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DOI: 10.1002/cmdc.200800136

Rational Design and Synthesis of 2,2-Bisheterocycle Tandem Derivatives as Non-Nucleoside Hepatitis B Virus Inhibitors

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Despite the existence of an effective vaccine against the hepatitis B virus (HBV), chronic infection still poses a huge health burden on the global community.[1] Current clinical therapies for the treatment of chronic hepatitis B include interferon α, and the nucleoside-derived, viral polymerase inhibitors lamivudine (3TC), adefovir, entecavir, and telbivudine. Unfortunately, resistance of the virus to nucleoside-derived inhibitors, and the adverse effects of interferon α, limit the clinical application of these drugs. Therefore, the development of potent non-nucleoside anti-HBV agents is urgently required.[2]

Natural products have been a rich source of medicines; their structural diversity has served as a source of inspiration in the search for pharmacologically active molecules.[3] Our strategy focuses on the discovery of therapeutic agents inspired by small-molecule natural products with unique structural motifs, such as heterocyclic tandem subunits.[4] Heterocyclic tandem pairs have been found in a few natural products, and play a pivotal role in their bioactivity through specific interactions with DNA or other targets.[5] Leucamide A is a cyclic heptapeptide containing a mixed 4,2-bisheterocycle tandem pair isolated from the Australian marine sponge *Leucetta microraphis*, and is moderately cytotoxic in several tumor cell lines.[6] The mixed 4,2-bisheterocycle tandem pair represents a novel scaffold worthy of further investigation. Following our first total synthesis of leucamide A,[7] we synthesized a library of 4,2-bisheterocycle tandem derivatives consisting of a methyloxazole and a thiazole subunit (Figure 1). These compounds were screened in several in vitro assays to determine their antiviral activities against influenza virus, herpes simplex virus (HSV), and HBV. Several compounds showed moderate activity against influenza A virus,[8] HSV-2, and HBV, whereas leucamide A itself showed no antiviral activity. Of these derivatives, compound **1**, which has an IC_{50} value of 76.4 μM against HBV DNA replication, represents a novel chemical scaffold unlike any known non-nucleoside inhibitors. Though compound **1** showed relatively weak inhibitory activity against HBV DNA replication, it was considered to be a good lead for further optimization. To diversify the bisheterocyclic scaffold, several series of compounds were designed and synthesized, including 2,4 and 2,2 tandem pairs in which the latter proved more potent (Figure 1).[9]

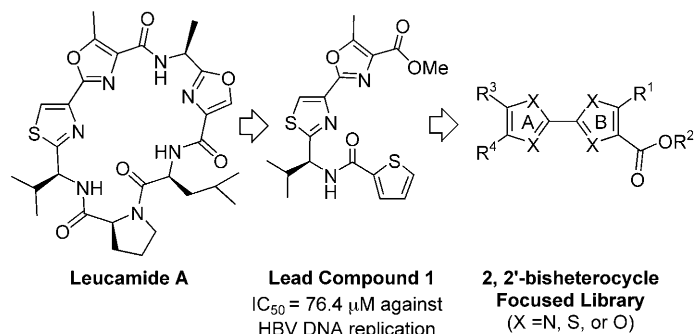
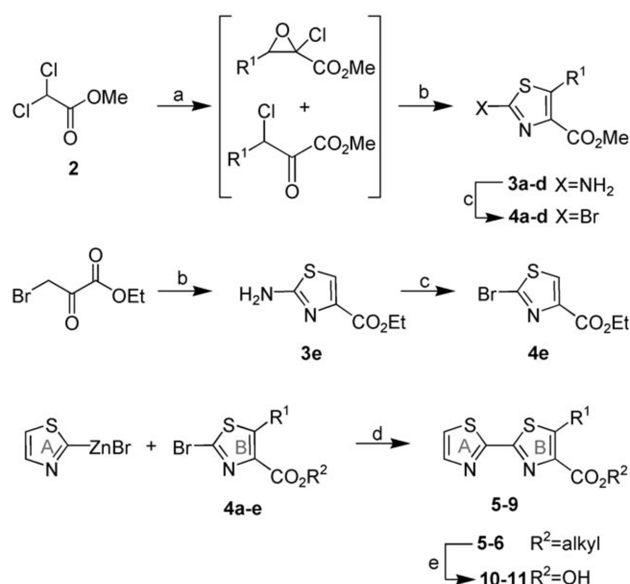


Figure 1. Discovery of the anti-HBV lead compound (**1**) from the natural product Leucamide A and the design of a focused library of tandem substituted 2,2′-bisheterocycles.



