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2,4-Diaryl-4,6,7,8-tetrahydroquinazolin-5(1*H*)-one derivatives as anti-HBV agents targeting at capsid assembly

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ABSTRACT

A series of novel 2,4-diaryl-4,6,7,8-tetrahydroquinazolin-5(1*H*)-one derivatives were designed and synthesized as potent inhibitors of HBV capsid assembly. These compounds arose from efforts to rigidify an earlier series of heteroaryldihydropyrimidines (HAPs), and compounds **12**, **13**, **20**, **24**, **30** and **32** showed potent inhibition of HBV capsid assembly, especially **24** with IC_{50} value at sub-micromolar range.

More than 350 million people around the world are chronically infected with the hepatitis B virus (HBV), and estimated one million patients die each year due to the long-term complications of liver cirrhosis or hepatocellular carcinomas. So far, six antiviral agents have been approved for treating chronic hepatitis B (CHB): two immune modulators (IFN-α and pegIFN-α), and four polymerase inhibitors (lamivudine, entecavir, telbivudine and adefovir).[1] However, there are several disadvantages of current treatments,[2] such as the emergence of resistant mutants, poor tolerability, and the inefficiency of eradicating HBV from CHB patients. Therefore, development of more effective therapeutic agents for HBV infection is still highly necessary.

Aside from the HBV polymerase, various potential new targets have recently been investigated to prevent HBV replication, such as the virus nucleocapsid assembly and the host cell ER glucosidases.[3,4] Some features involved in the capsid assembly make the disruption of this process an attractive target for antivirus discovery. First, encapsidation of HBV pregenomic RNA is a very important process in HBV life cycle. The assembly disruption will affect the virus replication.[4c,5] Furthermore, the encapsidation is an evolutionary constraint process.[6] Compared to the polymerase, the genetic stability makes capsid assembly process a better drug target against various genotypes of HBV resistant mutants.[7] Finally, only a few small molecular agents that inhibit the interaction between core proteins or misdirect the assembly of capsid have been reported so far[3,8,9] (Fig. 1). Heteroaryldihydropyrimidines (HAPs), first developed by Bayer investigators,[3,9] were shown to bind the HBV core proteins and misdirect the assembly of the capsid in vitro, rather than affect the protein synthesis.[3] Moreover, the HAP compounds were also effective in the transgenic mice

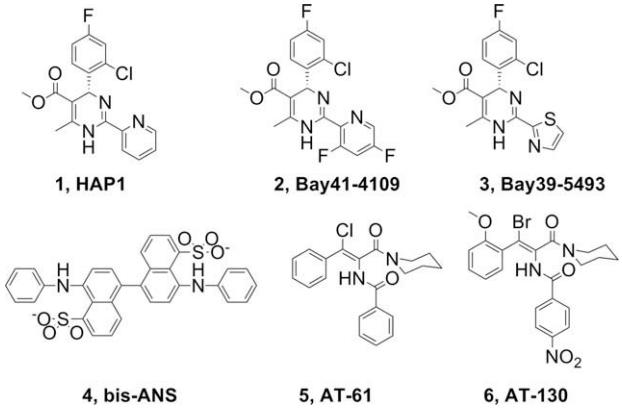

Figure 1. Representative HBV capsid assembly inhibitors.

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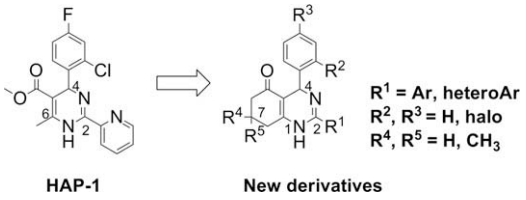


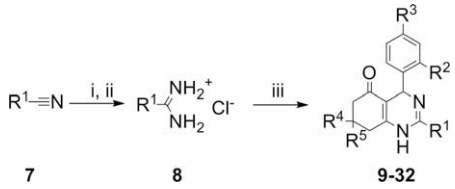
Figure 2. Design of 4,6,7,8-tetrahydroquinazolin-5(1*H*)-one derivatives.

model.[10] In 2006, the cocrystal structure of HBV capsid-HAP was solved by Bourne and coworkers.[11] HAP1 binds to a hydrophobic groove located at the interface between the capsid protein subunits.[12] However, due to the low resolution at 5 Å and only the 'apo' structure was released, it was difficult to perform the structure-based design of new capsid assembly inhibitors by using this crystal structure.

