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Design, Synthesis, and Antihepatitis B Virus Activities of Novel 2-Pyridone Derivatives

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A series of novel 2-pyridone derivatives were synthesized and evaluated for their antihepatitis B virus (HBV) activity and cytotoxicity in vitro. Moderate to good activity against HBV DNA replication was observed in these 2-pyridone analogues. The most active compounds were **5d** and **6l**, with good inhibitory activity against HBV DNA replication (IC_{50} = 0.206 and 0.12 μM, respectively) and remarkable high selectivity (selectivity indexes of >532 and 467, respectively). A pharmacophore model of the synthesized compounds was proposed by the GASP program. The pharmacophore model consists of three hydrophobic points, four HBA points, and one HBD point. The 2-pyridone derivatives represent a novel class of HBV inhibitors, which are worth further optimization.

Introduction

Hepatitis B virus (HBV[a]) is a cause of chronic liver disease worldwide, affecting more than 5% of the world's population.[1] HBV can cause both acute and chronic infections. Chronic HBV infection leads to liver damage, cirrhosis, and liver cancer with high mortality. There are approximately 350−400 million who are chronically infected, resulting in 0.5−1.2 million deaths annually.[2,3] HBV infection represents a serious global medical, social, and economic burden.

Important viral-encoded genes include the envelope protein, hepatitis B surface antigen (HBsAg), the primary serum marker of viral infection; a structural nucleocapsid, hepatitis B core antigen; and a soluble nucleocapsid protein, hepatitis B e antigen (HBeAg), which combined with serum levels of HBV DNA are serum markers of active viral replication.[2] The replication of HBV shares unusually similar features with retroviruses, and eradication of HBV infection turns out to be difficult because stable, covalently closed circular DNA (cccDNA), which is relatively resistant to antiviral action and immune clearance, becomes established in hepatocyte nuclei and integrated into the host genome.[2,4]

Seven drugs have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of HBV infection either as monotherapy or in combination. These drugs are categorized as interferons (interferon-α and pegylated interferon α-2a) and nucleotide analogues (lamivudine, telbivudine,[5] entecavir,[6] adefovir and tenofovir[3,7]) (Figure 1). Until now, the major therapeutic option for HBV carriers was α-interferon. Unfortunately, its use is limited because the success rate is low, the therapy is expensive, and serious side effects (such as influenza-like symptoms, depression, and insomnia) are observed.[8,9] Lamivudine has been used for the treatment of HBV infection for a few years. It is orally effective and well tolerated.[10] However, the emergence of resistance to lamivudine has been reported.[11] Adefovir dipivoxil was approved by the U.S. Food and Drug Administration in 2002 for the treatment of chronic hepatitis B infection. Adefovir dipivoxil can reduce serum HBV DNA levels significantly and can lead to seroconversion in 20−27% of the patients treated for 12 weeks at a daily dose of 30 mg or greater.[12] However, adefovir dipivoxil therapy is limited by dose-related side effects such as nephrotoxicity, lactic acidosis, and severe hepatomegaly with steatosis.[13] More recently, telbivudine and entecavir were marked with pharmacological profile. However, their chemical structures are similar to those of lamivudine and adefovir dipivoxil. They may act by the same biological mechanism and encounter the same problems as lamivudine and adefovir dipivoxil. Therefore, there is a tremendous clinical need to develop novel classes of antiviral agents for the treatment of HBV infection.

In the screening of an in-house library for anti-HBV activity, we identified 5-(2-hydroxy-4-methoxybenzoyl)-1-phenylpyridin-2(1*H*)-one (**5a**) as a modest inhibitor of HBV, with an IC_{50} value of 20 μM in inhibiting HBV DNA replication and low cytotoxicity (CC_{50} = 179 μM) in vitro. Because the chemical scaffold of compound **5a** differs from those of all reported anti-HBV agents, we were interested in synthesizing its derivatives and analyzing their structure−activity relationships (SARs). In the present investigation, we designed and synthesized a new series of 5-(2-hydroxy-4-methoxybenzoyl)pyridin-2-one derivatives with compound **5a** as the starting point. The anti-HBV activities of the compounds were investigated, and a pharmacophore model was built to guide further structural optimization.

Chemistry

The synthetic route of compound **4** was depicted in Scheme 1. 3-Iodo-7-methyloxy-4*H*-chromone (**3**) was synthesized by a reported procedure.[14] Coupling of compound **3**

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[a] Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; cccDNA, covalently closed circular DNA; SARs, structure−activity relationships; SI, selectivity index; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; vdW, van der Waals.

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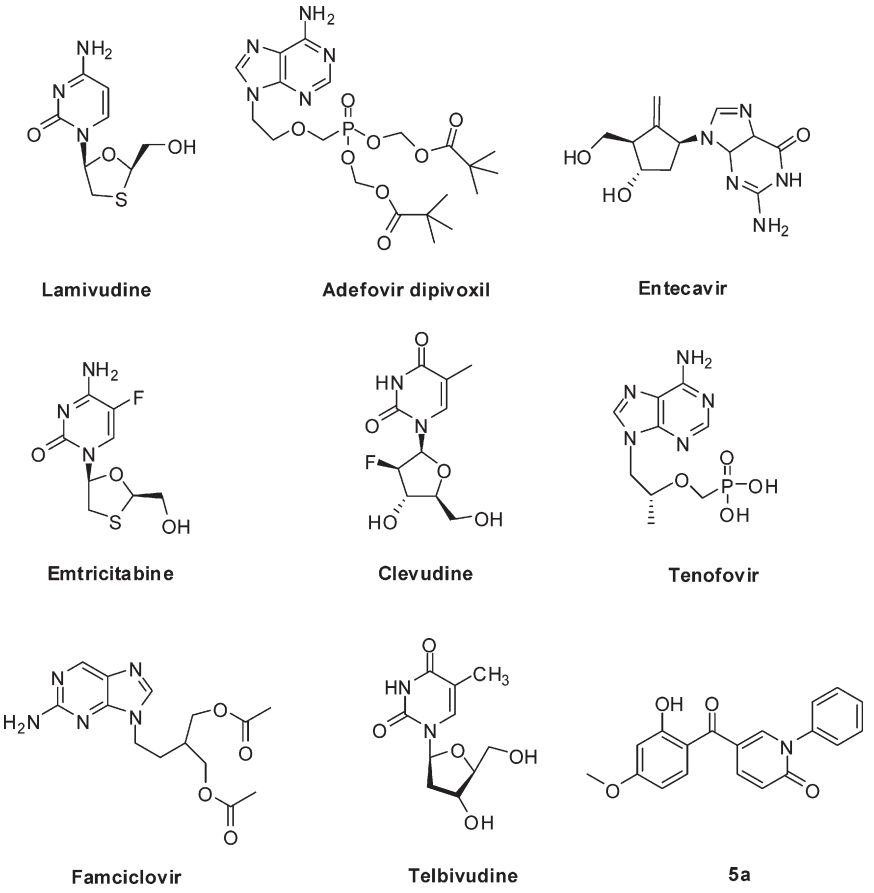
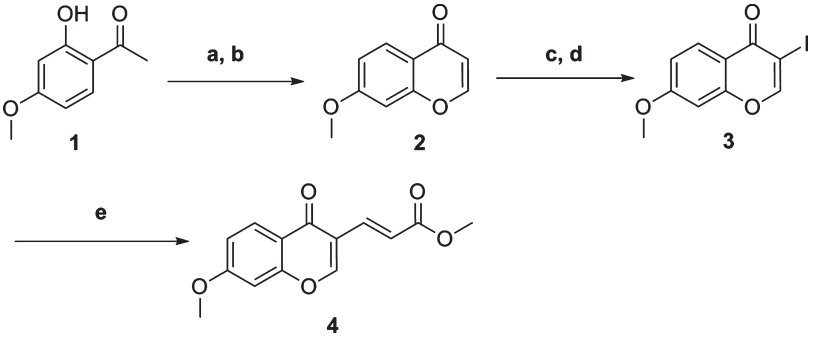


Figure 1. Chemical structures of the anti-HBV agents and lead compound **5a**.