Scheme 1. Synthesis of compounds **5**–**11**; Reagents and conditions: a) MeONa/MeOH, R^1CHO, Et_2O, 0 °C; b) $(H_2N)_2CS$, MeOH, reflux, 6 h, 40–83 %; c) $CuBr_2$, *t*BuONO, CH_3CN, 0 °C, 1 h, 45–81 %; d) $Pd(PPh_3)_4$, THF, reflux, 63–80 %; e) LiOH, $MeOH/H_2O$ (*v/v* 1:1), 0 °C to RT, 1 h, 97–98 %.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200800136.

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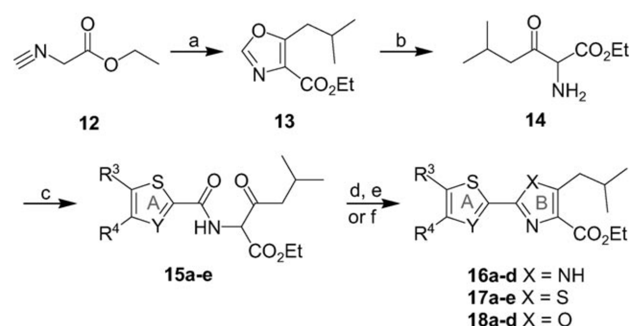
To understand their structure–activity relationship, simple 2,2-bisheterocycle compounds without substitutions on the thiazole A ring were synthesized (Scheme 1). The Darzens reaction of methyl dichloroacetate (**2**) with aldehydes produced a mixture of α-chloroglycidic esters and β-chloro-α-oxo-esters. These esters or ethyl bromopyruvate were reacted with thiourea to form the 2-aminothiazole-4-carboxylic esters (**3a**–**d**) or **3e**,[10] followed by diazotization and bromination at the 2-position to give the thiazole ester compound (**4a**–**e**).[11] Coupling of the 2-bromothiazole-4-carboxylates (**4a**–**e**) with 2-thiazolezinc bromide, which was prepared by treating 2-bromothiazole with activated zinc in THF[12] using the Negishi method afforded products **5**–**9**. Saponification of **5** and **6** with lithium hydroxide in aqueous methanol gave the corresponding carboxylic acids **10** and **11**, respectively.

The anti-HBV activity of these compounds was evaluated in HBV-transfected HepG2.2.15 cells (Table 1).[13] Of these compounds, derivative **6** showed dramatically increased inhibition of HBV DNA replication (IC_{50} = 2.4 μм), which was ~30 times more potent than the lead compound **1**. Conversely, compound **7**, which has a 5-benzyl group on the thiazole B ring, showed only a slight increase in inhibitory activity (IC_{50} = 31.3 μм), and compound **9**, with no substitution at the 5-position, had no activity against HBV DNA replication. These results suggest that a bulky aliphatic group at the 5-position of the heterocycle B ring has an advantageous effect on the activity of the compounds against HBV DNA replication. The inhibitory activities of acids **10** and **11** were substantially diminished compared with their ester analogues **5** and **6**, respectively (IC_{50} = 125 (**10**) and 60 (**11**) vs. 19.4 (**5**) and 2.4 μм (**6**)). This loss of activity might be partially explained by pharmacokinetic effects. These results strongly indicate that an ester group is crucial for the effective inhibition of HBV DNA replication. Compound **8**, which only differs structurally from compound **5** in the side chain where one CH_2 group is replaced by an oxygen atom to give the ether, had similar potency against HBV DNA replication, with an IC_{50} value of 2.5 μм. A cytotoxicity assay showed that 2,2-bisthiazole derivatives have relative low cytotoxicity, with CC_{50} values > 100.0 μм.

Initial SAR data for the B ring indicate that compound **6** (where R^1 is isobutyl and R^2 is an ester) is the most potent derivative with significant inhibitory activity against HBV DNA replication. We then investigated the effects of varying the heteroatom at position X on the B ring and the substitution of the A ring. Compounds **16a–d**, **17a–e**, and **18a–d** were synthesized, where R^1 and R^2 were fixed as 5-isobutyl and 4-ethoxycarbonyl, respectively (Scheme 2). Ethyl-5-isobutyl-oxazole-4-carboxylate **13** was easily obtained from ethyl isocyanoacetate **12**[14] by reaction with isobutyric anhydride in the presence of DBU. Subsequent acid hydrolysis gave the ethyl-2-amino-5-methyl-3-oxo-hexanoate **14** in good yield. The corresponding *N*-acyl derivatives **15a–e** were obtained by coupling compound **14** with thiazole-2-carboxylic acid,[15] thiophene-2-carboxylic acid, benzothiophene-2-carboxylic acid, 5-methylthiophene-2-carboxylic acid,[16] or 5-ethylthiophene 2-carboxylic acid, respectively. The *N*-acylamino ketones **15a–e** were converted into the three desired heterocyclic cores;[17] for example, the reaction of **15a** with ammonium acetate and sodium acetate resulted in its cyclization to the imidazole **16a**, thiation of **15a** using Lawesson's reagent gener-



Scheme 2. Synthesis of compounds **16a–d**, **17a–e and 18a–d** Reagents and conditions: a) isobutyric anhydride, DBU, THF; b) 3 м HCl/EtOH; c) EDC, DMAP, pyridine, different heterocycle 2-carboxylic acids; d) NH_4OAc, NaOAc, 140 °C; e) Lawesson's reagent, THF, reflux; f) $POCl_3$, reflux.