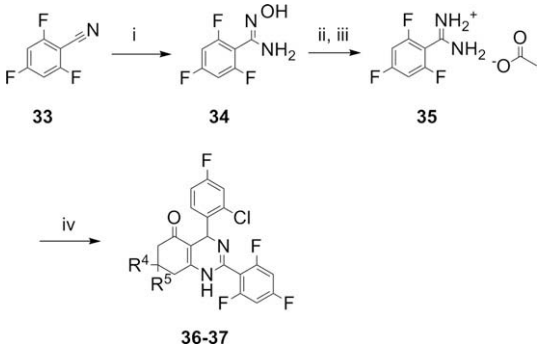
We reported herein the synthesis, characterization and in vitro antiviral activities of 2-aryl-4,6,7,8-tetrahydroquinazolin-5(1*H*)-one derivatives, a novel class of HBV capsid assembly inhibitor. The derivatives were designed by rigidifying an earlier series of HAPs (Fig. 2).

A general synthetic procedure is illustrated in Scheme 1. In most cases the appropriate commercially available nitrile **7** was converted to the carboxyamidine **8** via treatment with sodium methoxide in anhydrous methanol at room temperature, followed by reaction with ammonium chloride.[13] These carboxyamidines were then converted to the 2,4-diaryl-4,6,7,8-tetrahydroquinazolin-5(1*H*)-ones **9–32** by reacting with cyclohexane-1,3-dione and benzaldehyde under the Biginelli condition.[14]

Compounds **36** and **37** were prepared according to the method described in Scheme 2. 2,4,6-Trifluorobenzonitrile **33** was first treated with hydroxylamine hydrochloride to afford 2,4,6-trifluoro-*N*′-hydroxybenzimidamide **34**, and followed by hydrogenation in the presence of 5% Pd/C to give 2,4,6-trifluorobenzimidamide acetate **35**.[15] Coupling of **35** with cyclohexane-1,3-dione and 2-chloro-4-fluorobenzaldehyde afforded compounds **36** and **37**. All the new compounds described were characterized by ^{1}H NMR, MS and HRMS spectrums.[16]



Scheme 1. Reagents and conditions: (i) 0.05 equiv CH_3ONa, anhydrous MeOH, 0 °C, overnight; (ii) ammonium chloride, ambient temperature, 24 h; (iii) substituted cyclohexane-1,3-dione, substituted benzaldehyde, 1.5 equiv NaOAc, EtOH, reflux, 20 h.



Scheme 2. Reagents and conditions: (i) hydroxylamine hydrochloride, K_2CO_3, DMSO, ambient temperature, overnight; (ii) Ac_2O, AcOH, rt, 30 min; (iii) 5% Pd/C, H_2, 2 atm, 2.5 h; (iv) substituted cyclohexane-1,3-dione, 2-chloro-4-fluorobenzaldehyde, 1.5 equiv NaOAc, EtOH, reflux, 20 h.

Inhibition activities for HBV replication of the final compounds **9–32** and **36–37** were determined as described previously[17] in the HepG2.2.15 cells, which constitutively produces HBV genomes,