Scheme 1. Synthesis of the Key Intermediate **4**[a]



[a] Reagents and conditions: (a) ether, Na, ethyl formate, 0 °C, 8 h, yield 95%; (b) CH_3COOH, conc HCl, 100 °C, 30 min, yield 98%; (c) piperidine, methanol, reflux, 3 h, yield 99%; (d) dichloromethane, I_2, room temp, overnight, yield 97%; (e) $PdCl_2(Ph_3P)_2$, CuI, K_2CO_3, THF/H_2O, 100 °C, 10 h, yield 80%.

with methyl acrylate by a Heck reaction[15,16] afforded the key intermediate **4**. In order to establish a robust synthetic procedure of compound **4**, the reaction conditions were optimized. Various solvents, bases, metal catalysts, and temperatures were investigated. The mixed solvent (THF/H_2O = 9:1) was found to be the best choice with K_2CO_3 as the base and $PdCl_2(Ph_3P)_2$ as the metal catalyst. The optimal reaction temperature and time were 100 °C and 10 h, respectively.

In the presence of triethylamine, target compounds **5a**−**s** were obtained by reacting intermediate **4** with various substituted phenylamines and alkylamines (molar ratio of 1: 1) via three sequencing steps in one pot. First, the ester group was ammonolyzed by amines. Second, the chromone ring was opened by the attack of the amine group. Last, the 2-pyrinone ring was formed by a rearrangement process. When intermediate **4** was reacted with excess benzylamines and alkylamines, compounds **6a**−**o** were obtained. However, reacting compound **4** with excess phenylamines could not afford corresponding Schiff bases. In order to determine the configuration of the Schiff base, the crystal structure of compound **6c** was determined. Figure 2 shows the ORTEP drawing of compound **6c**, indicating that the Schiff base is in cis-configuration.

Results and Discussion

In Vitro Virological Examination and Preliminary Structure−**Activity Relationships (SARs).** The potential anti-HBV activity and cytotoxicity of the synthesized 2-pyridones were tested in HepG2 2.2.15 cells, and adefovir was used as a reference antiviral drug. The results are summarized in Table 1. The anti-HBV activity of each compound was

expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) of HBsAg, HBeAg, and HBV DNA replication. The cytotoxicity of each compound was expressed as the concentration of compound required to kill 50% (CC_{50}) of the HepG2 2.2.15 cells. The selectivity index (SI), an important pharmaceutical parameter that estimates possible future clinical development, was determined as the ratio of CC_{50} value to IC_{50} value. The bioactivity of each compound was evaluated by the combination of its IC_{50} values and SI.

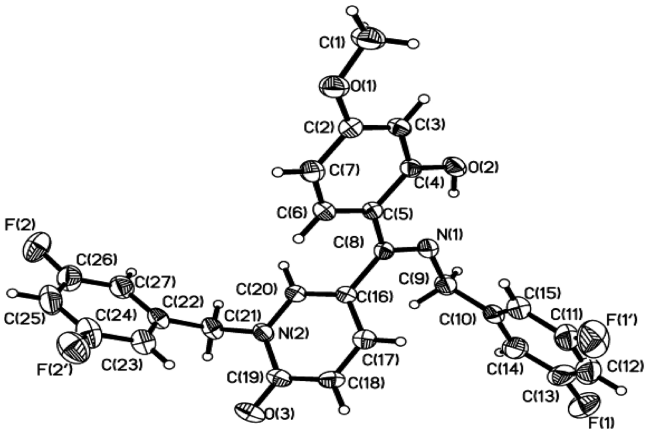


Figure 2. ORTEP drawing of compound **6c**.

Compounds **5a**−**l** have different patterns of substitution on the phenyl ring attached to the N atom of 2-pyridones. As shown in Table 1, these compounds generally exhibited moderate to good anti-HBV potency. Various substitutions on the phenyl ring are important for the antiviral activity. 4-Ethoxyl (compound **5d**) and 3-methyl (compound **5f**) analogues showed excellent anti-HBV activity with their IC_{50} values against HBV DNA replication of 0.206 and 0.8 μM, respectively, which were more potent than reference drug adefovir. They also showed higher inhibitory activity against HBsAg and HBeAg than adefovir. Moreover, compounds **5d** and **5f** were almost nontoxic against HepG2 2.2.15 cells, which resulted in their high SI values. When the 3-methyl group of compound **5f** was moved to position 2 (compound **5g**) and position 4 (compound **5e**), their anti-HBV activity was decreased by 6- to 14-fold. 2,5-2CH_3 derivative (compound **5h**) also showed good inhibitory activity against HBV DNA replication (IC_{50} = 0.5 μM). However, it was very toxic with a CC_{50} value of 0.5 μM (SI = 1). If the 4-methyl group of compound **5e** was replaced by a chlorine atom (compound **5i**), the inhibitory activity against HBV DNA replication was decreased slightly (IC_{50} = 6.2 μM).

Table 1. In Vitro Anti-HBV Activity and Cytotoxicity of the Target Compounds

		HBsAg		HBeAg		DNA replication	
compd	CC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]	IC_{50} (μM)[b]	SI[c]	IC_{50} (μM)[b]	SI[c]
5a	179	176	1.02	76	2.36	20	8.95
5b	346	> 113.9	*d*	222	1.56	5.2	66.54
5c	> 113.9	> 113.9	*d*	49	> 2.3	76	> 1.5
5d	> 109.6	> 109.6	*d*	55	> 1.99	0.206	> 532
5e	> 119.4	> 119.4	*d*	2	> 59.7	5	> 23.9
5f	159	29	5.48	0.3	530	0.8	198.75
5g	> 119.4	18	> 6.63	0.1	> 1194	11	> 10.85
5h	0.5	13	0.04	38	0.01	0.5	1.0
5i	164	61	2.69	1.6	102.5	6.2	26.45
5j	> 117.9	> 117.9	*d*	> 117.9	*d*	98.3	> 1.2
5k	177	> 109.3	< 1.07	> 109.3	< 1.07	NA[e]	*d*
5l	133	> 110.2	< 1.21	774	0.17	11.2	11.88
5m	15	99	0.15	0.73	20.55	99.6	0.15
5n	131	> 139.3	*d*	46.3	2.83	33.3	3.93
5o	67	> 132.7	< 0.5	> 132.7	< 0.5	28.6	2.34
5p	79	46	1.72	39	2.03	20	3.95
5q	696.1	846.2	0.82	> 846.2	< 0.82	170.3	4.09
5r	232	276	0.84	58	4.0	100.8	2.3
5s	244	77	3.17	> 97	< 2.5	> 363.7	< 0.67
6a	102	40	2.55	4.4	23.18	31.5	3.24
6b	51	72	0.71	30	1.70	92.6	0.55
6c	8	13	0.62	0.5	16.0	2.1	3.81
6d	10	> 86.9	< 0.12	> 86.9	< 0.12	64.5	0.15
6e	10	13	0.77	40	0.25	3	3.33
6f	201.4	496.5	0.41	> 402.8	< 0.5	97.5	2.07
6g	32	36	0.89	2	16.0	1	32
6h	20	> 117.8	< 0.17	> 117.8	< 0.17	21.7	0.92
6i	387	201	1.93	71	5.45	4	96.75
6j	164	123	1.33	10	16.4	> 462.4	< 0.35
6k	120	> 121.9	< 1	> 121.9	< 1	33.8	3.55
6l	56	111	0.50	56	1.0	0.12	466.67
6m	98	29	3.38	5	19.6	30	3.27
6n	> 121.9	> 121.9	*d*	54	> 2.26	> 121.9	*d*
6o	20	98	0.20	40	0.5	12	1.67
adefovir	540	305	1.77	286	1.89	0.517	1044.5

[a] CC_{50} is 50% cytotoxicity concentration in HepG2 2.2.15 cells. [b] IC_{50} is 50% inhibitory concentration. [c] Selectivity index (SI = CC_{50}/IC_{50}). [d] No SI can be obtained. [e] NA = not active.

The introduction of a nitro group (compound **5k**) or acetyl group (compound **5l**) on position 4 of the phenyl ring led to the loss of anti-HBV activity.