Table 1. Anti-HBV activity of 2,2-disubstituted thiazole-thiazole derivatives (**5–11**).[a]

Compound	R^1	R^2	CC_{50}[b] [μм]	IC_{50}[c] [μм]	SI[d]
1	–	–	> 500	76.4	6.5
5	n-butyl	Me	600.0	19.4	30.9
6	isobutyl	Me	600.0	2.4	248.9
7	benzyl	Me	173.6	31.3	5.6
8	$CH_3OCH_2CH_2$	Me	328.6	2.5	129.9
9	H	Et	611.4	NA[e]	
10	n-butyl	H	256.7	125.0	2.1
11	isobutyl	H	437.3	60.0	7.3
3TC	–	–	1714.7	0.26	6595

[a] The data shown here are from a representative experiment repeated three times with similar results. [b] Concentration of compounds required for 50% extinction of HepG 2.2.15 cells. [c] Concentration of compounds achieving 50% inhibition of extracellular HBV-DNA replication. [d] Selectivity index (SI) was determined as the CC_{50}/IC_{50} value. [e] NA = no activity up to 50% concentration of the CC_{50} value.

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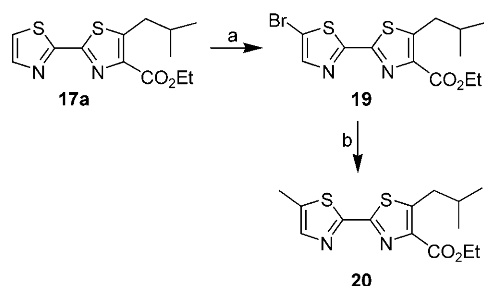
ated thiazole **17a**, dehydration of **15a** with phosphoryl chloride gave oxazole **18a**.

Imidazoles **16a–d**, thiazoles **17a–e**, and oxazoles **18a–d** were examined for their anti-HBV activity, and the results are summarized in Table 2. The nature of the heterocycle had a significant effect on antiviral potency. Oxazoles **18a–d** were completely inactive against HBV DNA replication, while imidazoles **16a–d** showed a slight decrease in activity compared with the corresponding thiazole congeners **17a–d**. These results suggest that thiazoles, where X is S, are the optimal B heterocycle. Comparing thiophene-thiazoles **17b**, **17d** and **17e**, the methyl-substituted thiophene-thiazole **17d** was ~13-fold more potent than the unsubstituted derivative **17b**, however, the ethyl-substituted compound **17e** showed significant loss of activity. These results indicate that a small substituent at the 5-position of ring A was tolerated in the thiophene-thiazole series.

To better understand the substitution effects of heterocycle A, the 2,2′-bisthiazole **20**, where the C3 position of the thiophene ring of compound **17d** was replaced with a nitrogen atom, was synthesized by the bromination of **17a**, followed by cross-coupling with methylzinc iodide[18] in the presence of 5 mol% $Cl_2Ni(PPh_3)_2$ (Scheme 3). Compound **20** showed the greatest potency (IC_{50} = 0.14 μM) in inhibiting HBV DNA replication, and was ~500-fold more potent than the lead compound (**1**, IC_{50} = 76.4 μM), and even more potent than Lamivudine (IC_{50} = 0.26 μM).



Scheme 3. Synthesis of compound **20**; Reagents and conditions: a) Br_2, CH_2Cl_2, 18 h, 63%; b) MeZnI, $Cl_2Ni(PPh_3)_2$, DMA, 3 h, RT, 96%.

In summary, we discovered a series of potent, novel, non-nucleoside HBV inhibitors, through structural diversification of a substructure of the natural product leucamide A. We established the primary structure–activity relationships, defining the structural requirements for biological activity through structural modifications. Notably, the compounds reported here were inactive at 200 $\mu g\,mL^{-1}$ in HBV polymerase assays, indicating no activity against known anti-HBV drug targets, such as polymerase. These results present an opportunity for the development of novel non-nucleoside small-molecule anti-HBV chemotherapeutic agents with a potentially new mechanism of action. Further SAR studies are needed for structural optimization of these compounds, as is the identification of their molecular targets; continued investigation into new bisheterocycle tandem pairs is actively underway in our laboratory.

