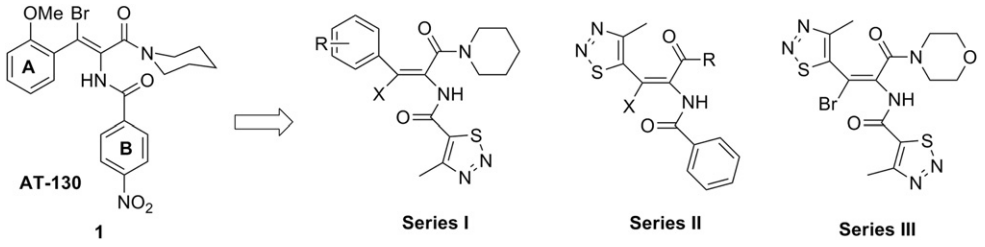
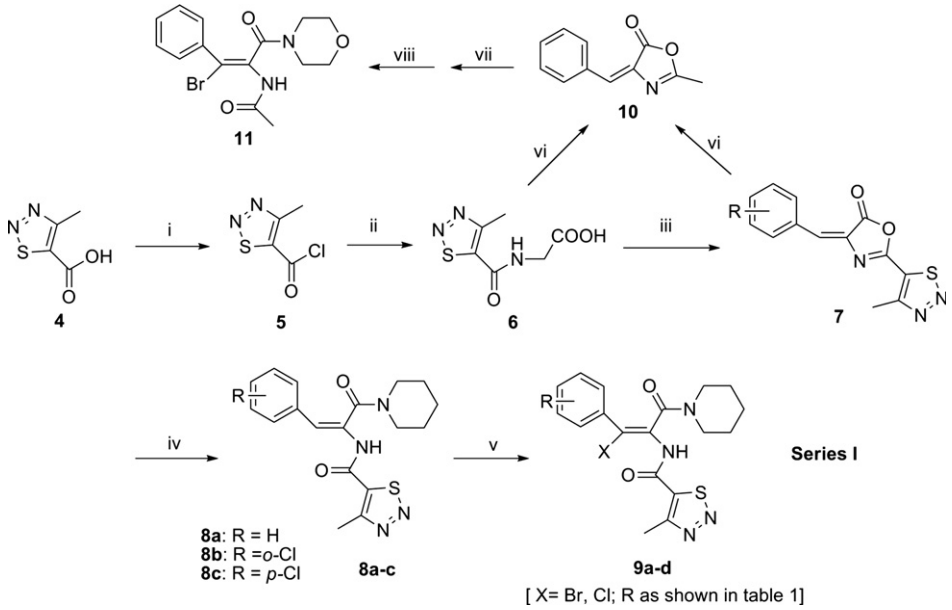


Fig. 2. The design of novel acrylamide derivatives containing 1,2,3-thiadiazole.



Scheme 1. Synthetic pathway to phenylacrylamide derivatives containing 1,2,3-thiadiazole derivatives **9a–d**. *Reagents and conditions*: (i) $SOCl_2$, reflux; (ii) glycine, NaOH, pH = 9–10; (iii) ArCHO, Ac_2O/NaOAc, ultrasonic/70 °C; (iv) piperidine, $CHCl_3$; (v) X_2, $CHCl_3$; (vi) ArCHO, Ac_2O/NaOAc, 140 °C; (vii) morpholine, $CHCl_3$; (viii) Br_2, $CHCl_3$.

assessed for their anti-HBV activity and cytotoxicity *in vitro*, using lamivudine as the reference drug. Compound **9c** showed the most potence anti-HBV activity within all of the tested compounds. Its IC_{50} was 3.59 μg/mL, which was about 3 times higher than that of the control lamivudine. Compound **9b** and **17a** also exhibited significant efficacy on the replication of HBV DNA (IC_{50}: 10.4, 9.0 μg/mL). In addition, compound **9d** exhibited excellent inhibitory activity against secretion of HBeAg (IC_{50} = 12.26 μg/mL).

5. Experimental

5.1. Chemistry

Thin-layer chromatography (TLC) was carried out on silica GF254 plates (Qingdao Haiyang Chemical Co., Ltd, China). Column chromatography was carried out on silica gel (200–300 mesh). Melting points were measured on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and were uncorrected. ^{1}H NMR spectra were obtained at 300 MHz on a Bruker Avance 300 spectrometer with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were obtained at 100 MHz on a Bruker Avance 400 spectrometer with tetramethylsilane (TMS) as an internal standard. All chemical shifts were reported in ppm. Elemental analysis was performed on a Vario EL III automatic elemental analyzer. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage LC/mass detector instrument. IR spectra were recorded on an Bruker550 spectrometer. X-ray diffraction data were collected on a Bruker SMART 1000 charge-coupled device (CCD) diffractometer. Reagent-grade solvents were used without further purification unless otherwise specified.