Decreased anti-HBV activity was observed with the replacement of the substituted phenyl groups of compounds **5a**−**l** by the alkyl groups (compounds **5m**−**s**). Compound **5m**, with a cyclohexyl group attached to the N atom of 2-pyridone, was almost inactive against HBV DNA replication (IC_{50} = 99.6 μM). The replacement of the cyclohexyl group of compound **5m** by a propyl group (compounds **5n**) or pentyl group (compounds **5p**) yielded 3- to 5-fold improvement in anti-HBV activity. However, the introduction of a 2-(naphthalene-1-yl-amino)ethenyl group at this position resulted in the loss of antiviral activity, suggesting that a steric group larger than the phenyl group was not tolerated at this position.

Compounds **6a**−**o** have the same substitutions on both the N atom of 2-pyridone and the imino group. Compound **6a** showed moderate anti-HBV activity. The IC_{50} values of compound **6a** against HBV DNA replication, HBsAg, and HBeAg are 31.5, 40, and 4.4 μM, respectively. Introducing a fluorine atom on the position 2 of the benzyl group (compound **6b**, IC_{50} = 92.6 μM) led to a decrease of antiviral activity. However, moderate anti-HBV activity was observed for compound **6e** (HBV DNA replication, IC_{50} = 3 μM) after the addition of another fluorine atom on the position 4 of the benzyl group of compound **6b**. Moreover, the 3-F derivative (compound **6c**) also revealed good activity against HBV DNA replication, HBsAg, HBeAg (IC_{50} = 2.1, 13, and 0.5 μM, respectively). The most potent compound in this subseries is compound **6g** (HBV DNA replication, IC_{50} = 1 μM), indicating that the methoxyl at position 4 of the benzyl group is important for the antiviral activity.

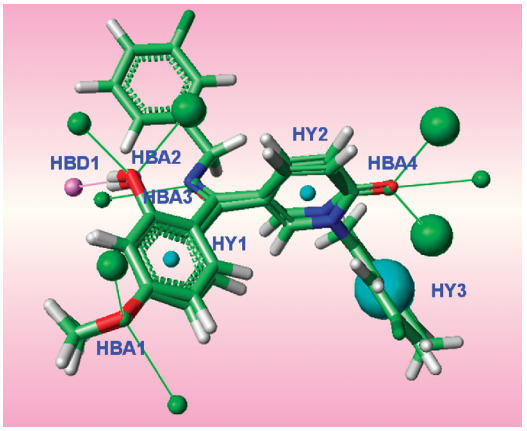
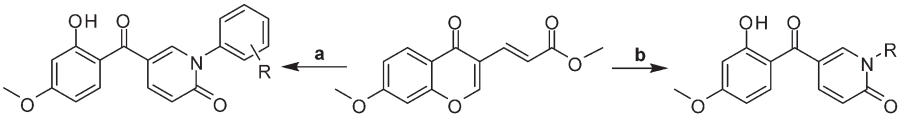


Figure 3. Pharmacophore model of the 2-pyrinone derivatives. HY1, HY2, and HY3 are hydrophobic points (shown as cyan balls) located around the centers of the aromatic rings. HBA1, eHBA2, HBA3, and HBA4 are hydrogen bond (HB) acceptor points (shown as green balls). HBD1 is a HB donor point (shown as purple balls). The complementary HB donor and acceptor points of the receptor are also represented as balls with the same color as the acceptor or donor atom of the template molecules in the direction of lone pair or hydrogen. The green lines show directions of the HB interactions.

Compared with compounds **6a**−**g**, the dialkyl substituted derivatives (compounds **6i**−**o**) generally showed lower antiviral activity. The loss of antiviral activity was observed for the propyl, cyclopropyl, and isopropyl derivatives (compounds **6k**, **6j**, and **6n**). The addition of a double bond to the propyl group afforded compound **6o**, which showed moderate activity against HBV DNA replication (IC_{50} = 12 μM). When the propyl group of compound **6k** was extended to the butyl group (compound **6l**), the anti-HBV activity was increased obviously. It is worth noting that compound **6l** is the most potent one among the synthesized compounds. The IC_{50} value of compound **6l** against HBV DNA replication is 0.12 μM, which is 4-fold more potent than adefovir. Moreover, compound **6l** also showed high SI value (SI = 466.67), suggesting that it is a good starting point for further optimization.

From the biological data, preliminary SARs of the synthesized compounds are as follows. For compounds **5a**−**s**, the *N*-aryl derivatives showed better anti-HBV activity than the *N*-alkyl derivatives. The substitutions on the phenyl ring were important for the antiviral activities. An electron withdrawing group was not favorable at position 4 of the phenyl ring. The introduction of an electron-donating group (such as methyl or methoxyl) enhanced the antiviral activity, especially in the inhibition of HBV DNA replication. The dibenzyl or dialkyl substituted derivatives (compounds **6a**−**o**) did not show improved anti-HBV activity, suggesting that the imino groups are not important for their anti-HBV activities. Because the present study is a preliminary structural modification and the target of these compounds remains unknown, the SARs of 2- pyridones remain to be further studied.

Scheme 2. Synthesis of the Target Compounds **5a**−**s**[a]



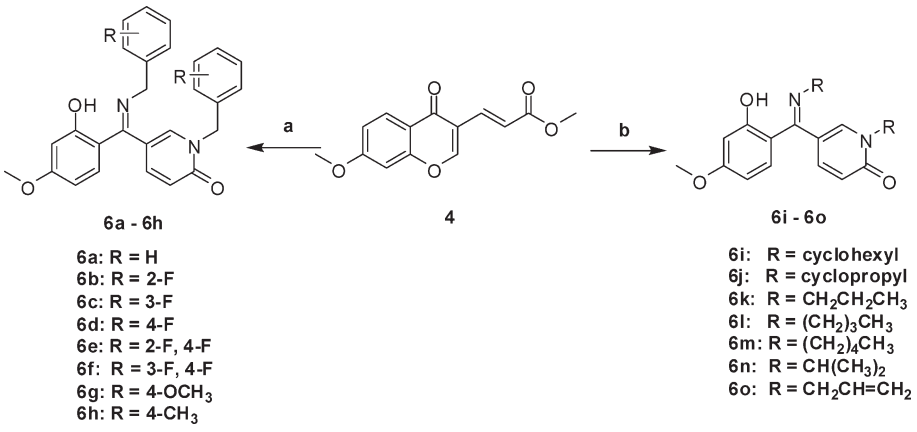
5a - 5l **4** **5m - 5s**

5a: R = H
5b: R = 4-OCH_3
5c: R = 2-OCH_3
5d: R = 4-OCH_2CH_3
5e: R = 4-CH_3
5f: R = 3-CH_3
5g: R = 2-CH_3
5h: R = 2-CH_3, 5-CH_3
5i: R = 4-Cl
5j: R = 4-I
5k: R = 4-NO_2
5l: R = 4-$COCH_3$

5m: R = cyclohexyl
5n: R = $CH_2CH_2CH_3$
5o: R = $(CH_2)_3CH_3$
5p: R = $(CH_2)_4CH_3$
5q: R = $CH(CH_3)_2$
5r: R = $CH_2CH{=}CH_2$
5s: R = (*E*)-2-(naphthalen-1-ylamino)ethenyl

[a] Reagents and conditions: (a) methanol, substituted aniline, triethylamine, reflux, 8 h; (b) methanol, alkylamine, triethylamine, reflux, 8 h, yield 35−89%.

Scheme 3. Synthesis of the Target Compounds **6a**−**o**[a]



[a] Reagents and conditions: (a) methanol, substituted benzylamine, triethylamine, reflux, overnight; (b) methanol, alkylamine, triethylamine, reflux, overnight, yield 55−90%.

Pharmacophore Model of 2-Pyridones. The synthesized 2-pyridones represent a new class of anti-HBV compounds. However, the target of 2-pyridone derivatives is unknown. Therefore, the construction a pharmacophore model is a useful method for further structural optimization. Moreover, the pharmacophore model can also be used in virtual screening and identifying new lead structures. According to the chemical diversity and anti-HBV potency, compounds **5f** and **6c** were selected as template molecules. Their low-energy conformations were obtained by energy minimization and simulated annealing. Then the pharmacophore model was derived by means of a genetic algorithm similarity program GASP.[17] Several pharmacophore points were identified (Figure 3), including three hydrophobic, four hydrogen bond (HB) acceptor, and one HB donor points. Three phenyl groups (HY1, HY2, and HY3) of compound **5f** were identified as hydrophobic points. HY1 and HY2 are identical among the compounds. HY3 highlighted the importance of the substituted phenyl group attached to the N atom of 2-pyrinones, which was consistent with the SAR results. Another phenyl group of compound **6c** was not recognized as a hydrophobic point, indicating that the substituted imino group was not important for the anti-HBV activity. All four oxygen atoms of compound **5f** were identified as HB acceptor points (HBA1−HBA4). Moreover, the hydrogen atoms of the hydroxyl groups of compounds **5f** and **6c** were recognized as a HB donor point (HBD1). However, the HBA and HBD points are common structural elements among the synthesized compounds. Their function needs to be demonstrated by further structural modifications.