Experimental Section

Synthesis

General experimental details including reagent grades, instrument and software details, HPLC conditions, and general methods including intermediate compounds and work-up procedures referred to in the Experimental Section, are in the Supporting Information.

General procedure (5–9): A suspension of activated Zn (3.0 mmol) in THF (1.5 mL) was treated slowly under N_2 with a solution of 2-bromothiazole (2.8 mmol) in THF (2.8 mL) and heated at reflux for 3 h. The reaction was cooled to RT before $Pd(PPh_3)_4$ (5 mol%) and the appropriate thiazole (**4a–e**, 2 mmol) were added, and then stirred at reflux until completion. The reaction was concentrated in vacuo and the crude material was partitioned between aq EDTA and EtOAc. The organic phase was processed in the usual way (Supporting Information) and purification by flash chromatography gave compounds **5–9**.

Methyl-5-butyl-[2, 2′]bithiazolyl-4-carboxylate (5): White power (70%); mp: 116–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, J = 7.5 Hz, 3H), 1.45 (tq, J = 7.5 Hz, 2.0 Hz, 2H), 1.70 (tt, J = 7.5 Hz, 2.0 Hz, 2H), 3.25 (t, J = 7.5 Hz, 2H), 3.92 (s, 3H), 7.43 (d, J = 3.3 Hz, 1H), 7.84 ppm (d, J = 3.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.6, 22.2, 27.3, 33.5, 52.2, 121.3, 141.2, 143.7, 153.1, 157.4, 160.8, 162.4 ppm; HRMS (EI) calcd for $C_{12}H_{14}N_2O_2S_2$ [M^+] 282.0497; found 282.0498.

Methyl-5-isobutyl-[2,2′]bithiazolyl-4-carboxylate (6): White power (75%); mp: 102–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99 (d, J = 6.6 Hz, 6H), 1.95–1.23 (m, 1H), 3.15 (d, J = 6.9 Hz, 2H), 3.96 (s, 3H), 7.46 (d, J = 3.3 Hz, 1H), 7.86 ppm (d, J = 3.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.9, 30.6, 35.8, 51.9, 121.3, 141.5, 143.5, 151.2, 157.3, 160.4, 162.1 ppm; HRMS (EI) calcd for $C_{12}H_{14}N_2O_2S_2$ [M^+] 282.0497; found 282.0487.

Methyl-5-benzyl-[2,2′]bithiazolyl-4-carboxylate (7): Yellow power (63%); mp: 124–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.98 (s, 3H), 4.60 (s, 2H), 7.25–7.35 (m, 5H), 7.43 (d, J = 3.3 Hz, 1H), 7.82 ppm (d, J = 3.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 33.8, 52.3, 121.5, 127.2, 128.7, 128.9, 138.7, 141.2, 143.8, 152.3, 158.3, 160.6, 162.5 ppm; HRMS (EI) calcd for $C_{15}H_{12}N_2O_2S_2$ [M^+] 316.0340; found 316.0334.

Methyl-5-(2-methoxyethyl)-[2,2′]bithiazolyl-4-carboxylate (8): White power (70%); mp: 120–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.41 (s, 3H), 3.55 (t, J = 6.6 Hz, 2H), 3.68 (t, J = 6.6 Hz, 2H), 3.96 (s, 3H), 7.46 (d, J = 3.3 Hz, 1H), 7.88 ppm (d, J = 3.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.6, 52.6, 58.9, 71.7, 121.7, 142.0, 144.1, 149.2, 159.1, 161.2, 162.9 ppm; HRMS (EI) calcd for $C_{11}H_{12}N_2O_3S_2$ [M^+] 284.0289; found 284.0283.

Ethyl-[2,2′]bithiazolyl-4-carboxylate (9): White power (80%); mp: 128–130 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (t, J = 7.2 Hz, 3H), 4.40 (q, J = 6.9 Hz, 2H), 7.48 (d, J = 3.3 Hz, 1H), 7.86 (d, J = 3.3 Hz, 1H), 8.20 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.4, 61.8, 122.2, 128.9, 144.0, 148.2, 160.5, 161.1, 162.2 ppm; HRMS (EI) calcd for $C_9H_8N_2O_2S_2$ [M^+] 240.0027; found 240.0031.

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Table 2. Anti-HBV activity of 2,2′-tandem five-member heterocycle derivatives.[a]

Compound	Structure	CC_{50}[b] [μM]	IC_{50}[c] [μM]	SI[d]
16a		785.7	NA[e]	
17a		> 100	1.1	> 90.9
18a		> 100	NA[e]	
16b		> 100	NA[e]	
17b		> 100	9.9	> 10.1
18b		> 100	NA[e]	
16c		> 100	13.7	> 7.3
17c		> 88.1	1.3	> 66.7
18c		> 100	NA[e]	
16d		120.6	41.3	2.9
17d		97.7	0.78	125.3
18d		> 50	NA[e]	
17e		> 50	34.9	> 1.4
20		118.2	0.14	844.3
3TC	–	1714.7	0.26	6595

[a] The data shown here are from a representative experiment repeated three times with similar results. [b] Concentration of compounds required for 50% extinction of HepG 2.2.15 cells. [c] Concentration of compounds achieving 50% inhibition of extracellular HBV-DNA replication. [d] Selectivity index (SI) was determined as the CC_{50}/IC_{50} value. [e] NA = no activity up to 50% concentration of the CC_{50} value.