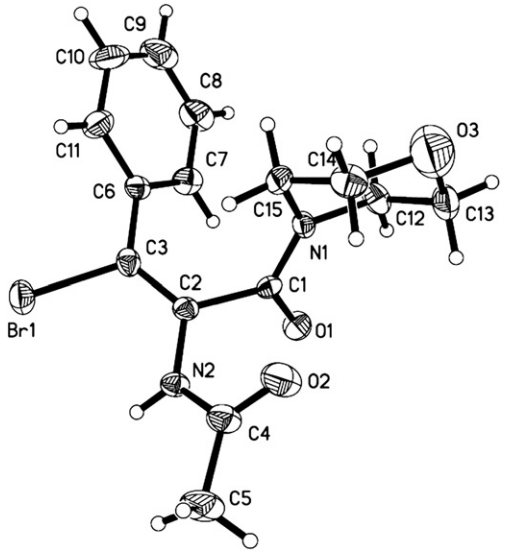


Fig. 3. Crystal structure of compound **11**.

5.1.1. 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (**4**)

Compound **4** was prepared as a white solid using commercially available acetyl ethyl acetate as the starting material according to the literature [24]. Yield = 98.2%; m.p. 174–178 °C (m.p. 174–175 °C [25]).

5.1.2. 4-Methyl-1,2,3-thiadiazole-5-carbonyl chloride (**5**)

In a 50 mL round-bottomed flask, **4** (7.07 g, 49 mmol) and 28 mL of thionyl chloride were placed. The resulting mixture was heated at 70–80 °C for 4 h and then concentrated *in vacuo* to obtain the crude residue **5**, which was used in the next step without further characterization.

5.1.3. 2-(4-Methyl-1,2,3-thiadiazole-5-carboxamido)acetic acid (**6**)

In a 100 mL three-neck round-bottomed flask, glycine (3.0 g, 40 mmol) and 8 mL water were placed. The solution was stirred until the mixture became clear, and then it was neutralized to pH 10 with 4 N NaOH solution. The crude acid chloride **5** was added slowly to the solution under a salt-ice bath. The reaction mixture was stirred for 3 h and left at room temperature overnight. It was filtered and washed with ethyl acetate. The filtered solution was acidified to pH 2 and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to afford **6**

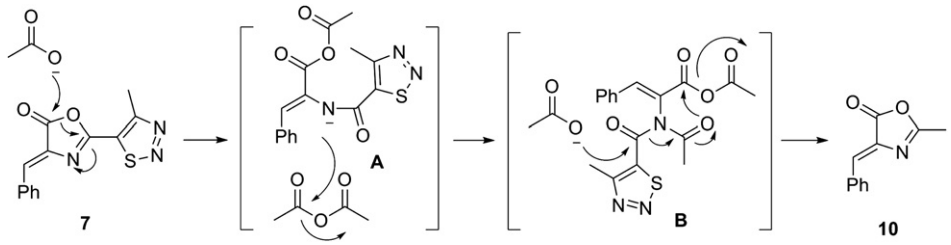


Fig. 4. The possible reaction mechanism of rearranged product **10**.

(6.97 g, 69.0% yield) as a yellow solid, m.p. 141–143 °C; ^{1}H NMR (300 MHz, D_6-Acetone): δ 3.47 (s, 3H, CH_3), 4.10 (d, J = 3.9 Hz, 2H, CH_2), 7.34 (s, 1H, NH).

*5.1.4. General procedure for the preparation of 4-substituted benzylidene-2-(4-methyl-1,2,3-thiadiazol-5-yl)oxazol-5(4H)-one (**7a–c**)*

Compound **6** (8.0 g, 38 mmol) and NaAc (3.28 g, 40 mmol) were porphyrized and added to a 100 mL conical flask, then substituted benzaldehydes (40 mmol) and acetic anhydride (12.72 g, 120 mmol) were added. The mixture was rocked rapidly at room temperature, which was subsequently treated under ultrasound irradiation at 70 °C for 2 h, 15 mL ethanol was added and rocked, and then the product was precipitated out of solution. The resulting precipitate was filtered and dried to obtain the intermediates **7**, which were used in the next step without further characterization.

*5.1.5. General procedure for the preparation of intermediates **8a–c***

In a 25 mL round-bottomed flask, compound **7** (2.2 mmol) and 30 mL of $CHCl_3$ were placed. After cooling in a ice bath, a solution of piperidine (0.196 g, 2.3 mmol) in $CHCl_3$ (6 mL) was added. The resulting solution was stirred at room temperature overnight and then concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 5/1) to afford compounds **8a–c**.