Conclusion

In summary, a series of novel 2-pyridone analogues based on compound **5a** were synthesized and tested for their in vitro anti-HBV activity, using adefovir as a reference control. Most of the compounds revealed moderate to good anti-HBV activity. Compounds **5d**, **5f**, **5h**, **6g**, and **6l** were active with IC_{50} values against HBV DNA replication in the range of 0.12−1.0 μM. The most promising results were observed for compounds **5d** and **6l**, with IC_{50} values against HBV DNA replication of 0.206 and 0.12 μM, respectively, with high selectivity (SI of $>$532 and 467, respectively). A pharmacophore model of the synthesized compounds was constructed, which consists of three hydrophobic points, four HBA points, and one HBD point. The pharmacophore model can provide useful information for further structural modification. The present findings make them attractive candidates for further optimization as well as the investigation of the mechanism of action.

Experimental Section

Chemistry. General Methods. Melting points were measured on an uncorrected X-5 digital melting point apparatus (Gongyi City Yuhua Instrument Co., Ltd.; China). IR spectra were recorded on a PE Spectrum One FI-IR spectrometer as KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are given in ppm (δ). Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within 0.4%. The mass spectra were recorded on an Esquire 3000 LC−MS mass spectrometer. Crystal structure was determined on a Bruker SMART CCD (Bruker Company, Germany). Silica gel thin-layer chromatography was performed on precoated plates GF-254 (Qingdao Haiyang Chemical, China). All solvents and reagents were analytically pure, and no further purification was needed. All starting materials were commercially available. The purity of final compounds was assessed on the basis of analytical HPLC, and the results were greater than 95%.

(*E*)-Methyl 3-(5-Hydroxy-7-methoxy-4-oxo-4*H*-chromen-3-yl)acrylate (4). To a 500 mL round-bottom flask fitted with mechanical stirrer, a mixed solvent of DMF and H_2O (225 mL of DMF and 25 mL of H_2O), 3-iodo-7-methyloxy-4*H*-chromone (**3**) (30.2 g, 0.1 mol), methyl acrylate (11.1 g, 0.15 mol), Pd-$(Ph_3P)_2Cl_2$ (0.7 g, 0.001 mol), CuI (1.9 g, 0.01 mol), and K_2CO_3 (13.8 g, 0.1 mol) were added successively. The mixture was heated to 70 °C and stirred for 4 h. Then the mixture was filtered. The filtrate was poured into 800 mL of ice−water and then extracted with EtOAc (200 mL × 3). The extract was washed with saturated NaCl solution (50 mL × 3), dried over anhydrous $MgSO_4$, and concentrated to a volume of approximately 300 mL. Brown crystals (**4**) were collected after the concentrated solution was cooled down to 4 °C and maintained overnight. Yield 21 g (80.5%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.78 (s, 3H), 3.86 (s, 3H), 6.84 (d, 1H, J = 2.4 Hz), 6.99 (dd, 1H, J_1 = 2.4 Hz, J_2 = 9 Hz), 7.24 (d, 1H, J = 15.9 Hz), 7.37 (d, 1H, J = 15.9 Hz), 8.04 (s, 1H), 8.16 (d, 1H, J = 9 Hz). ESI-MS (m/z): 261.1 [M + 1]. Anal. ($C_{14}H_{12}O_5$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-phenylpyridin-2(1*H*)-one (5a). A solution of compound **4** (1.04 g, 0.004 mol), aniline

(0.41 g, 0.0044 mol), and triethylamine (3 drops) in MeOH (45 mL) was stirred under reflux for 8 h. After the mixture was cooled to room temperature and the solvent removed, the crude product was purified by chromatography over silica gel (eluent petroleum ether/acetic ether, 9:1 to 3:1 v/v) to give compound **5a** as a yellow crystal (Scheme 2). Yield 0.95 g (76%). Mp: 179−179.7 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.86 (s, 3H), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.51 (d, 1H, J = 2.7 Hz), 6.70 (d, 1H, J = 9.3 Hz), 7.41 (m, 2H), 7.53 (m, 2H), 7.56 (m, 2H), 7.81 (dd, 1H, J_1 = 9.3 Hz, J_2 = 2.7 Hz), 7.93 (d, 1H, J = 2.7 Hz), 12.23 (s, 1H). ESI-MS (m/z): 322 [M + 1]. Anal. ($C_{19}H_{15}NO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-(4-methoxyphenyl)pyridin-2(1*H*)-one (5b). The titled compound was prepared from **4** and 4-methoxyaniline according to the method of compound **5a**. Yield 85%, yellow crystal. Mp: 152.2−152.6 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.86 (s, 3H), 3.87 (s, 3H), 6.45 (dd, 1H, $J1$ = 9 Hz, J_2 = 2.7 Hz), 6.52 (d, 1H, J = 2.7 Hz), 6.69 (d, 1H, J = 9.6 Hz), 7.00 (m, 2H), 7.32 (m, 2H), 7.53 (d, 1H, J = 9 Hz), 7.77 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.93 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ESI-MS (m/z): 352.3 [M + 1]. Anal. ($C_20H_{17}NO_5$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-(2-methoxyphenyl)pyridin-2(1*H*)-one (5c). The titled compound was prepared from **4** and 2-methoxyaniline according to the method of compound **5a**. Yield 84%, white needle crystal. Mp: 161.0−162.9 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.85 (s, 3H), 3.87 (s, 3H), 6.44 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.51 (d, 1H, J = 2.7 Hz), 6.72 (m, 1H), 7.06 (m, 2H), 7.31 (dd, 2H, J_1 = 8.1 Hz, J_2 = 1.8 Hz), 7.42 (m, 1H), 7.59 (d, 1H, J = 9 Hz), 7.81 (m, 2H), 12.27 (s, 1H). ESI-MS (m/z): 352.3 [M + 1]. Anal. ($C_{20}H_{17}NO_5$) C, H, N.

1-(4-Ethoxyphenyl)-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5d). The titled compound was prepared from **4** and 4-ethoxyaniline according to the method of compound **5a**. Yield 80%, white crystal. Mp: 178.5−178.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (t, 3H, J = 7.5 Hz), 3.67 (q, 3H), 6.45 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.52 (d, 1H, J = 2.7 Hz), 6.69 (d, 1H, J = 9.6 Hz), 7.00 (t, 2H), 7.32 (t, 2H), 7.53 (d, 1H, J = 9 Hz), 7.77 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.93 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ESI-MS (m/z): 366.3 [M + 1]. Anal. ($C_{21}H_{19}NO_5$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-*p*-tolylpyridin-2(1*H*)-one (5e). The titled compound was prepared from **4** and 4-methylaniline according to the method of compound **5a**. Yield 79%, yellow crystal. Mp: 189.1−192.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.42 (s, 3H), 3.86 (s, 3H), 6.45 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.51 (d, 1H, J = 2.4 Hz), 6.70 (d, 1H, 9.6 Hz), 7.27 (m, 3H), 7.53 (d, 1H, J = 9 Hz), 7.79 (dd, 1H, J_1 = 9.6H, J_2 = 2.4 Hz), 7.93 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ESI-MS (m/z): 336.3 [M + 1]. Anal. ($C_{20}H_{17}NO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-*m*-tolylpyridin-2(1*H*)-one (5f). The titled compound was prepared from **4** and 3-methylaniline according to the method of compound **5a**. Yield 81%, yellow crystal. Mp: 123.8−125.8 °C. IR (KBr) ν (cm^{-1}): 3070.34, 3033.05, 2975.80, 2947.44, 1684.65, 1633.84, 1579.98, 1273.79. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.43 (s, 3H), 3.87 (s, 3H), 6.45 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.52 (d, 1H, J = 2.4 Hz), 6.71 (d,1H, J = 9.6 Hz), 7.19 (m, 2H), 7.28 (m,1H), 7.39 (m, 1H), 7.53 (d,1H, J = 9 Hz), 7.78 (dd, 1H, J_1 = 9.6H, J_2 = 2.4 Hz), 7.93 (d, 1H, J = 2.7 Hz), 12.24 (s,1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.30, 55.66, 101.48, 107.66, 112.58, 117.50, 120.78, 123.38, 126.99, 129.38, 129.94, 133.27, 139.67, 139.78, 140.06, 142.91, 161.80, 165.84, 166.24, 193.80. ESI-MS (m/z): 336.3 [M + 1]. Anal. ($C_{20}H_{17}NO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-*o*-tolylpyridin-2(1*H*)-one (5g). The titled compound was prepared from **4** and 2-methylaniline according to the method of compound **5a**. Yield 83%, light-yellow crystal. Mp: 146.2−147.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 3H), 3.86 (s, 3H), 6.45 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.51 (d, 1H, J = 2.4 Hz), 6.74 (d, 1H, J = 9.6 Hz), 7.25 (m, 1H), 7.38 (m, 3H), 7.39 (m, 1H), 7.52 (d,1H, J = 9 Hz), 7.81 (dd, 1H, J_1 = 9.6H, J_2 = 2.4 Hz), 7.86 (d, 1H, J = 2.7 Hz), 12.23 (s, 1H). ESI-MS (m/z): 326.2 [M + 1]. Anal. ($C_{20}H_{17}NO_4$) C, H, N.