General procedure (10–11): A solution of ester (**5**–**6**, 0.25 mmol) in MeOH/H_2O (4:1, 1 mL) was cooled to 0 °C, treated with LiOH·H_2O (1 mmol) and stirred while warming to RT for 1 h and stirring was continued until complete. The reaction was concentrated and the residue was partitioned between EtOAc and H_2O. The organic phase was separated, and the aqueous phase was acidified to pH 2 with aq HCl (10%) and then extracted with EtOAc. The combined organic phases were then processed in the usual way to obtain the carboxylic acid (**10**–**11**).

5-Butyl-[2,2′]bithiazolyl-4-carboxylic acid (10): White solid (98%); mp: 135–137 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.76$ (t, $J = 7.5$ Hz, 3H), 1.13 (m, 2H), 1.33–1.41 (m, 2H), 2.47 (t, $J = 7.5$ Hz, 2H), 7.36 (d, $J = 3.3$ Hz, 1H), 7.74 ppm (d, $J = 3.3$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 13.5$, 21.7, 26.6, 33.2, 123.0, 142.4, 144.1, 151.5, 156.3, 160.1, 162.7 ppm; HRMS (EI) calcd for $C_{11}H_{12}N_2O_2S_2$ [M^+] 268.0340; found 268.0346.

5-Isobutyl-[2,2′]bithiazolyl-4-carboxylic acid (11): Yellow power (97%); mp: 133–134 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.01$ (d, $J = 6.6$ Hz, 6H), 1.98–2.07 (m, 1H), 3.33 (d, $J = 6.6$ Hz, 2H), 7.40 (d, $J = 3.0$ Hz, 1H), 7.80 ppm (d, $J = 3.0$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 21.9$, 30.6, 35.3, 123.0, 143.1, 144.1, 149.8, 156.7, 160.1, 162.9 ppm; HRMS (EI) calcd for $C_{11}H_{12}N_2O_2S_2$ [M^+] 268.0340; found 268.0340.

General procedure (16a–d): Compound **15** (**a**–**d**, 0.26 mmol) was mixed with ammonium acetate (1.0 g) and sodium acetate (500 mg), and the mixture was heated at 140 °C for 6 h. After cooling, the residue was dissolved in water (50 mL) and extracted with EtOAc; the EtOAc phase was processed in the usual way (Supporting Information). Purification by flash chromatography (petroleum ether/EtOAc) afforded compound **16** (**a**–**d**).

Ethyl-5-isobutyl-2-thiazol-2-yl-1(3)*H*-imidazole-4-carboxylate (16a): White power (37%); mp: 135–137 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.86$ (d, $J = 6.6$ Hz, 6H), 1.34 (t, $J = 7.2$ Hz, 3H), 1.90–1.98 (m, 1H), 2.79 (d, $J = 7.2$ Hz, 2H), 4.33 (q, $J = 7.2$ Hz, 2H), 7.39 (d, $J = 3.3$ Hz, 1H), 7.78 ppm (d, $J = 3.3$ Hz, 1H); HRMS (EI) calcd for $C_{18}H_{20}N_2O_2S$ [M^+] 279.1041; found 279.1033.

Ethyl-5-isobutyl-2-thiophen-2-yl-1(3)*H*-imidazole-4-carboxylate (16b): White soild (38%); mp: 245–246 °C; ^{1}H NMR (300 MHz,

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$CDCl_3$): $\delta = 0.95$ (d, $J = 5.7$ Hz, 6H), 1.35 (t, $J = 6.9$ Hz, 3H), 2.04–2.12 (m, 1H), 2.83 (br s, 2H), 4.34 (q, $J = 6.9$ Hz, 2H), 7.26 (d, $J = 0.6$ Hz, 1H), 7.78 (d, $J = 3.6$ Hz, 1H), 755–7.60 ppm (m, 1H); HRMS (EI) calcd for $C_{18}H_{20}N_2O_2S$ [M^+] 278.1089; found 278.1087.