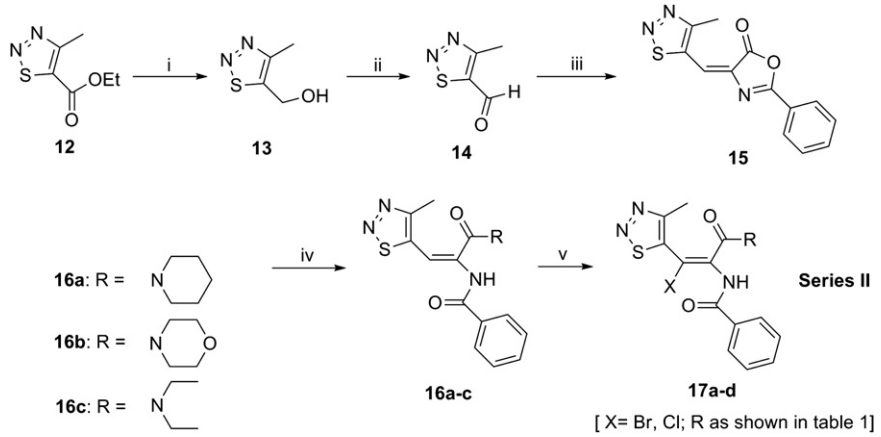
*5.1.5.1. 4-Methyl-N-(3-oxo-1-phenyl-3-(piperidin-1-yl)prop-1-en-2-yl)-1,2,3-thiadiazole-5-carboxamide (**8a**).* White solid, yield 88.0%, m.p. 163–165 °C; IR (KBr, cm^{-1}): 3139 (NH), 2956, 2936, 2859 (CH aliph.), 1665, 1649 (C=O), 1609, 1521, 1474 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65–1.68 (m, 6H, piperidine-3CH_2), 2.86 (s, 3H, thiadiazole-CH_3), 3.53–3.71 (m, 4H, piperidine-2CH_2), 6.07 (s, 1H, C=C–H), 7.36–7.44 (m, 5H, Ph-H), 9.41 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.95, 161.05, 158.48, 141.83, 133.30, 129.13 (2C), 128.73, 128.63 (2C), 128.42, 124.14, 49.24, 43.43, 25.68, 25.26, 24.51, 13.92. ESI-MS (m/z): 355.12 (M − H)$^-$. Anal. calcd for $C_{18}H_{20}N_4O_2S$: C, 60.65; H, 5.66; N, 15.72; found: C, 60.47; H, 5.52; N, 16.01.

*5.1.5.2. N-(1-(2-Chlorophenyl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**8b**).* White solid, yield 98.0%, m.p. 175–177 °C; IR (KBr, cm^{-1}): 3145 (NH), 2944, 2932, 2859 (CH aliph.), 1674, 1651 (C=O), 1597, 1522, 1473 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.66–1.72 (m, 6H, piperidine-3CH_2), 2.78 (s, 3H, thiadiazole-CH_3), 3.58–3.79 (m, 4H, piperidine-2CH_2), 6.40 (s, 1H, C=C–H), 7.30–7.55 (m, 4H, Ph-H), 8.97 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.10, 160.47, 158.06, 141.95, 133.75, 131.98, 130.18–129.70 (4C), 126.78, 121.83, 49.33, 43.51, 25.81, 25.38, 24.47, 13.79. ESI-MS (m/z): 389.18 (M − H)$^-$. Anal. calcd for $C_{18}H_{19}ClN_4O_2S$: C, 55.31; H, 4.90; N, 14.33; found: C, 55.44; H, 4.74; N, 14.34.

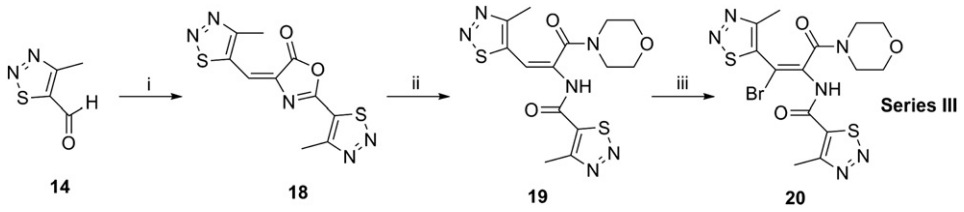
*5.1.5.3. N-(1-(4-Chlorophenyl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**8c**).* White solid, yield: 97.9%, m.p. 176–178 °C; IR (KBr, cm^{-1}): 3142 (NH), 2937, 2856 (CH aliph.), 1671, 1646 (C=O), 1600, 1525, 1489 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49–1.54 (m, 6H, piperidine-3CH_2), 2.90 (s, 3H, thiadiazole-CH_3), 3.05–3.59 (m, 4H, piperidine-2CH_2), 6.85 (s, 1H, C=C–H), 7.09–7.24 (m, 4H, Ph-H), 8.89 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.74, 161.27, 158.45, 141.45, 134.56, 131.69, 130.33 (2C), 128.89 (2C), 128.54, 122.36, 49.32, 43.57, 25.64, 25.31, 24.48, 13.94. ESI-MS (m/z): 389.07 (M − H)$^-$. Anal. calcd for $C_{18}H_{19}ClN_4O_2S$: C, 55.31; H, 4.90; N, 14.33; found: C, 55.37; H, 4.86; N, 14.47.

*5.1.6. General procedure for the preparation of derivatives **9a–d***

Bromine liquid (0.225 g, 1.4 mmol) was added to a solution of compounds **8a–c** (1.4 mmol) in $CHCl_3$ (15 mL) under a salt-ice bath.



