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Synthesis and in vitro anti-hepatitis B virus activities of 4-aryl-6-chloro-quinolin-2-one and 5-aryl-7-chloro-1,4-benzodiazepine derivatives

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ABSTRACT

A series of 4-aryl-6-chloro-quinolin-2-ones and 5-aryl-7-chloro-1,4-benzodiazepine were synthesized and assayed for their in vitro anti-hepatitis B virus activities and cytotoxicities for the first time. Some of the tested compounds were active against HBsAg and HBeAg secretion in Hep G2.2.15 cells. Compound **5c** showed IC_{50} of 0.074 and 0.449 mM on HBsAg and HBeAg secretions, respectively, which were 10 times higher than that of its analog **4c** and led to better selective index (SI) values (SI = 23.2 and 3.4, respectively).

Hepatitis B virus (HBV) infection is still a major health problem around the world, particularly in Asia. Approximately 350 million people are chronic carriers of HBV worldwide, even though effective vaccines have been available for the last 20 years.[1] Worldwide deaths from liver cancer caused by HBV infection probably exceed 1 million per year.[2] Nowadays, at least two different treatment options, including interferon and nucleoside analogs such as lamivudine, are considered as antiviral therapy for chronic hepatitis B infection.[3] However, the side effects of interferon and the viral resistance of nucleoside analogs make the current treatment regimens far from satisfactory.[4–6] To circumvent the existing therapeutic difficulties, novel compounds with unique modes of actions are still urgently needed.

4-Aryl-quinoline-2-ones are inhibitors of acyl coenzyme A and cholesterol acyltransferase and are potent openers of the high conductance, calcium-activated K^+-channels.[7] As a part of our continuous search for active anti-HBV leads from natural sources and synthetic compounds,[8–10] a rational screening suggested that 4-aryl-6-chloro-quinolin-2-one (**4a**, Fig. 1) possessed moderate activity to inhibit the production of HBV surface antigen (HBsAg) in HBV-infected Hep G2.2.15 cells with selective index (SI) value of 2.6 (IC_{50} = 0.458 mM). Considering the diversity of the structures of non-nucleoside HBV inhibitors,[11–13] we decided to investigate the biological properties of 4-aryl-6-chloro-quinolin-2-ones and their analogs as potential anti-HBV agents.

In this paper, we report the synthesis of several 4-aryl-6-chloro-quinolin-2-ones and 5-aryl-7-chloro-1,4-benzodiazepines, which were mainly modified at C-3 of **4a** to 3-substituted-quinolin-2-ones, and at ring A to ring expanded 5-aryl-7-chloro-1,4-benzodiazepines (Fig. 1). The synthesized compounds were evaluated for their in vitro anti-HBV activities for the first time.

The synthetic route of two kinds of target compounds was summarized in Scheme 1. 4-Aryl-6-chloro-quinolin-2-ones and 5-aryl-7-chloro-1,4-benzodiazepines shared the same synthetic precursor 2-aminobenzophenones (**3**, Scheme 1). The synthesis of compounds **3** by a variety of methods has been reviewed.[14] Based on the complex-induced proximity effect,[15] the majority of compounds **3** were prepared by the reaction of aryl esters with *ortho*-lithiated Boc protected 3-chloro-aniline (**2**) via the formation

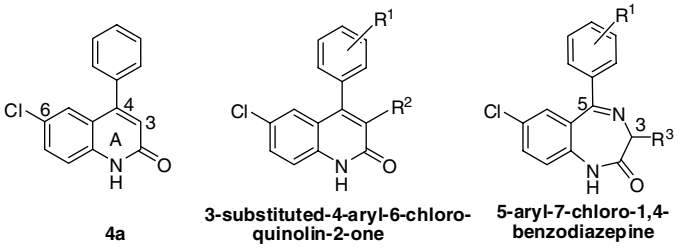

Figure 1. Structures of **4a**, 3-substituted-quinolin-2-one, and 5-aryl-1,4-benzodiazepine.