Ethyl-2-benzo[b]thiophen-2-yl-5-isobutyl-1(3)*H*-imidazole-4-carboxylate (16c): White power (41%); mp: 217–218 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.80$ (d, $J = 6.6$ Hz, 6H), 1.23 (t, $J = 6.9$ Hz, 3H), 1.85–1.94 (m, 1H), 2.66 (d, $J = 7.2$ Hz, 2H), 4.19 (q, $J = 7.2$ Hz, 2H), 7.16–7.19 (m, 2H), 7.60–7.67 ppm (m. 3H); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$): $\delta = 14.0$, 22.1, 29.1, 29.5, 60.3, 121.9, 122.2, 123.9, 124.5, 125.0, 127.6, 128.7, 131.0, 132.3, 139.8, 141.8, 163.4 ppm; HRMS (EI) calcd for $C_{18}H_{20}N_2O_2S$ [M^+] 328.1245; found 328.1238.

Ethyl-5-isobutyl-2-(5-methylthiophen-2-yl)-1(3)*H*-imidazole-4-carboxylate (16d): White soild (32%); mp: 133–134 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.90$ (d, $J = 6.6$ Hz, 6H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.96–2.05 (m, 1H), 2.46 (s, 3H), 2.78 (d, $J = 7.2$ Hz, 2H), 4.30 (q, $J = 7.2$ Hz, 2H), 6.67 (d, $J = 3.6$ Hz, 1H), 7.37 ppm (d, $J = 3.6$ Hz, 1H); HRMS (EI) calcd for $C_{15}H_{20}N_2O_2S$ [M^+] 292.1245; found 292.1252.

General procedure (17a–e): Compound **15** (**a**–**e**, 0.14 mmol) was dissolved in THF (5 mL) and Lawesson's reagent (0.22 mmol) was added. The mixture was stirred at reflux for 5 h. The reaction was concentrated in vacuo and redissolved in EtOAc (100 mL) then washed with saturated aq $NaHCO_3$, H_3PO_4 (1 N), and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by flash chromatography (petroleum ether/EtOAc) gave compounds **17a–e**.

Ethyl-5-isobutyl-[2,2′]bithiazolyl-4-carboxylic acid ethyl ester (17a): White solid (75%); mp: 118–119 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.00$ (d, $J = 6.6$ Hz, 6H), 1.44 (t, $J = 6.9$ Hz, 3H), 1.96–2.04 (m, 1H), 3.15 (d, $J = 6.9$ Hz, 2H), 4.43 (q, $J = 6.9$ HZ, 2H), 7.46 (d, $J = 3.3$ Hz, 1H), 7.87 ppm (d, $J = 3.3$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.4$, 22.3, 31.0, 36.2, 61.3, 121.6, 142.4, 143.8, 150.9, 157.6, 160.9, 162.1 ppm; HRMS (EI) calcd for $C_{13}H_{16}N_2O_2S_2$ [M^+] 296.0653; found 296.0663.

Ethyl-5-isobutyl-2-thiophen-2-ylthiazole-4-carboxylate (17b): Yellow oil (65%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.00$ (d, $J = 6.6$ Hz, 6H), 1.43 (t, $J = 7.2$ Hz, 3H), 1.94–2.00 (m, 1H), 3.12 (d, $J = 6.9$ Hz, 2H), 4.41 (q, $J = 7.2$ Hz, 2H), 7.07 (dd, $J = 3.6$ Hz, 4.8 Hz, 1H), 7.40 (dd, $J = 0.9$ Hz, 4.8 Hz, 1H), 7.49 ppm (d, $J = 3.9$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.3$, 22.2, 30.9, 35.9, 61.0, 127.1, 127.7, 128.0, 136.5, 141.8, 148.3, 157.9, 162.2 ppm; HRMS (EI) calcd for $C_{15}H_{19}NO_2S_2$ [M^+] 295.0701; found 295.0712.

Ethyl-2-benzo[b]thiophen-2-yl-5-isobutylthiazole-4-carboxylate (17c): White solid (78%); mp: 80–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.00$ (d, $J = 6.6$ Hz, 6H), 1.44 (t, $J = 7.2$ Hz, 3H), 1.99 (dq, $J = 6.6$ Hz, 6.9 Hz, 1H), 3.13 (d, $J = 7.2$ Hz, 2H), 4.43 (q, $J = 7.2$ Hz, 2H), 7.34–7.39 (m, 2H), 7.40 (s, 1H), 7.76–7.79 (m, 1H), 7.81–7.84 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.5$, 22.5, 31.2, 36.2, 61.4, 122.7, 123.7, 124.4, 125.0, 125.9, 136.5, 139.7, 140.5, 142.3, 149.6, 158.1, 162.4 ppm; HRMS (EI) calcd for $C_{18}H_{19}NO_2S_2$ [M^+] 345.0857; found 345.0852.