Scheme 2. Synthetic pathway to 1,2,3-thiadiazolylacrylamide derivatives **17a–d**. *Reagents and conditions*: (i) KBH_4, EtOH; (ii) PCC, CH_2Cl_2; (iii) Hippuric acid, Ac_2O/NaOAc, 100 °C; (iv) Secondary amines, $CHCl_3$; (v) X_2, $CHCl_3$.



Scheme 3. Synthetic pathway to 1,2,3-thiadiazolylacrylamide derivative **20**. *Reagents and conditions*: (i) 2-(4-methyl-1,2,3-thiadiazole-5-carboxamido)acetic acid **6**, Ac_2O/NaOAc, 100 °C; (ii) morpholine, $CHCl_3$; (iii) Br_2, $CHCl_3$.

The mixture was stirred at room temperature for 3 h and then $CaCO_3$ (0.07 g, 0.7 mmol) was added, the resulting solution was stirred at room temperature overnight. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 5/1) to afford compounds **9a**–**c**.

The solution of **8a** (0.5 g, 1.4 mmol) in CCl_4 (15 mL) was stirred under a salt-ice bath. A solution of Cl_2 (0.1 g, 1.4 mmol) in CCl_4 was added and stirred at room temperature for 5 h, after which $CaCO_3$ (0.07 g, 0.7 mmol) was added, the resulting solution was stirred at room temperature overnight. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 5/1) to afford compound **9d**.

*5.1.6.1. N-(1-Bromo-3-oxo-1-phenyl-3-(piperidin-1-yl)prop-1-en-2-yl) -4-methyl-1,2,3-thiadiazole-5-carboxamide (**9a**).* White solid, yield 83.4%, m.p. 180–182 °C; IR (KBr, cm^{-1}): 3155 (NH), 2942, 2857 (CH aliph.), 1675, 1659 (C=O), 1608, 1517, 1484 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.53–1.51 (m, 6H, piperidine-3CH_2), 3.04 (s, 3H, thiadiazole-CH_3), 3.02–3.48 (m, 4H, piperidine-2CH_2), 7.34–7.53 (m, 5H, Ph-H), 7.95 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.53, 159.96, 157.13, 142.48, 135.97, 129.77, 129.52, 129.16 (2C), 128.27 (2C), 116.24, 47.79, 42.65, 24.52, 24.46, 23.93, 14.14. ESI-MS (m/z): 434.93 $(M - H)^-$. Anal. calcd for $C_{18}H_{19}BrN_4O_2S$: C, 49.66; H, 4.40; N, 12.87; found: C, 49.72; H, 4.44; N, 12.91.

*5.1.6.2. N-(1-Bromo-1-(2-chlorophenyl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**9b**).* White solid, yield 99.0%, m.p. 173–175 °C; IR (KBr, cm^{-1}): 3154 (NH), 2936, 2858 (CH aliph.), 1675, 1665 (C=O), 1621, 1517, 1466 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98–1.50 (m, 6H, piperidine-3CH_2), 3.08 (s, 3H, thiadiazole-CH_3), 3.17–3.48 (m, 4H, piperidine-2CH_2), 7.32–7.49 (m, 4H, Ph-H), 8.04 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.96, 161.20, 159.13, 156.34, 142.98, 134.42, 131.70, 131.19, 130.25, 127.41, 126.97, 108.64, 47.81, 42.70, 25.12, 24.81, 24.07, 14.34. ESI-MS (m/z): 470.93 $(M + H)^+$. Anal. calcd for $C_{18}H_{18}BrClN_4O_2S$: C, 46.02; H, 3.86; N, 11.93; found: C, 45.99; H, 3.73; N, 12.05.

*5.1.6.3. N-(1-Bromo-1-(4-chlorophenyl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**9c**).* White solid, yield 73.9%, m.p. 178–180 °C; IR (KBr, cm^{-1}): 3146 (NH), 2939, 2864 (CH aliph.), 1674, 1655 (C=O), 1614, 1520, 1484 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98–1.50 (m, 6H, piperidine-3CH_2), 3.08 (s, 3H, thiadiazole-CH_3), 3.17–3.48 (m, 4H, piperidine-2CH_2), 7.32–7.49 (m, 4H, Ph-H), 8.04 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.96, 159.79, 156.77, 142.43, 135.83, 134.42, 130.53 (2C), 130.18, 128.59 (2C), 113.09, 47.80, 42.70, 24.75, 24.55, 23.97, 14.22. ESI-MS (m/z): 468.86 $(M - H)^-$. Anal. calcd for $C_{18}H_{18}BrClN_4O_2S$: C, 46.02; H, 3.86; N, 11.93; found: C, 45.81; H, 3.74; N, 12.05.