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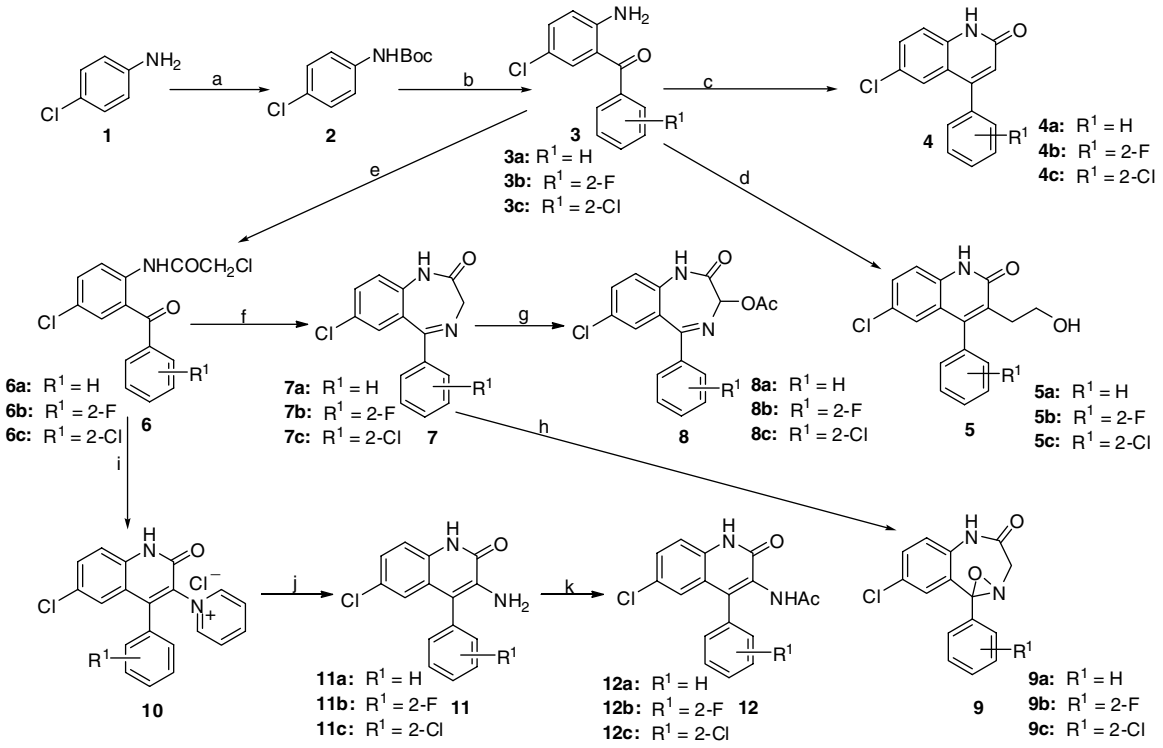

Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) $(Boc)_2O$, DIEA, DCM; (b) −78 °C, 2.2 equiv *tert*-BuLi, then add R^1-PhCOOMe at −40 °C and warm up to 0 °C, then 3 N HCl, EtOH, reflux, in three steps; (c) 0 °C, LiHMDS, EtOAc; (d) 0 °C, LiHMDS, γ-valerolactone; (e) $ClCH_2COCl$, DIEA, toluene; (f) HMTA, NH_4OAc, EtOH, reflux; (g) I_2, $K_2S_2O_8$, KOAc, AcOH, 70 °C; (h) *m*-CPBA, DCM; (i) pyridine, reflux; (j) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux; (k) pyridine, Ac_2O, 70 °C.

of dianion species with *tert*-BuLi (2.2 equiv) followed by deprotection of Boc group (Scheme 1).[16] A tandem amidation/Knoevengel condensation of readily prepared compounds **3** with ethyl acetate or γ-valerolactone gave compounds **4** and 3-hydroxyl ethyl substituted compounds (**5**) in good yields, respectively.[17]