Ethyl-5-isobutyl-2-(5-methylthiophen-2-yl)-thiazole-4-carboxylate (17d): White power (60%); mp: 65–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.95$ (d, $J = 6.6$ Hz, 6H), 1.38 (t, $J = 7.2$ Hz, 3H), 1.87–1.96 (m, 1H), 2.46 (s, 3H), 3.05 (d, $J = 7.2$ Hz, 2H), 4.36 (q, $J = 7.2$ Hz, 2H), 6.67 (d, $J = 3.6$ Hz, 1H), 7.24 ppm (d, $J = 3.6$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.4$, 15.6, 22.3, 31.1, 36.0, 61.2, 126.2, 127.3, 134.2, 141.6, 143.3, 148.0, 158.3, 162.5 ppm; HRMS (EI) calcd for $C_{15}H_{19}NO_2S_2$ [M^+] 309.0857; found 309.0859.

Ethyl-2-(5-ethylthiophen-2-yl)-5-isobutylthiazole-4-carboxylate (17e): Yellow oil (91%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.96$ (d, $J = 6.9$ Hz, 6H), 1.29 (t, $J = 7.8$ Hz, 3H), 1.39 (t, $J = 7.2$ Hz, 3H), 1.88–1.96 (m, 1H), 2.82 (q, $J = 7.8$ Hz, 2H), 3.06 (d, $J = 7.2$ Hz, 2H), 4.37 (q, $J = 7.2$ Hz, 2H), 6.71 (d, $J = 3.6$ Hz, 1H), 7.27 ppm (d, $J = 3.6$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.4$, 15.9, 22.4, 23.8, 31.1, 36.1, 61.2, 124.4, 127.2, 133.9, 141.7, 148.0, 151.1, 158.5, 162.6 ppm; HRMS (EI) calcd for $C_{15}H_{19}NO_2S_2$ [M^+] 323.1014; found 323.0995.

General procedure (18a–d): Compound **15** (**a**–**d**, 0.1 mmol) was dissolved in $POCl_3$ (0.5 mL), and the mixture was stirred at reflux for 2 h. The solution washed with saturated aq $NaHCO_3$, and extracted with EtOAc. The organic phases were then processed in the usual way (Supporting Information) and purified by flash chromatography (petroleum ether/EtOAc) to afford compound **18** (**a**–**d**).

Ethyl-5-isobutyl-2-thiazol-2-yloxazole-4-carboxylate (18a): White power (80%); mp: 80–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.96$ (d, $J = 6.9$ Hz, 6H), 1.36 (t, $J = 6.9$ Hz, 3H), 2.12–2.19 (m, 1H), 2.99 (d, $J = 7.5$ Hz, 2H), 4.39 (q, $J = 7.2$ Hz, 2H), 7.51 (d, $J = 3.3$ Hz, 1H), 7.94 ppm (d, $J = 3.0$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.2$, 22.2, 28.3, 34.8, 61.1, 122.3, 129.4, 144.4, 154.0, 154.2, 160.4, 161.6 ppm; HRMS (EI) calcd for $C_{18}H_{19}NO_3S$ [M^+] 280.0882; found 280.0890.

Ethyl-5-isobutyl-2-thiophen-2-yloxazole-4-carboxylate (18b): White power (92%); mp: 66–67 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.99$ (d, $J = 6.6$ Hz, 6H), 1.41 (t, $J = 7.2$ Hz, 3H), 2.01–2.08 (m, 1H), 2.96 (d, $J = 7.2$ Hz, 2H), 4.40 (q, $J = 7.2$ Hz, 2H), 7.10 (dd, $J = 3.9$ Hz, 4.8 Hz, 1H), 7.44 (dd, $J = 1.2$ Hz, 5.1 Hz, 1H), 7.10 ppm (dd, $J = 1.2$ Hz, 3.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.3$, 22.3, 28.3, 34.7, 61.2, 128.1, 128.3, 128.5, 129.3, 129.6, 156.2, 158.9, 161.8 ppm; HRMS (EI) calcd for $C_{18}H_{19}NO_3S$ [M^+] 279.0929; found 279.0922.

Ethyl-2-benzo[b]thiophen-2-yl-5-isobutyloxazole-4-carboxylate (18c): White power (76%); mp: 102–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.00$ (d, $J = 6.6$ Hz, 6H), 1.40 (t, $J = 7.2$ Hz, 3H), 2.13 (dq, $J = 6.6$ Hz, 6.9 Hz, 1H), 2.97 (d, $J = 6.9$ Hz, 2H), 4.40 (q, $J = 7.2$ Hz, 3H), 7.33–7.39 (m, 2H), 7.79–7.84 (m, 2H), 7.93 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.4$, 22.4, 28.4, 34.9, 61.2, 122.6, 124.7, 125.0, 125.3, 126.1, 128.7, 129.4, 139.3, 140.7, 156.0, 159.6, 162.2 ppm; HRMS (EI) calcd for $C_{18}H_{19}NO_3S$ [M^+] 329.1086; found 329.1092.