*5.1.6.4. N-(1-Chloro-3-oxo-1-phenyl-3-(piperidin-1-yl)prop-1-en-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**9d**).* White solid, yield 80.9%, m.p. 176–179 °C; IR (KBr, cm^{-1}): 3150 (NH), 2943, 2856 (CH aliph.), 1671, 1662 (C=O), 1611, 1519, 1486 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.40–1.39 (m, 6H, piperidine-3CH_2), 2.91 (s, 3H, thiadiazole-CH_3), 3.08–3.38 (m, 4H, piperidine-2CH_2), 7.25–7.42 (m, 5H, Ph-H), 7.75 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.59, 159.98, 156.98, 142.45, 134.51, 129.86, 128.62 (2C), 128.31 (2C), 127.25, 123.99, 47.84, 42.63, 24.48, 24.42, 23.98, 14.08. ESI-MS (m/z): 389.04 $(M - H)^-$. Anal. calcd for $C_{18}H_{19}ClN_4O_2S$: C, 55.31; H, 4.90; N, 14.33; found: C, 55.41; H, 4.82; N, 14.27.

*5.1.7. 4-Benzylidene-2-methyloxazol-5(4H)-one (**10**)*

Compound **6** (0.50 g, 2.5 mmol), benzaldehyde (0.25 g, 2.5 mmol), NaAc (0.21 g, 2.5 mmol) and acetic anhydride (10 mL) were added to a 25 mL round-bottomed flask. The mixture was heated at 140 °C for 2 h. After being cooled to room temperature,

Table 1
Anti-HBV activities of title compounds (μg/mL).

No.	X	R	MW	TC_{50}[a]	HBsAg		HBeAg		DNA replication	
					IC_{50}[b]	SI[c]	IC_{50}	SI	IC_{50}	SI
9a	Br	H	435.34	111.11	–[d]	–	–	–	–	–
9b	Br	*o*-Cl	469.78	86.23	–	–	–	–	10.4	8.29
9c	Br	*p*-Cl	469.78	17.81	–	–	–	–	3.59	4.96
9d	Cl	H	390.89	64.15	–	–	12.26	5.23	–	–
16a	H	piperidine	356.44	231.12	–	–	–	–	–	–
17a	Br	piperidine	435.34	192.45	–	–	–	–	9.00	21.38
17b	Br	Morpholine	437.31	333.33	–	–	–	–	30.51	10.92
17c	Br	Diethylamine	423.33	86.23	–	–	–	–	–	–
17d	Cl	Morpholine	392.86	577.35	–	–	–	–	–	–
20	Br	Morpholine	459.34	47.72	–	–	–	–	–	–
Lamivudine			229.26	961.50	–	–	–	–	14.80	64.97

[a] TC_{50} is 50% cytotoxic concentration in 2.2.15 cells.
[b] IC_{50} is 50% inhibitory concentration.
[c] Selectivity Index (SI:TC_{50}/IC_{50}).
[d] No antiviral activity at a concentration lower than its TC_{50}.

30 mL CH_2Cl_2 was added and rocked. The resulting precipitate was filtered and washed with water, the filtered solution was concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 3/1) to afford the crude **10**, which was used in the next step without further characterization.

*5.1.8. N-(1-Bromo-3-morpholino-3-oxo-1-phenylprop-1-en-2-yl)acetamide (**11**)*

Compound **11** was prepared in a similar manner as described for the synthesis of **9a–c** except morpholine was used in place of piperidine. The compound **11** was confirmed by X-ray diffraction analysis.

*5.1.9. Ethyl 4-methyl-1,2,3-thiadiazole-5-carboxylate (**12**)*

Compound **12** was prepared as a yellow oil using commercially available acetyl ethyl acetate as the starting material according to the literature [24]. Yield = 67.1%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H, $CH_2\underline{CH_3}$), 2.95 (s, 3H, thiadiazole-CH_3), 4.49 (q, 2H, $\underline{CH_2}CH_3$) [26].

*5.1.10. 5-Hydroxymethyl-4-methyl-1,2,3-thiadiazole (**13**)*

Compound **13** was prepared according to the literature [27] as a yellow oil. Yield = 41.9%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.62 (s, 3H, thiadiazole-CH_3), 3.35 (s, 1H, OH), 5.00 (s, 2H, CH_2) [26].

*5.1.11. 4-Methyl-1,2,3-thiadiazole-5-carboxaldehyde (**14**)*

Compound **14** was prepared according to the literature [28] as a yellow oil. Yield = 43.7%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.03 (s, 3H, thiadiazole-CH_3), 10.25 (s, 1H, CHO) [26].

*5.1.12. 4-((4-Methyl-1,2,3-thiadiazol-5-yl)methylene)-2-phenyloxazol-5(4H)-one (**15**)*

Benzoylglycine (3.36 g, 17.9 mmol), **14** (2.32 g, 17.9 mmol), anhydrous NaAc (1.54 g, 17.9 mmol), and acetic anhydride (5.47 g, 53.6 mmol) were placed in a 50 mL three-necked round-bottomed flask equipped with a reflux condenser bearing a calcium chloride tube. The mixture was heated at 138 °C for 10 min, then cooled to 95–100 °C for 2 h. After being cooled to 80 °C, the reaction mixture was treated with ethanol (3 mL) and cooled to room temperature. The product was precipitated out of the solution, and after standing overnight, the solid was collected, washed with ethanol (2 × 10 mL) and hot water (3 × 5 mL), and then dried to obtain **15** (2.81 g, 57.0% yield) as a red solid, m.p. 233–235 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90 (s, 3H, thiadiazole-CH_3), 7.40 (s, 1H, C=C–H), 7.57–8.30 (m, 5H, Ph-H).