1-(2,5-Dimethylphenyl)-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5h). The titled compound was prepared from **4** and 2,5-dimethylaniline according to the method of compound **5a**. Yield 83%, white crystal. Mp: 128−129.3 °C. ^{1}H NMR ($CDCl_3$, 300 MHz,): δ 2.16 (s, 3H), 2.37 (s, 3H), 3.86 (s, 3H), 6.45 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.51 (d, 1H, J = 2.7 Hz), 6.72 (d, 1H, J = 9.6 Hz), 7.05 (s, 1H), 7.24 (m, 2H), 7.52 (d, 1H, J = 9 Hz), 7.81 (m, 2H), 12.24 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 17.15, 20.77, 55.65, 101.49, 107.64, 112.58, 117.44, 120.82, 127.42, 130.44, 131.12, 131.49, 133.20, 137.36, 139.19, 139.54, 163.04, 161.57, 165.81, 166.22, 193.73. ESI-MS (m/z): 350.13 [M + 1]. Anal. ($C_{21}H_{19}NO_4$) C, H, N.

1-(4-Chlorophenyl)-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5i). The titled compound was prepared from **4** and 4-chloroaniline according to the method of compound **5a**. Yield 65%, light-yellow crystal. Mp: 173.8−175.6 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.87 (s, 1H), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.52 (d, 1H, J = 2.7 Hz), 6.70 (d, 1H, J = 9.6 Hz), 7.36 (d, 2H, J = 6.6 Hz), 7.49 (m, 3H), 7.78 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.4 Hz), 7.90 (d, 1H, J = 2.4 Hz), 12.20 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 55.70, 101.54, 107.80, 112.51, 117.96, 120.88, 127.80, 129.80, 133.20, 135.22, 138.46, 139.61, 142.32, 161.54, 165.94, 166.39, 193.62. ESI-MS (m/z): 356.68 [M + 1]. Anal. ($C_{19}H_{14}ClNO_4$) C, H, N.

1-(4-Fluorophenyl)-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5j). The titled compound was prepared from **4** and 4-fluoroaniline according to the method of compound **5a**. Yield 78%, yellow crystal. Mp: 178.8−180.4 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.87 (s, 3H), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.52 (d, 1H, J = 2.4 Hz), 6.71 (d, 1H, J = 9.6 Hz), 7.19 (m, 2H), 7.39 (m, 2H), 7.52 (d, 1H, J = 9 Hz), 7.78 (dd, 1H, J_1 = 9.6H, J_2 = 2.4 Hz), 7.92 (d, 1H, J = 2.7 Hz), 12.21 (s, 1H). ESI-MS (m/z): 340.8 [M + 1]. Anal. ($C_{19}H_{14}FNO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-(4-nitrophenyl)pyridin-2(1*H*)-one (5k). The titled compound was prepared from **4** and 4-nitroaniline according to the method of compound **5a**. Yield 35%, yellow solid. Mp: 158.6−161.1 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 1H), 6.47 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 6.62 (d, 1H, J = 2.4 Hz), 6.73 (d, 1H, J = 9.3 Hz), 7.51 (d, 1H, J = 9 Hz), 7.65 (m, 2H), 7.80 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.91 (d, 1H, J = 2.4 Hz), 8.40 (m, 2H). ESI-MS (m/z): 367.68 [M + 1]. Anal. ($C_{19}H_{14}N_2O_6$) C, H, N.

1-(4-Acetylphenyl)-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5l). The titled compound was prepared from **4** and 4-acetylaniline according to the method of compound **5a**. Yield 39%, yellow crystal. Mp: 196.4−196.7 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.62 (s, 3H), 3.77 (s, 3H), 6.49 (m, 2H), 6.59 (d, 1H, J = 9.6 Hz), 7.53 (d, 1H, J = 8.4 Hz), 7.66 (d, 2H, J = 6.6 Hz), 7.84 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 8.02 (d, 1H, J = 2.4 Hz), 8.08 (d, 2H, J = 6.6 Hz), 11.17 (s, 1H). ESI-MS (m/z): 364 [M + 1]. Anal. ($C_{21}H_{17}NO_5$) C, H, N.

1-Cyclohexyl-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5m). The titled compound was prepared from **4** and cyclopropanamine according to the method of compound **5a**. Yield 80%, white crystal. Mp: 199.9−201.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (m, 1H), 1.48 (m, 4H), 1.75 (m, 1H), 1.97 (m, 4H), 3.88 (s, 3H), 4.9 (m, 1H), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.53 (d, 1H, J = 2.7 Hz), 6.59 (d, 1H, J = 9.6 Hz), 7.45 (d, 1H, J = 9 Hz), 7.66 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.92 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ESI-MS (m/z): 328.1 [M + 1]. Anal. ($C_{19}H_{21}NO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-propylpyridin-2(1*H*)-one (5n). The titled compound was prepared from **4** and propylamine according to the method of compound **5a**. Yield 78%, yellow crystal. Mp: 128.5−129.5 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.98 (t, 3H, J=7.5 Hz), 1.80 (q, 2H, J=7.5 Hz), 3.88 (s, 1H), 3.95 (t, 2H, J=7.5 Hz), 6.46 (dd, 1H, J_1=9 Hz, J_2=2.7 Hz),

6.53 (d, 1H, J = 2.7 Hz), 6.59 (d, 1H, J = 9.6 Hz), 7.48 (d, 1H, J = 9 Hz), 7.69 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.86 (d, 1H, J = 2.7 Hz), 12.23 (s, 1H). ESI-MS (m/z): 288.23[M + 1]. Anal. ($C_{16}H_{17}NO_4$) C, H, N.