Ethyl-5-isobutyl-2-(5-methylthiophen-2-yl)-oxazole-4-carboxylate (18d): Yellow oil (77%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.94$ (d, $J = 6.9$ Hz, 6H), 1.36 (t, $J = 7.2$ Hz, 3H), 2.03–2.10 (m, 1H), 2.47 (s, 3H), 2.90 (d, $J = 7.2$ Hz, 2H), 4.34 (q, $J = 7.2$ Hz, 2H), 6.70 (d, $J = 3.9$ Hz, 1H), 7.47 ppm (d, $J = 3.9$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.4$, 15.5, 22.4, 28.3, 34.7, 61.0, 126.3, 126.5, 128.6, 128.8, 144.2, 156.1, 158.6, 162.4 ppm; HRMS (EI) calcd for $C_{15}H_{19}NO_3S$ [M^+] 293.1086; found 293.1091.

Ethyl-5-isobutyl-5′-methyl-[2,2′]bithiazolyl-4-carboxylate (20): A solution of intermediate **19** (48 mg, 0.13 mmol) and $Cl_2Ni(PPh_3)_2$ (4.2 mg, 5 mol%) in anhyd DMA (2 mL) was treated with a solution of MeZnI[18] in DMA (0.25 mL, 0.26 mmol, 1 N) and the mixture was stirred for 3 h at RT. The reaction was quenched with aq HCl (1 N), and the mixture was extracted with EtOAc (50 mL). The organic phase was washed with brine, dried (Na_2SO_4), filtered and concen-

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trated. Purification by flash chromatography gave the cross-coupling product **20** as a pale yellow power (38 mg, 96%); mp:74–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.97$ (d, $J = 6.6$ Hz, 6H), 1.40 (t, $J = 7.2$ Hz, 3H), 1.93–2.00 (m, 1H), 2.50 (s, 3H), 3.12 (d, $J = 6.9$ Hz, 2H), 4.40 (q, $J = 7.2$ Hz, 2H), 7.48 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.3$, 14.4, 22.4, 31.1.9, 36.3, 61.4, 137.0, 141.8, 142.4, 150.5, 158.1, 159.1, 162.3 ppm; HRMS (EI) calcd for $C_{18}H_{19}NO_3S$ (M^+) 310.0810; found 310.0800.

Cell culture and antiviral assays

The antiviral procedure and the growth conditions for HepG 2.2.15 cells have been previously described.[13] Briefly, confluent cultures in 96-well culture plates were treated with varying doses of the compounds in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Fresh MEM with the same concentration of compounds was replaced at day 4, and the supernatants were harvested at day 8, then the extracellular (virion) HBV DNA copies were measured by real-time fluorescent PCR. The purity of the compounds was assessed by HPLC before biological testing (Supporting Information).

Cytotoxicity measurements: Cytotoxicity was assessed using a MTT assay as previously described.[13] Briefly, HepG 2.2.15 cells were cultured for 8 days with the test compound. Cells incubated with media alone were used as controls. MTT (5 g L^{-1}) reagent was added 4 h before the end of culture, and then cells were lysed with 10% sodium dodecyl sulfate (SDS), 50% DMF, pH 7.2. O.D. values were read at 570 nm 6 h later and the percent cell death was calculated.

Real-time fluorescent PCR: The supernatants of HepG2.2.15 cells, collected on day 8 after the compounds were added, were extracted for HBV DNA and quantified by real-time fluorescent PCR. Briefly, 50 μL of the supernatants were added into the extraction buffer, heated at 100 °C for 10 min and centrifuged for 5 min. Aliquots (2 μL) were used for the fluorescent PCR. PCR primers were: P1: 5′-ATC CTG CTG CTA TGC CTC ATCTT-3′, P2: 5′-ACA GTG GGG AAA GCC CTA CGAA-3′. The probe was 5′-TGG CTA GTT TAC TAG TGC CAT TTTG-3′. PCR reactions were run at MJ Research PTC-200, and results were analyzed using OpticonMonitor v2.01.

Acknowledgements

This work was supported by the Major State Hi-tech Research and Development Program (Grant 2006AA09z442, 2007AA02z4A2), the National Natural Science Foundation of China (grant 30572242, 30725049 to F.-J.N., and grant 30672492, 30701033 to J.-P.Z.), the Chinese Academy of Sciences (grant KSCX1-YW-10-03) and Shanghai Commission of Science and Technology (grant 05dz19331).

Keywords: antiviral agents · leucamide A · 2,2′-bisheterocycle tandem · hepatitis B virus · non-nucleoside inhibitors

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Received: May 5, 2008
Revised: June 6, 2008
Published online on July 28, 2008

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