*5.1.13. General procedure for the preparation of intermediates **16a–c***

A solution of **15** (1.4 g, 5.1 mmol) in $CHCl_3$ (20 mL) was stirred under an ice bath, a solution of appropriate secondary amines (5.6 mmol) in $CHCl_3$ (10 mL) was added dropwise, and then the mixture was stirred at room temperature for 1.5 h. After standing overnight, the resulting solution was filtered and evaporated to afford the product.

*5.1.13.1. N-(1-(4-Ethyl-1,2,3-thiadiazol-5-yl)-3-oxo-3-(piperidin-1-yl)-prop-1-en-2-yl)benzamide (**16a**).* Yellow solid, yield: 69.4%, m.p. 175–177 °C; IR (KBr, cm^{-1}): 3236 (NH), 3060 (CH, olefinic), 2925, 2855 (CH aliph.), 1733, 1671 (C=O), 1618, 1533, 1474 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92–1.75 (m, 6H, piperidine-$3CH_2$), 2.59 (s, 3H, thiadiazole-CH_3), 3.29–3.72 (m, 4H, piperidine-$2CH_2$), 6.25 (s, 1H, C=C–H), 7.24–7.81 (m, 5H, Ph-5H), 9.15(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.06, 164.11, 157.01, 142.90, 136.39, 132.70, 132.37, 128.43 (2C), 127.38 (2C), 102.84, 47.69, 42.86, 25.77, 24.64, 24.20, 12.63. ESI-MS (*m*/*z*): 355.15 $(M-H)^-$. Anal. calcd for $C_{18}H_{20}N_4O_2S$: C, 60.65; H, 5.66; N, 15.72; found: C, 60.76; H, 5.45; N, 15.76.

*5.1.13.2. N-(1-(4-Methyl-1,2,3-thiadiazol-5-yl)-3-morpholino-3-oxo-prop-1-en-2-yl)benzamide (**16b**).* Yellow solid, yield 99.6%, m.p. 187–189 °C; IR (KBr, cm^{-1}): 3198 (NH), 2923, 2855 (CH aliph.), 1732, 1681 (C=O), 1604, 1509, 1474 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.67 (s, 3H, thiadiazole-CH_3), 3.40–3.79 (m, 8H, morpholine-$4CH_2$), 6.15 (s, 1H, C=C–H), 7.39–7.85 (m, 5H, Ph-5H), 9.28 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.83, 166.49, 157.54, 141.38, 134.79, 132.68, 131.61, 128.63 (2C), 127.81 (2C), 109.84, 66.37 (2C), 48.74, 42.90, 12.97. ESI-MS (*m*/*z*): 357.08 $(M-H)^-$. Anal. calcd for $C_{17}H_{18}N_4O_3S$: C, 56.97; H, 5.06; N, 15.63; found: C, 56.92; H, 5.00; N, 15.73.

*5.1.13.3. N-(3-(Diethylamino)-1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-oxoprop-1-en-2-yl)benzamide (**16c**).* Red solid, yield: 91.7%, m.p. 164–166 °C; IR (KBr, cm^{-1}): 3169 (NH), 2973, 2874 (CH aliph.), 1731, 1669 (C=O), 1618, 1514, 1477 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19 (br, 6H, 2 $CH_2\underline{CH_3}$), 2.62 (s, 3H, thiadiazole-CH_3), 3.40–3.71 (2br, 4H, 2 $\underline{CH_2}CH_3$), 6.14 (s, 1H, C=C–H), 7.32–7.83 (m, 5H, Ph-5H), 9.96 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.08, 166.72, 157.05, 141.88, 135.92, 132.20, 131.65, 128.29 (2C), 127.95 (2C), 109.12, 43.77, 39.88, 13.62, 12.82, 11.99. ESI-MS (*m*/*z*): 343.07 $(M-H)^-$. Anal. calcd for $C_{17}H_{20}N_4O_2S$: C, 59.28; H, 5.85; N, 16.27; found: C, 59.06; H, 5.76; N, 16.26.

*5.1.14. General procedure for the preparation of derivatives **17a–d***

Bromine liquid (0.32 g, 2.0 mmol) was added to a solution of **16** (1.96 mmol) in $CHCl_3$ (20 mL) under a salt-ice bath. The mixture was stirred at room temperature for 0.5 h, after which $CaCO_3$ (0.1 g, 1 mmol) was added. The resulting solution was stirred at room temperature overnight. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 5/1) to afford compound **17a–c**.

A solution of **16b** (0.5 g, 1.39 mmol) in $CHCl_3$ (10 mL) was stirred under a salt-ice bath, a solution of Cl_2 (0.1 g, 1.4 mmol) in CCl_4 was added. The mixture was stirred at room temperature for 2 h, and then $CaCO_3$ (0.07 g, 0.7 mmol) was added. The resulting solution was stirred at room temperature overnight. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 5/1) to afford compound **17d**.