As shown in Scheme 1, chloroacetylation of compounds **3** with chloroacetyl chloride in the presence of *N*,*N*-diisopropylethylamine (DIEA) in dichloromethane (DCM) gave the corresponding *N*-(chloroacetyl)-2-aminobenzophenone derivatives (**6**). Due to our interest to examine whether the ring expanded 1,4-benzodiazepine could have an effect on potential anti-HBV activities, compounds **7** were afforded by the reaction of intermediates **6** with hexamethylenetetramine (HMTA) in EtOH in the presence of NH_4OAc. In addition, compounds **7** were further transferred to compounds **8** as racemates and compounds **9** by acetoxylation reaction of 3-position of 1,4-benzodiazepine ring[18] and epoxidation of the C=N double bond,[19] respectively.

Upon heating a solution of compounds **6** in anhydrous pyridine at reflux for 30 min, the initially formed α-pyridinium salt underwent cyclodehydration to afford intermediates **10**, which were further hydrolyzed with hydrazine hydrate in ethanol at reflux for 2 h to provide the desired compounds **11**. The acetamide derivatives (**12**) of **11** were further prepared by a protocol using excess Ac_2O in pyridine at 70 °C.

Target compounds **4**–**5**, **7**–**9**, and **11**–**12** were evaluated for their cytotoxicities and anti-HBV activities, namely the ability to inhibit the secretion of HBsAg and HBV e antigen (HBeAg) in HBV-infected Hep G2.2.15 cells using lamivudine (3TC) as a positive control.

Although some of the synthesized compounds were already known or published to have other biological activities before,[20–30] 14 of them exhibited inhibitory effect on the secretion of HBsAg (SI > 1), and five compounds possessed inhibitory effect on the secretion of HBeAg (Table 1). 4-Aryl-6-chloro-quinolin-2-ones derivatives **4a**–**c** showed lower cell cytotoxicities, and their IC_{50} values on inhibition of HBsAg secretion were 0.485, 2.863, and 0.752 mM, respectively (SI = 2.6, >1.9, 8.0). In addition, compound

Table 1
Anti-HBV activity, cytotoxicity, and selectivity index of compounds **4**–**5**, **7**–**9**, and **11**–**12**[a]

Compound	CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
		IC_{50}[e] (mM)	SI[f]	IC_{50} (mM)	SI
4a[17]	1.25	0.485	2.6	>4.14	<1
4b	>5.46	2.84	>1.9	2.22	>2.4
4c	6.00	0.752	8.0	>6.00	<1
5a[20]	>4.91	0.300	>16	0.950	>5.2
5b	>2.99	0.187	>16	0.545	>5.5
5c	1.72	0.074	23	0.449	3.4
7a	0.221	0.255	<1	0.835	<1
7b	0.183	0.294	<1	5.36	<1
7c	0.076	0.076	<1	4.08	<1
8a[18]	0.678	0.292	2.3	>4.26	<1
8b	0.344	0.160	2.2	1.22	<1
8c	0.154	0.167	<1	1.94	<1
9a[21,22]	0.946	0.728	1.3	>3.28	<1
9b[23]	0.346	0.195	1.8	>3.26	<1
9c[23]	0.302	0.355	<1	4.49	<1
11a[24–26]	0.399	0.067	6.0	>3.99	<1
11b[27]	0.767	0.056	14	>3.61	<1
11c[28]	0.341	0.341	1.0	>5.47	<1
12a[28–30]	0.239	0.208	1.1	1.87	<1
12b	0.044	0.141	<1	0.079	<1
12c	0.208	0.129	1.6	0.080	2.6
3TC[g]	30.0	11.7	2.6	25.9	1.2

[a] All values are the mean of two independent experiments.
[b] CC_{50}: 50% cytotoxic concentration.
[c] HBsAg: HBV surface antigen.
[d] HBeAg: HBV e antigen.
[e] IC: 50% effective concentration.
[f] SI (selective index) = CC_{50}/IC_{50}.
[g] 3TC: lamivudine, an antiviral agent used as positive control.