Table 1
In vitro anti-HBV evaluation of compounds **9–32**, and **36–37**

Compds	R^1	R^2	R^3	R^4	R^5	DNA replication IC_{50}[a] (μM)	Cytotoxicity IC_{50}[a] (μM)	T.I.[b]
9	Pyridin-2-yl	H	H	H	H	6.60	571.31	86.56
10	Pyridin-2-yl	Cl	H	H	H	na	589.20	—
11	Pyridin-2-yl	H	F	H	H	na	515.62	—
12	Pyridin-2-yl	Cl	F	H	H	4.30	619.50	144.07
13	Pyrazin-2-yl	Cl	F	H	H	2.30	777.21	337.92
14	Pyrazin-2-yl	Cl	H	H	H	na	1470.40	—
15	Pyridin-3-yl	Cl	F	H	H	25.10	82.80	9.25
16	Pyridin-3-yl	Cl	H	H	H	na	1124.62	—
17	Pyridin-3-yl	H	F	H	H	na	280.50	—
18	Pyridin-3-yl	H	H	H	H	na	368.90	—
19	Furan-2-yl	Cl	F	H	H	na	717.0	—
20	3-Fluoropyridin-2-yl	Cl	F	H	H	1.10	1369.42	1244.93
21	3-Fluoropyridin-2-yl	Cl	F	CH_3	CH_3	na	371.10	—
22	Pyrazin-2-yl	Cl	F	CH_3	CH_3	14.20	773.41	54.46
23	Pyrazin-2-yl	Cl	H	CH_3	CH_3	na	622.94	—
24	Pyridin-3-yl	Cl	F	CH_3	CH_3	0.44	623.80	1417.73
25	Pyridin-3-yl	Cl	H	CH_3	CH_3	26.20	67.32	2.56
26	Pyridin-3-yl	H	F	CH_3	CH_3	25.30	145.10	5.73
27	Pyridin-3-yl	H	H	CH_3	CH_3	22.40	173.23	7.74
28	Pyridin-4-yl	Cl	F	H	H	16.47	142.39	8.60
29	Pyridin-4-yl	Cl	F	CH_3	CH_3	4.92	134.80	27.28
30	3,5-Difluoropyridin-2-yl	Cl	F	CH_3	CH_3	1.05	41.17	39.21
31	Thiazol-2-yl	Cl	F	H	H	8.75	47.68	5.73
32	Thiazol-2-yl	Cl	F	CH_3	CH_3	3.12	56.99	19.16
36	2,4,6-Trifluorophenyl	Cl	F	H	H	na	122.60	—
37	2,4,6-Trifluorophenyl	Cl	F	CH_3	CH_3	na	5.60	—
Bay41-4109						0.78	567	727
Lamivudine						0.22		

[a] Values are means of three experiments, na = not active at the maximum nontoxic concentration.
[b] In vitro therapeutic index (IC_{50} cytotoxicity/IC_{50} complement inhibition).

and secretes virus-like particles.[18] Bay41-4109 and Lamivudine were used as positive control. To ascertain the cytotoxic effects of all the tested compounds, the cell viability was determined after the cells had been exposed to the compounds for 48 h[17] (Table 1).

Based on the SAR results of HAPs, the effects of substitutions on the 4-phenyl rings were first examined. 4-(2-Chloro-4-fluorophenyl) substitution (**9**, IC_{50} = 6.6 μM) led to the best antiviral effect. Single substitution on the 4-phenyl ring such as 2-chloro (**10**) or 4-fluoro (**11**) attenuated the activity. The activities were susceptible to alterations on 4-phenyl rings. It was consistent with literatures reported by Bayer investigators.[9]

Compound **12** was identified as a potent anti-HBV agent. Replacement of the 2-pyridin-2-yl substitution in **12** by pyrazin-2-yl, pyridin-3-yl, furan-2-yl, 3-fluoropyridin-2-yl, pyridin-4-yl, thiazol-2-yl and 2,4,6-trifluorophenyl resulted in compounds **13**, **15**, **19**, **20**, **28**, **31** and **36**. Compounds **13** (IC_{50} = 2.3 μM) and **20** (IC_{50} = 1.1 μM) showed increased activities against HBV replication, while compounds **28** (IC_{50} = 16.47 μM) and **31** (IC_{50} = 8.75 μM) showed decreased activities, and compounds **15**, **19** and **36** lost their activities. At the same time, 4-(2-chlorophenyl) (**14**, **16**, **20**), 4-(4-fluorophenyl) (**17**) and 4-phenyl (**18**) analogs were prepared. Compared to their parent compounds, all these substitutions attenuated the activities. This result further confirmed that the antiviral activity has little tolerance to the changes of 4-(2-chloro-4-fluorophenyl).[12]

As revealed by the crystal structure, the 6-methyl group of the HAP-1 core faces a hydrophobic tunnel.[12] Installation of longer hydrophobic substitutions at this position is expected to be tolerated or even enhance the binding affinity. This observation prompted us to synthesize the 7,7-dimethyl substituted compounds **21–27**, **29–30**, **32** and **37**. Among this new series, compounds **24** (IC_{50} = 0.44 μM) and **30** (IC_{50} = 1.05 μM) showed good inhibition of HBV replication, which was comparable to Bay41-4109. Although compounds **29** (IC_{50} = 4.92 μM) and **32** (IC_{50} = 3.12 μM) just showed moderate inhibition of HBV replication, they were more potent than the 7-nonsubstituted compounds **28** and **31**. It indicated that 7,7-dimethyl substitution was favorable for the antiviral activity.