*5.1.14.1. N-(1-Bromo-1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)benzamide (**17a**).* White solid, Yield 43.0%, m.p. 192–193 °C; IR (KBr, cm^{-1}): 3317 (NH), 2934, 2856 (CH aliph.), 1725, 1683 (C=O), 1633, 1512, 1466 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90–1.88 (m, 6H, piperidine-$3CH_2$), 2.58 (s, 3H, thiadiazole-CH_3), 3.49–3.65 (m, 4H, piperidine-$2CH_2$), 7.22–7.53 (m, 5H, Ph-H), 9.11 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.79, 160.99, 159.17, 143.96, 136.96, 133.11, 131.98, 129.05 (2C), 127.52 (2C), 90.00, 47.72, 42.69, 25.09, 24.61, 24.05, 13.57. ESI-MS (*m*/*z*): 434.79 $(M-H)^-$. Anal. calcd for $C_{18}H_{19}BrN_4O_2S$: C, 49.66; H, 4.40; N, 12.87; found: C, 49.73; H, 4.42; N, 13.00.

*5.1.14.2. N-(1-Bromo-1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-morpholino-3-oxoprop-1-en-2-yl)benzamide (**17b**).* White solid, yield 58.6%, m.p. 187–188 °C; IR (KBr, cm^{-1}): 3167 (NH), 2986, 2924, 2849 (CH aliph.), 1678, 1625 (C=O), 1581, 1515, 1474 (C=C, phenyl). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.54 (s, 3H, thiadiazole-CH_3), 3.40–4.23 (m, 8H, morpholine-$4CH_2$), 7.24–7.51 (m, 5H, Ph-H), 9.26 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.19, 163.80, 156.84, 146.96, 134.37, 132.60, 130.89, 128.55 (2C), 127.57 (2C), 98.86, 66.06 (2C), 47.36, 42.26, 13.94. ESI-MS (*m*/*z*): 458.91 $(M+Na)^+$. Anal. calcd for $C_{17}H_{17}BrN_4O_3S$: C, 46.69; H, 3.92; N, 12.81; found: C, 46.61; H, 4.04; N, 12.98.

*5.1.14.3. N-(1-Bromo-3-(diethylamino)-1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-oxoprop-1-en-2-yl)benzamide (**17c**).* White solid, yield 56.8%, m.p. 181–182 °C; IR (KBr, cm^{-1}): 3242 (NH), 2988, 2941, 2873 (CH aliph.), 1729, 1670 (C=O), 1633, 1519, 1480 (C=C, phenyl). ^1H NMR (300 MHz, $CDCl_3$): δ 0.91 (t, 3H, $CH_2\underline{CH_3}$), 1.01 (t, 3H, $CH_2\underline{CH_3}$), 2.75 (s, 3H, thiadiazole-CH_3), 3.14–3.42 (m, 4H, $\underline{CH_2}CH_3$), 7.50–7.90 (m, 5H, Ph-H), 8.38 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.52, 161.52, 159.36, 143.82, 137.27, 133.07, 129.06 (2C), 128.39, 127.50 (2C), 89.45, 42.86, 38.48, 13.67, 12.65, 11.44. ESI-MS (*m*/*z*): 422.89 $(M-H)^-$. Anal. calcd for $C_{17}H_{19}BrN_4O_2S$: C, 48.23; H, 4.52; N, 13.23; found: C, 48.20; H, 4.52; N, 13.33.

*5.1.14.4. N-(1-Chloro-1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-morpholino-3-oxoprop-1-en-2-yl)benzamide (**17d**).* White solid, yield 60.3%, m.p. 196–198 °C; IR (KBr, cm^{-1}): 3202 (NH), 2924, 2855 (CH aliph.), 1723, 1680 (C=O), 1621, 1507, 1478 (C=C, phenyl). ^1H NMR (300 MHz, $CDCl_3$): δ 2.61 (s, 3H, thiadiazole-CH_3), 3.61–4.05 (m, 8H, morpholine-$4CH_2$), 7.44–7.89 (m, 5H, Ph-H), 9.16 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.63, 163.14, 159.45, 145.44, 134.63, 131.36, 128.99, 128.49, 127.85, 127.67, 127.55, 109.99, 66.36, 66.11, 47.27, 42.33, 14.18. ESI-MS (*m*/*z*): 391.19 $(M-H)^-$. Anal. calcd for $C_{17}H_{17}ClN_4O_3S$: C, 51.97; H, 4.36; N, 14.26; found: C, 51.78; H, 4.36; N, 14.46.