1-Butyl-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5o). The titled compound was prepared from **4** and butylamine according to the method of compound **5a**. Yield 87%, white crystal. Mp: 96.8−97.2 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.95 (t, 3H, J = 7.2 Hz), 1.37 (m, 2H), 1.75 (m, 2H), 3.88 (s, 1H), 3.98 (t, 2H, J = 7.2 Hz), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.53 (d, 1H, J = 2.7 Hz), 6.59 (d, 1H, J = 9.6 Hz), 7.48 (d, 1H, J = 9 Hz), 7.69 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.87 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ESI-MS (m/z): 302.3 [M + 1]. Anal. ($C_{17}H_{19}NO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-pentylpyridin-2(1*H*)-one (5p). The titled compound was prepared from **4** and pentylamine according to the method of compound **5a**. Yield 88%, white crystal. Mp: 74−74.6 °C. IR (KBr) ν (cm^{-1}): 3095.73, 3079.71, 3021.74, 2953.41, 2934.19, 2859.94, 1671.19, 1627.07, 1615.70, 1578.74, 1384.13. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.95 (t, 3H, J = 7.2 Hz), 1.27 (m, 4H), 1.65 (m, 2H), 3.83 (t, 2H, J = 7.2 Hz), 3.88 (s, 1H), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.53 (d, 1H, J = 2.7 Hz), 6.59 (d, 1H, J = 9.6 Hz), 7.48 (d, 1H, J = 9 Hz), 7.69 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.87 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 13.87, 22.25, 28.73, 28.79, 50.61, 55.66, 101.48, 107.55, 112.64, 117.38, 119.92, 133.30, 138.86, 142.54, 161.99, 165.81, 166.17, 193.92. ESI-MS (m/z): 316.2 [M + 1]. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

5-(2-Hydroxy-4-methoxybenzoyl)-1-isopropylpyridin-2(1*H*)-one (5q). The titled compound was prepared from **4** and isopropylamine according to the method of compound **5a**. Yield 79%, yellow crystal. Mp: 143.8−144.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (d, 6H, J = 6.9 Hz), 5.28 (q, 1H, J = 6.9 Hz), 6.46 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.53 (d, 1H, J = 2.7 Hz), 6.63 (d, 1H, J = 9.6 Hz), 7.46 (d, 1H, J = 9 Hz), 7.67 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.93 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ESI-MS (m/z): 288.2 [M + 1]. Anal. ($C_{16}H_{17}NO_4$) C, H, N.

1-Allyl-5-(2-hydroxy-4-methoxybenzoyl)pyridin-2(1*H*)-one (5r). The titled compound was prepared from **4** and 2-propen-1-ylamine according to the method of compound **5a**. Yield 89%, Yellow crystal. Mp: 131.7−132.6 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.87 (s, 1H), 4.62 (m, 2H), 5.25 (m, 2H), 5.92 (m, 1H), 6.56 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.52 (d, 1H, J = 2.7 Hz), 6.62 (d, 1H, J = 9.6 Hz), 7.46 (d, 1H, J = 9 Hz), 7.70 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.7 Hz), 7.86 (d, 1H, J = 2.7 Hz), 12.24 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 30.91, 51.62, 55.68, 101.45, 107.63, 112.56, 117.68, 119.78, 119.97, 131.65, 133.31, 139.10, 142.02, 161.78, 165.85, 166.22, 193.84. IR (KBr) ν (cm^{-1}): 3085.15, 3071.62, 2987.67, 2971.99, 2940.53, 2845.23, 1667.92, 1616.20, 1585.08, 1536.00, 1507.79, 1736.73, 1271.19,1111.47, 840.54, 606.96. ESI-MS (m/z): 286.1 [M + 1]. Anal. ($C_{16}H_{15}NO_4$) C, H, N.

(*E*)-5-(2-Hydroxy-4-methoxybenzoyl)-1-(2-(naphthalen-1-ylamino)vinyl)pyridin-2(1*H*)-one (5s). The titled compound was prepared from **4** and (*E*)-N^1-(naphthalen-1-yl)ethene-1,2-diamine according to the method of compound **5a**. Yield 88%, yellow crystal. Mp: 203.1−205.1 °C. IR (KBr) ν (cm^{-1}): 3440.21, 3049.82, 2922.37, 2854.85, 1667.34, 1583.46, 1532.23, 1383.58, 1350.42, 1235.41. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.66 (s, 3H), 5.50 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 638 (d, 1H, J = 2.4 Hz), 6.56 (d, 1H, J = 9.3 Hz), 7.04 (d, 1H, J = 9 Hz), 7.13 (d, 1H, J = 8.7 Hz), 7.24 (t, 1H, J = 8.1 Hz), 7.41 (m, 2H), 7.77 (m, 2H), 8.00 (d, 1H, J = 2.4 Hz), 8.10 (d, 1H, J = 7.8 Hz), 11.36 (s, 1H). ESI-MS (m/z): 415.65 [M + 1]. Anal. ($C_{25}H_{20}N_2O_4$) C, H, N.

(*E*)-1-Benzyl-5-((benzylimino)(2-hydroxy-4-methoxyphenyl)-methyl)pyridin-2(1*H*)-one (6a). A solution of compound **4** (1.04 g, 0.004 mol), benzylamine (1.07 g, 0.01 mol), and triethylamine (6 drops) in MeOH (45 mL) was stirred under reflux overnight. After the mixture was cooled to room temperature and the solvent removed, the crude product was purified by chromatography over silica gel (eluent petroleum ether/acetic ether, 6:1 to 2:1 v/v) to give compound **6a** (Scheme 3). Yield: 68%, yellow crystal. Mp: 173.1−174.3 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.80 (s, 3H), 4.58 (s, 2H), 5.12 (s, 2H), 6.21 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.42 (d, 1H, J = 2.7 Hz), 6.70 (d, 1H, J = 9.6 Hz), 6.84 (d, 1H, J = 9 Hz), 7.16 (m, 4H), 7.24 (m, 2H), 7.30 (m, 3H), 7.34 (m, 3H). ESI-MS (m/z): 425.15 [M + 1]. Anal. ($C_{27}H_{24}N_2O_3$) C, H, N.

(*E*)-1-(3-Fluorobenzyl)-5-((2-fluorobenzylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6b). The titled compound was prepared from **4** and 2-fluorobenzylamine according to the method of compound **6a**. Yield 63%, yellow crystal. Mp: 97.7−99.7 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.81 (s, 3H), 4.87 (m, 2H), 4.98 (m, 2H), 6.27 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.46 (d, 1H, J = 2.7 Hz), 6.74 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.4 Hz), 6.87 (m, 4H), 7.18 (m, 3H), 7.30 (m, 4H), 15.62 (s, 1H). ESI-MS (m/z): 461.16 [M + 1]. Anal. ($C_{27}H_{22}F_2N_2O_3$) C, H, N.

(*E*)-1-(3-Fluorobenzyl)-5-((3-fluorobenzylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridine-2(1*H*)-one (6c). The titled compound was prepared from **4** and 3-fluorobenzylamine according to the method of compound **6a**. Yield 83%, yellow crystal. Single crystals for X-ray diffraction were obtained by slow crystallization of a solution in petroleum ether/AcOEt. Mp: 142.3−144.3 °C. IR (KBr) ν (cm^{-1}): 3061.26, 2934.59, 2847.51, 1665.10, 1592.58, 1226.49, 1019.84, 831.76, 781.16. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.81 (s, 3H), 4.60 (m, 2H), 5.03 (m, 2H), 6.26 (dd, 1H, $J1$ = 9 Hz, J_2 = 2.7 Hz), 6.45 (d, 1H, J = 2.7 Hz), 6.72 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.4 Hz), 6.86 (m, 4H), 7.19 (m, 3H), 7.28 (m, 4H), 15.62 (s, 1H), ^{13}C NMR (300 MHz, $CDCl_3$): δ 51.7, 54.0, 55.43, 101.78, 106.45, 111.86, 113.89, 114.14, 114.42, 114.96, 115.36, 115.64, 121.66, 122.53, 123.63, 130.29, 130.40, 130.77, 132.11, 136.65, 138.96, 161.39, 164.14, 169.57. IR (KBr) ν (cm^{-1}): 3061.26, 2934.59, 2847.51, 1665.10, 1592.58, 1226.49, 1019.84, 831.76, 781.16. ESI-MS (m/z): 461.47 [M + 1]. Anal. ($C_{27}H_{22}F_2N_2O_3$) C, H, N.

(*E*)-1-(4-Fluorobenzyl)-5-((4-fluorobenzylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6d). The titled compound was prepared from **4** and 4-fluorobenzylamine according to the method of compound **6a**. Yield 70%, yellow crystal. Mp: 154.0−155.3 °C. IR (KBr) ν (cm^{-1}): 3101.82, 3067.29, 3047.88, 2954.01, 2940.03, 2871.62, 1665.49, 1307.58, 1508.73, 1382.74, 1223.25. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.79 (s, 3H), 4.57 (m, 2H), 5.15 (m, 2H), 6.23 (dd, 1H, J_1 = 9 Hz), J_2 = 2.4 Hz), 6.43 (d, 1H, J = 2.7 Hz), 6.71 (d, 1H, J = 9 Hz), 6.83 (d, 1H, J = 9 Hz), 7.02 (m, 3H), 7.12 (m, 4H), 7.24 (m, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 51.57, 53.61, 55.38, 76.58, 77.01, 77.43, 101.80, 106.31, 110.79, 112.77, 115.51, 115.79, 115.90, 116.19, 121.56, 128.54, 128.64, 130.06, 130.17, 131.52, 132.01, 134.23, 136.53, 138.86, 161.42, 164.12, 167.29, 169.25. ESI-MS (m/z): 461.16 [M + 1]. Anal. ($C_{27}H_{22}F_2N_2O_3$) C, H, N.