In summary, we have described the successful structural modification of HAPs to 4,6,7,8-tetrahydroquinazolin-5(1*H*)-ones, a novel class of HBV capsid assembly inhibitor. These newly developed derivatives demonstrated excellent in vitro activities against HBV replication. The results indicated that the design of new HBV capsid assembly inhibitor by rigidifying structures of HAPs is a feasible and promising strategy. The active compounds **24** and **30** could be used as the lead compounds for further modification.

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References and notes

1. Ferir, G.; Kaptein, S.; Neyts, J. *Rev. Med. Virol.* **2008**, *18*, 19.
2. (a) Ghany, M.; Liang, T. J. *Gastroenterology* **2007**, *132*, 1574; (b) Shaw, T.; Bartholomeusz, A. *J. Hepatol.* **2006**, *44*, 593; (c) Clercq, D. E. *Exp. Opin. Emerg. Drugs* **2008**, *13*, 393.
3. (a) Deres, K.; Schroder, C. H. *Science* **2003**, *299*, 893; (b) Hacker, H. J.; Deres, K. *Biochem. Pharmacol.* **2003**, *66*, 2273; (c) Stray, S. J.; Zlotnick, A. *J. Mol. Rec.* **2006**, *19*, 542.
4. (a) Block, T. M.; Lu, X.; Mehta, A. S.; Ferir, G. *Nat. Med.* **1998**, *4*, 610; (b) Block, T. M.; Jordan, R. *Antiviral. Chem. Chemother.* **2001**, *12*, 317; (c) Wen, Y.-M.; Lin, X.; Ma, Z.-M. *Curr. Drug Targets* **2003**, *3*, 241.
5. Le Pogam, S.; Yuan, T-T.; Sahu, G. K. *J. Virol.* **2000**, *74*, 9099.
6. Chain, B.; Myers, R.; Neyts, J. *BMC Microb.* **2005**, *5*, 33.
7. Choi, I.-G.; Yu, Y. G. *Infect. Disorders—Drug Targets* **2007**, *7*, 251.
8. (a) Zlotnick, A.; Ceres, P.; Singh, S. *J. Virol.* **2002**, *76*, 4848; (b) King, R. W.; Ladner, S. K.; Miller, T. J. *Antimicrob. Agents Chemother.* **1998**, *42*, 3179.
9. (a) Stoltefuss, J.; Goldmann, S. WO Patent 9,954,326, 1999.; (b) Goldmann, S.; Kramer, T. WO Patent 0,058,302, 2000.; (c) Goldmann, S.; Stoltefuss, J. DE Patent 10,012,824A1, 2001.; (d) Goldmann, S.; Stoltefuss, J.; U. Niewohner. DE Patent 10,013,126A1, 2001.; (e) Goldmann, S.; Stoltefuss, J. U.S. Patent 6,503,913B1, 2003.
10. Weber, O.; Schlemmer, K. H.; Hartmann, E. *Antiviral. Res.* **2002**, *54*, 69.
11. Bourne, C.; Finn, M. G.; Zlotnick, A. *J. Virol.* **2006**, *80*, 11055.
12. Bourne, C.; Lee, S.; Venkataiah, B. *J. Virol.* **2008**, *82*, 10262.
13. Slee, H. D.; Chen, Y.; Zhang, X. *J. Med. Chem.* **2008**, *51*, 1719.
14. Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937.
15. Judkins, B. D.; Allen, D. G.; Cook, T. A. *Synth. Commun.* **1996**, *26*, 4351.
16. *Selected data for compound* **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04–2.05 (2H, m, CH_2); 2.34–2.76 (4H, m, CH_2); 5.79–5.97 (1H, BR, CH); 7.21–7.30 (3H, m, ArH); 7.39–7.43 (3H, m, ArH); 7.83 (1H, m, ArH); 8.34–8.42 (1H, m, ArH); 8.54–8.55 (1H, m, ArH); 8.78 (1H, s, NH); MS(EI) 303.2(M^+); HRMS (*m*/*z*) calcd for $C_{19}H_{17}N_3O$: 1372; found 303.1370.
Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13–2.15 (2H, m, CH_2); 2.45 (2H, s, CH_2); 2.65–2.85 (2H, m, CH_2); 6.14–6.22 (1H, m, CH); 6.90–9.92 (1H, m, ArH); 7.16–7.26 (1H, m, ArH); 8.19–8.54 (2H, m, ArH); 8.77 (1H,s,NH); MS(EI) 355.2 (M^+); HRMS (*m*/*z*) calcd for $C_{19}H_{15}N_3OFCl$: 355.0888; found 373.0889.
Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.14–2.19 (2H, m, CH_2); 2.47 (2H, s, CH_2); 2.72–2.87 (2H, m, CH_2); 6.14–6.23 (1H, m, CH); 6.91–6.95 (1H, m, ArH); 7.13–7.26 (2H, m, ArH); 8.21–8.52 (2H, m, ArH); 8.71 (1H, m, ArH); 9.58(1H, s, NH); MS (EI) 356.2 (M^+); HRMS (*m*/*z*) calcd for $C_{18}H_{14}N_4OFCl$: 356.0840; found 356.0839.
Compound **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.11–2.17 (2H, m, CH_2); 2.39–2.50 (3H, s, CH_3); 2.64–2.78 (2H, m, CH_2); 6.18 (1H, s, CH); 6.89–6.93 (1H, m, ArH); 7.12–7.14 (1H, m, ArH); 7.24–7.28(1H, m, Ar); 7.42- 7.46 (1H, m, ArH); 8.39–8.40 (1H, m, ArH); MS(EI)373.1 (M^+); HRMS (*m*/*z*) calcd for $C_{19}H_{14}N_3OClF_2$: 373.0793; found 373.0792.
Compound **24**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (3H, s, CH_3); 1.16 (3H, s, CH_3); 2.31(2H, s, CH_2); 2.53–2.69(2H, m, CH_2); 6.07(1H, s, CH); 6.94- 6.96 (1H, m, ArH); 7.14–7.17 (1H, m, ArH); 7.23–7.26 (1H, m, ArH); 7.34- 7.38 (1H, m, ArH); 8.07–8.09 (1H, m, ArH); 8.69–8.71 (1H, m, ArH); 8.89(1H, m, ArH); MS (EI) 383.1 (M^+); HRMS (*m*/*z*) calcd for $C_{21}H_{19}N_3OFCl$: 383.1201; found 383.1198.
Compound **30**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30–8.29 (m, 1H, ArH); 8.05 (s, 1H, NH); 7.32–7.29 (t, 1H, J_1 = 2.4 Hz, J_2 = 8.0 Hz, ArH); 7.29–7.26 (m, 1H, ArH); 7.14- 7.11 (d, 1H, J_1 = 2.4 Hz, J_2 = 8.0 Hz, ArH); 6.94–6.90 (t, 1H, J_1 = 2.4 Hz, J_2 = 8.0 Hz, ArH); 6.16 (s, 1H, CH); 2.70–2.53 (m, 2H, CH_2); 2.30 (m, 4H, CH_2); 1.16 (s, 3H, CH_3); 1.15 (s, 3H, CH_3); HRMS (*m*/*z*) calcd for $C_{21}H_{17}ClF_3N_3O$: 419.1012; found 419.1010(M^+).
Compound **32**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95–7.85 (m, 1H, ArH); 7.64–7.47 (m, 1H, ArH); 7.34–7.24 (m, 1H, ArH); 7.13–7.11 (m, 1H, ArH); 6.11 (s, 1H, CH); 2.70–2.52 (m, 2H, CH_2); 2.43–2.26 (m, 2H, CH_2); 1.15 (s, 6H, CH3); HRMS (*m*/*z*) calcd for $C_{19}H_{17}ClFN_3OS$: 389.0765; found 389.0765(M^+).
17. Korba, B. E.; Gerin, J. L. *Antiviral. Res.* **1992**, *19*, 55.
18. (a) Sells, M. A.; Chen, M. L.; Acs, G. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1005; (b) Sells, M. A.; Zelent, A. Z.; Shvartsman, M. *J. Virol.* **1988**, *62*, 2836.