*5.1.15. 2-(4-Methyl-1,2,3-thiadiazol-5-yl)-4-((4-methyl-1,2,3-thiadiazol-5-yl)methylene)oxazol-5(4H)-one (**18**)*

Compound **6** (4.21 g, 21.0 mmol), **14** (2.68 g, 21.0 mmol), anhydrous NaAc (1.72 g, 21.0 mmol), and acetic anhydride (6.41 g, 62.3 mmol) were placed in a 50 mL three-necked round-bottomed flask equipped with a reflux condenser bearing a calcium chloride tube. The mixture was rocked rapidly at room temperature. After this, the reaction mixture was treated under ultrasound irradiation at 70 °C for 2 h. The product was precipitated out of the solution. The solid was collected, washed with ethanol (3 × 10 mL), hot water (3 × 5 mL), and petroleum ether (2 × 10 mL), and dried to obtain compound **18** (3.90 g, 63.4% yield) as a yellow solid, m.p. 166–168 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.94 (s, 3H, thiadiazole-CH_3), 3.24 (s, 3H, thiadiazole-CH_3), 7.58 (s, 1H, C=C–H).

*5.1.16. 4-Methyl-N-(1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-morpholino-3-oxoprop-1-en-2-yl)-1,2,3-thiadiazole-5-carboxamide (**19**)*

A solution of **18** (1.33 g, 4.44 mmol) in $CHCl_3$ (20 mL) was stirred under an ice bath, a solution of morpholine (0.39 g, 4.44 mmol) in $CHCl_3$ (10 mL) was added dropwise. The mixture was stirred at room temperature for 4 h. After standing overnight, the residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 4/1) to afford compound **19** (0.73 g, 43.3% yield) as a white solid, m.p. 195–198 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.71 (s, 3H, thiadiazole-CH_3), 2.78 (s, 3H, thiadiazole-CH_3), 3.75–3.81 (m, 8H, morpholine-$4CH_2$), 6.20 (s, 1H, C=C–H), 9.92 (br, 1H, NH).

*5.1.17. N-(1-Bromo-1-(4-methyl-1,2,3-thiadiazol-5-yl)-3-morpholino-3-oxoprop-1-en-2-yl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (**20**)*

Bromine liquid (0.31 g, 1.92 mmol) was added to a solution of **19** (0.73 g, 1.92 mmol) in $CHCl_3$ (20 mL) under a salt-ice bath. The mixture was stirred at room temperature for 2.5 h, and then $CaCO_3$ (0.1 g, 1 mmol) was added. The resulting solution was stirred at room temperature overnight. The residue was subjected to silica gel column chromatography (petroleum ether/ethyl acetate: 4/1) to afford compound **20** (0.6 g, 67.9% yield) as a white solid, m.p. 181–183 °C; IR (KBr, cm^{-1}): 3076 (NH), 2921, 2856 (CH aliph.), 1671, 1665 (C=O), 1611, 1508, 1477 (C=C, phenyl). ^1H NMR (300 MHz, $CDCl_3$): δ 2.55 (s, 3H, thiadiazole-CH_3), 2.72 (s, 3H, thiadiazole-CH_3), 3.69–3.97 (m, 8H, morpholine-$4CH_2$), 9.63 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.42, 162.20, 159.09, 158.25, 140.27, 140.07, 132.96, 112.63, 66.34, 66.37, 29.70 (2C), 13.99, 13.00. ESI-MS (*m*/*z*): 379.10 $(M-Br)^-$. Anal. calcd for $C_{14}H_{15}BrN_6O_3S_2$: C, 36.61; H, 3.29; N, 18.30; found: C, 36.47; H, 3.38; N, 18.41.

5.2. Crystal data for **11**

The crystal data of the rearranged product are as follows. $C_{15}H_{17}BrN_2O_3$, $M = 353.22$, Monoclinic, $a = 12.201(3)$, $b = 14.079(4)$, $c = 18.852(5)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 3238.5(15)$ $Å^3$, $T = 293(2)$ K, space group P2(1)2(1)2(1), $Z = 8$, $Dc = 1.449$ g/cm^3, μ (Mo-K_α) = 0.71073 mm^{-1}, $F(000) = 1440$ reflections measured, 6650 unique ($R_{int} = 0.0519$), which were used in all calculation. Fine $R_1 = 0.0567$, wR (F^2) = 0.1092 (all data). The full crystallographic details of the rearranged product have been deposited at the Cambridge Crystallographic Data Center and were allocated the deposition number CCDC 662310.

5.3. Biological assay

5.3.1. In vitro anti-HBV activity assay

The anti-HBV activities of the target compounds were evaluated in 2.2.15 cells by previously reported methods [29,30]. This assay included 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 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. The cell control was set up. Medium was changed daily with fresh test compounds and positive control for 8 days. The excretion of HBsAg and HBeAg in the cell supernatant was detected by radioimmunoassay (RIA) using commercial kits (HBsAg, HBeAg, Beijing North Institute of Biological Technology). The DNA replication of HBV in the cell was detected by dot blot hybridization. The IC_{50} and selected index of the evaluated compounds and lamivudine were calculated, respectively.

5.3.2. Cytotoxicity assay

The cytotoxicities of the test compounds to 2.2.15 cells were assessed by MTT assay [31]. Briefly, 2.2.15 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 absorbance at 510 nm relative to untreated cells, 9 days of treatment.

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