(*E*)-1-(3,4-Difluorobenzyl)-5-((2,4-difluorobenzylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6e). The titled compound was prepared from **4** and 2,4-difluorobenzylamine according to the method of compound **6a**. Yield 55%, yellow crystal. Mp: 102.5−103.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.80 (s, 3H), 4.54 (m, 2H), 5.05 (m, 2H), 6.29 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.49 (d, 1H, J = 2.7 Hz), 6.78 (d, 1H, J = 9 Hz), 6.95 (m, 2H), 7.12 (m, 2H), 7.25 (m, 3H), 15.44 (s, 1H). ESI-MS (m/z): 497.14 [M + 1]. Anal. ($C_{27}H_{20}F_4N_2O_3$) C, H, N.

(*E*)-1-(3,4-Difluorobenzyl)-5-((3,4-difluorobenzylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6f). The titled compound was prepared from **4** and 3,4-difluorobenzylamine according to the method of compound **6a**. Yield 75%, yellow crystal. Mp: 129−130.8 °C. IR (KBr) ν (cm^{-1}): 3445.28, 3049.49, 3005.44, 2968.00, 2934.85, 2913.94, 2838.74, 1667.72, 1597.44, 1520.12, 1457.75. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 3H), 4.55 (m, 2H), 5.09 (m, 2H), 6.28 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.47 (d, 1H, J = 2.7 Hz), 6.76 (d, 1H, J = 9 Hz), 6.89

(m, 2H), 7.03 (m, 2H), 7.16 (m, 3H), 15.44 (s, 1H). ESI-MS (*m*/*z*): 497.14 [M + 1]. Anal. ($C_{27}H_{20}F_4N_2O_3$) C, H, N.

(*E*)-5-((2-Hydroxy-4-methoxyphenyl)(4-methoxybenzylimino)-methyl)-1-(4-methoxybenzyl)pyridin-2(1*H*)-one (6g). The titled compound was prepared from **4** and 4-methoxybenzylamine according to the method of compound **6a**. Yield 67%, yellow crystal. Mp: 128.5−129.9 °C. IR (KBr) ν (cm^{-1}): 3444.64, 3063.50, 3030.28, 2999.59, 2951.76, 2930.54, 2833.12, 1666.27, 1611.78, 1514.40, 1257.94, 1227.13, 1174.57. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.80 (t, 9H), 4.51 (s, 2H), 5.07 (s, 2H), 6.20 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.42 (d, 1H, J = 2.9 Hz), 6.72 (m, 1H), 6.85 (m, 5H), 7.03 (m, 2H), 7.16 (m, 2H), 7.22 (m, 2H), 15.49 (s, 1H). ESI-MS (*m*/*z*): 485.2 [M + 1]. Anal. ($C_{29}H_{28}N_2O_5$) C, H, N.

(*E*)-5-((2-Hydroxy-4-methoxyphenyl)(4-methylbenzylimino)-methyl)-1-(4-methylbenzyl)pyridin-2(1*H*)-one (6h). The titled compound was prepared from **4** and 4-methylbenzylamine according to the method of compound **6a**. Yield 66%, yellow needle crystal. Mp: 158.8−159.5 °C. IR (KBr) ν (cm^{-1}): 3446.44, 3454.69, 3049.93, 3001.00, 2981.32, 2922.59, 2854.95, 1659.67, 1595.70, 1384.37. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.34 (s, 6H), 3.80 (s, 3H), 4.54 (m, 2H), 5.09 (m, 2H), 6.20 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.41 (d, 1H, J = 2.7 Hz), 6.69 (d, 1H, J = 9.9 Hz), 6.82 (d, 1H, J = 9 Hz), 7.04 (m, 2H), 7.10 (m, 2H), 7.15 (m, 4H), 7.18 (m, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.07, 21.14, 51.93, 53.68, 55.35, 76.58, 77.00, 77.43, 101.89, 106.18, 111.41, 112.64, 120.11, 121.27, 127.03, 128.34, 128.57, 129.46, 129.77, 132.05, 132.59, 135.39, 136.90, 138.28, 138.84, 161.61, 164.20, 138.50, 169.02. ESI-MS (*m*/*z*): 453.21 [M + 1]. Anal. ($C_{29}H_{28}N_2O_3$) C, H, N.

(*E*)-1-Cyclohexyl-5-((cyclohexylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6i). The titled compound was prepared from **4** and cyclohexylamine according to the method of compound **6a**. Yield 79%, yellow crystal. Mp: 182−184.2 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.20 (m, 2H), 1.25 (m, 2H), 1.32 (m, 4H), 1.75 (m, 4H), 1.93 (m, 4H), 3.35 (m, 1H), 3.80 (s, 3H), 4.94 (m, 1H), 6.18 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.39 (d, 1H, J = 2.7 Hz), 6.67 (d, 1H, J = 9.3 Hz), 6.80 (d, 1H, J = 9 Hz), 7.15 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 7.28 (d, 1H, J = 2.7 Hz). ESI-MS (*m*/*z*): 409 [M + 1]. Anal. ($C_{25}H_{32}N_2O_3$) C, H, N.

(*E*)-1-Cyclopropyl-5-((cyclopropylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6j). The titled compound was prepared from **4** and cycloprpylamine according to the method of compound **6a**. Yield 75%, yellow crystal. Mp: 178.4−180.0 °C. IR (KBr) ν (cm^{-1}): 3446.29, 3421.37, 3079.42, 2938.77, 1663.68, 1588.65, 1287.39. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.94 (m, 2H), 0.97 (m, 4H), 1.17 (m, 2H), 2.86 (m, 1H), 3.41 (m, 1H), 3.80 (s, 3H), 6.29 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.44 (d, 1H, J = 2.7 Hz), 6.65 (d, 1H, J = 9.3 Hz), 6.87 (d, 1H, J = 9 Hz), 7.07 (m, 3H), 7.27 (m, 1H), 7.33 (m, 1H), 14.79 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 6.96, 10.27, 32.59, 33.53, 55.33, 101.63, 105.71, 112.29, 113.41, 120.83, 131.18, 136.97, 139.06, 162.88, 163.02, 164.48, 166.46. ESI-MS (*m*/*z*): 325.15 [M + 1]. Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

(*E*)-5-((2-Hydroxy-4-methoxyphenyl)(propylimino)methyl)-1-propylpyridin-2(1*H*)-one (6k). The titled compound was prepared from **4** and propylamine according to the method of compound **6a**. Yield 71%, yellow crystal. Mp: 129.5−131 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.99 (m, 6H), 1.69 (m, 2H), 1.82 (m, 2H), 3.38 (t, 2H, J = 0.72 Hz), 3,81 (s, 3H), 3.95 (t, 2H, J=0.72), 6.20 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 6.39 (d, 1H, J = 2.4 Hz), 6.67 (d, 1H, J = 9.3 Hz), 6.85 (d, 1H, J = 9 Hz), 7.21 (m, 2H). ^{13}C NMR (300 MHz $CDCl_3$) δ 11.00, 11.75, 22.41, 23.99, 51.17, 51.84, 55.31, 102.15, 106.05, 111.02, 112.17, 121.31, 131.87, 137.40, 138.74, 161.50, 164.47, 168.30, 170.49. ESI-MS (*m*/*z*): 329.1 [M + 1]. Anal. ($C_{19}H_{24}N_2O_3$) C, H, N.