4b showed inhibitory activity on HBeAg secretion with an SI value of >2.4 (IC_{50} = 2.22 mM). Compounds **5a–c**, derived from hydroxyl ethyl introduction to C-3 of compounds **4**, exhibited increased suppressant properties of the secretion of HBsAg and HBeAg. Especially, compound **5c** showed IC_{50} of 0.074 and 0.449 mM on HBsAg and HBeAg secretion, respectively, which were 10 times lower than its analog **4c** and led to greatly increased SI values (SI_{HBsAg} = 23, SI_{HBeAg} = 3.4). It was worth noting that compounds **5a–c** all possessed relative low cell cytotoxicities and good SI values on inhibitory effect of HBeAg secretion.

Compared with the quinolin-2-one derivatives, 1,4-benzodiazepines **7–9** showed increased cell cytotoxicities with slightly changed activities of inhibition on HBsAg secretion and reduced suppressant properties on the secretion of HBeAg. Thus, their anti-HBV SI values were suboptimal and only compounds **8a–b** and **9a–b** exhibited SI_{HBsAg} values of 2.3, 2.2, 1.3, and 1.8. Compounds **7–9** were all inactive to inhibit HBeAg secretion. Interestingly, according to Helena and colleagues' research, the 3-amino substituted 1,4-benzodiazepines, analogs of compounds **8**, were identified as anti-hepatitis C infection agents.[31]

The introduction of amino group to C-3 of compounds **4** increased both cytotoxicities and activities on inhibition of HBsAg secretion (compound **11a** vs **4a**, **11b** vs **4b**, **11c** vs **4c**). The SI_{HBsAg} values of compounds **11a** and **11b** were 6.0 and 13.6, respectively (IC_{50} = 0.067 and 0.056 mM). However, compounds **11** lost the properties to inhibit HBeAg secretion compared with compounds **5**. *N*-acetylation of compounds **11a–c** gave derivatives 1**2a–c**. Compounds **12b** (IC_{50} = 0.079 mM) and **12c** (IC_{50} = 0.080 mM) were the most active analogs to inhibit the secretion of HBeAg. However, these two compounds were more toxic in Hep G2.2.15 cells, and only compound **12b** showed an SI_{HBeAg} value of 2.6.

In summary, a series of 4-aryl-6-chloro-quinolin-2-ones and 5-aryl-7-chloro-1,4-benzodiazepines were synthesized and examined for their in vitro anti-HBV activities and cytotoxicities and several tested compounds were active against HBV in Hep G2.2.15 cells. Based on the above structure and activity relationship results, the following conclusion can be drawn: (i) 4-aryl-6-chloro-quinolin-2-ones exhibited better SI values compared with benzodiazepines. (ii) For compounds **5a–c**, a hydroxyl ethyl group introduced to C-3 of 4-aryl-6-chloro-quinolin-2-ones increased the properties of inhibition of both HBsAg and HBeAg secretion, and this kind of analogs showed low cytotoxicities. (iii) As to compounds **11a–c**, an amino group introduced to C-3 of 4-aryl-6-chloro-quinolin-2-ones led to increased cytotoxicities and activities on inhibition of HBsAg secretion. But these compounds were inactive to inhibit the production of HBeAg. (iv) The anti-HBV SI values of ring expanded benzodiazepines were suboptimal because of the increased cytotoxicities. Moreover, as the first report on 4-aryl-6-chloro-quinolin-2-ones and 5-aryl-7-chloro-1,4-benzodiazepines serving as anti-HBV agents, these results provided a lead (compounds **5**) in the research and development of new non-nucleoside anti-HBV medicine.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.065.

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