(*E*)-1-Butyl-5-((butylimino)(2-hydroxy-4-methoxyphenyl)methyl)pyridin-2(1*H*)-one (6l). The titled compound was prepared from **4** and butylamine according to the method of compound **6a**. Yield 73%, yellow crystal. Mp: 85.6−86.6 °C. IR (KBr) ν (cm^{-1}): 3447.08, 3065.86, 3000.35, 2954.68, 2932.74, 2871.64, 2838.84, 1667.75, 1614.25, 1592.11, 1541.96, 1528.65, 1384.16, 1224.30. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.926(m, 6H), 1.40 (m, 4), 1.63 (m, 2H), 1.75 (m, 2H), 3.41 (t, 2H, J = 6.9 Hz), 3.81 (s, 3H), 3.99 (t, 2H, J = 6.9 Hz), 6.20 (dd, 1H, J_1 = 9.3 Hz, J_2 = 2.4 Hz), 6.40 (d, 1H, J = 2.4 Hz), 6.67 (d, 1H, J = 9 Hz), 6.85 (d, 1H, J = 9 Hz), 7.21 (m, 2H), 16.24 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 13.68, 19.80, 20.30, 31.22, 32.75, 49.16, 50.00, 55.29, 102.15, 105.99, 111.05, 112.23, 121.28, 131.84, 137.30, 138.68, 161.46, 164.42, 168.24, 170.38. ESI-MS (*m*/*z*): 357.21 [M + 1]. Anal. ($C_{21}H_{28}N_2O_3$) C, H, N.

(*E*)-5-((2-Hydroxy-4-methoxyphenyl)(pentylimino)methyl)-1-pentylpyridin-2(1*H*)-one (6m). The titled compound was prepared from **4** and pentylamine according to the method of compound **6a**. Yield 90%, yellow crystal. Mp: 95.8−97.4 °C. IR (KBr) ν (cm^{-1}): 3446.24, 3005.10, 2952.81, 2927.19, 2858.28, 1669.93, 1615.19, 1525.84, 1229.60. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.89 (m, 6H), 1.34 (m, 8H), 1.67 (m, 2H), 1.78 (m, 2H), 3.39 (m, 2H), 3.80 (s, 3H), 3.97 (m, 2H), 6.19 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.39 (d, 1H, J = 2.7 Hz), 6.66 (d, 1H, J = 9.6 Hz), 6.85 (d, 1H, J = 9 Hz), 7.22 (m, 2H), 16.26 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 13.90, 22.28, 28.75, 28.87, 29.32, 30.42, 49.50, 52.28, 55.31, 76.58, 77.00, 77.43, 102.17, 106.02, 111.06, 112.25, 121.31, 131.86, 137.33, 138.69, 161.47, 164.45, 168.23, 170.40. ESI-MS (*m*/*z*): 385.65 [M + 1]. Anal. ($C_{23}H_{32}N_2O_3$) C, H, N.

(*E*)-5-((2-Hydroxy-4-methoxyphenyl)(isopropylimino)methyl)-1-isopropylpyridin-2(1*H*)-one (6n). The titled compound was prepared from **4** and isopropylamine according to the method of compound **6a**. Yield 71%, light-yellow crystal. Mp: 152.8−155.6 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.261 (d, 6H, J = 6.3 Hz), 1.37 (d, 6H, J = 6.9 Hz), 3.67 (m, 1H), 3.81 (s, 3H), 5.34 (m, 1H), 6.21 (dd, 1H, J_1 = 9 Hz, J_2 = 2.7 Hz), 6.42 (d, 1H, J = 2.7 Hz), 6.67 (d, 1H, J = 9.3 Hz), 6.81 (d, 1H, J = 9H), 7.17 (dd, 1H, J_1 = 9.3 Hz, J_2 = 2.4 Hz), 7.26 (d, 1H, J = 2.4 Hz), 16.17 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.96, 24.21, 46.63, 50.58, 55.32, 102.06, 105.95, 111.75, 112.40, 120.97, 131.87, 132.29, 137.91, 161.16, 164.16, 166.58, 169.34. ESI-MS (*m*/*z*): 328.1 [M + 1]. Anal. ($C_{19}H_{24}N_2O_3$) C, H, N.

(*E*)-1-Allyl-5-((allylimino)(2-hydroxy-4-methoxyphenyl)methyl)-pyridin-2(1*H*)-one (6o). The titled compound was prepared from **4** and 2-propen-1-ylamine according to the method of compound **6a**. Yield 73%, yellow crystal. Mp: 139.2−141.6 °C. IR (KBr) ν (cm^{-1}): 3445.94, 3421.28, 3061.74, 2923.71, 2851.53, 1665.02, 1613.79, 1592.52, 1522.85, 1383.92. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 3H), 4.08 (s, 2H), 4.62 (s, 2H), 5.17 (m, 1H), 5.20 (m, 3H), 5.98 (m, 2H), 6.24 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 6.44 (d, 1H, J = 2.7 Hz), 6.90 (d, 1H, J = 9.6 Hz), 6.72 (d, 1H, J = 9 Hz), 7.24 (m, 2H), 16.93 (s, 1H). ESI-MS (*m*/*z*): 325.16 [M + 1]. Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

In Vitro Cytotoxicity Study of Target Compounds. Cytotoxicity of all synthesized compounds was assessed by MTT assays as previously described.[18] The compounds, dissolved in DMSO, were serially diluted in cell culture media and then added onto cell monolayers. HepG2 2.2.15 cells were maintained in 96-well tissue culture plates for 48 h before being treated with compounds. The cells were subsequently incubated at 37 °C for 9 days. Cells with media alone were used as the blank control, while adefovir was used as positive controls. MTT reagents were added at a concentration of 5 g/L at 4 h before the cells were harvested and lysed with 10% sodium dodecyl sulfate (SDS) and 50% *N*,*N*-dimethylformamide (DMF), pH 7.2. The OD values of cell lysates were read at a wavelength of 570 nm, and the percentages of cell death were determined.[19]

General Method for the HBsAg, HBeAg, and HBV DNA Inhibition Assays. HBV antigen and HBV DNA inhibitory efficacies of these compounds were evaluated in cultured HepG2 2.2.15 cells. Nine days after treatment with different dosages of the compounds, cell culture supernatants were collected, and HBV antigen activities were tested by enzyme linked immunosorbent assay (ELISA). Levels of HBV-DNA in the culture

media were quantified by fluorescent PCR as previously described.[20] PCR primers used were as follows: forward, 5′-TGTCCTGGTTATCGCTGG-3′; reverse, 5′-CAAACGGGCAACATA-CCTT-3′. Probe used was 5′-(FAM)-TGTGTCTGCGGCGTTTTATCAT-(TAMRA)-3′. PCR reactions were run on a MJ Research PTC-200, and data were analyzed by OpticonMonitor, version 2.01, software. The antiviral activities of the compounds are summarized in Table 1.

Computational Details. GASP[17] and SYBYL molecular modeling software[21] were used to construct the pharmacophore model. The three-dimensional structures of compounds **5f** and **6c** were built from the SYBYL fragment library. Energy minimization was performed using the Tripos force field, Powell optimization method, and MAXIMIN2 minimizer with a convergence criterion of 0.001 kcal/(mol·Å). Charges were calculated by the Gasteiger−Hückel method. Simulated annealing was then performed. The system was heated to 1000 K for 1.0 ps and then annealed to 250 K for 1.5 ps. The annealing function was exponential; 50 such cycles of annealing were run, and the resulting 50 conformers were optimized using methods described above. The lowest energy conformation was selected.

The GASP program uses a genetic algorithm for determining the correspondence between functional groups in the superimposed ligands and the alignment of these groups in a common geometry for receptor binding. A population of chromosomes is randomly constructed with each chromosome representing a possible alignment. Torsion angles for the rotatable bonds are adjusted when searching for molecule alignment. The fitness score of a given alignment is a weighted sum of three terms: the number and similarity of the overlaid elements, the common volume of the molecules, and the internal van der Waals (vdW) energy of each molecule. The calculation terminates when the fitness of the population does not further improve by a specified value or when the preset number of genetic operations is completed. The GASP settings were as follows. The GASP parameters were set to the following default values: population size 100; selection pressure 1.1; maximum number of operations 100 000; operation increment 6500; fitness increment 0.01; point cross weight 95.0; allele mutate weight 95.0; full mutation weight 0.0; full cross weight 0.0; internal vdW energy coefficient 1; HB weight coefficient 750; vdW contact cutoff 0.8.

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Supporting Information Available: HPLC analysis data of compounds **4**, **5a**−**s**, and **6a**−**o**. This material is available free of charge via the Internet at http://pubs.acs.org.

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