European Journal of Medicinal Chemistry 46 (2011) 307–319

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Structure–activity relationships study of 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one derivatives as novel non-nucleoside anti-hepatitis B virus agents

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ARTICLE INFO

Article history:
Received 19 July 2010
Received in revised form
6 November 2010
Accepted 11 November 2010
Available online 19 November 2010

Keywords:
Anti-hepatitis B virus activity
Quinolin-2(1*H*)-one
Structure–activity relationships

ABSTRACT

A series of novel 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one derivatives were synthesized and evaluated for anti-hepatitis B virus (anti-HBV) activities in vitro to explore their structure–activity relationships (SARs). Most of the synthesized compounds possessed potent anti-HBV activity, of which the promising compound **44** exhibited significantly inhibitory potency against the secretion of hepatitis surface antigen (HBsAg) (IC_{50} = 0.010 mM, SI > 135), hepatitis e antigen (HBeAg) (IC_{50} = 0.026 mM, SI > 51) and the replication of HBV DNA (IC_{50} = 0.045 mM). Preliminary mechanism study suggested compound **44** could mainly enhance the transcript activity of HBV ENI (enhancer I), EN-II (enhancer II).

1. Introduction

Hepatitis B virus, belonging to hepadnavirus (hepatotropic DNA viruses) family, causes acute and chronic infection of liver [1,2], and constitutes the world's ninth leading cause of death [3]. Chronic HBV infection can lead to cirrhosis of liver, liver failure, and hepatocellular carcinoma (HCC) [4]. An estimated 350–400 million people are chronically infected by HBV throughout the world with 0.5–1.2 million global deaths per year [5]. Currently only two interferons-alphas (interferon alpha-2a and PEGylated interferon alpha-2a) and five nucleoside inhibitors (lamivudine, adefovir dipivoxil, entecavir, telbivudine, tenofovir disoproxil fumarate) have been approved by the FDA as single-agent treatments for HBV infection [6,7].

However, use of α-interferon is limited for its lower success rate and serious side effects, and nucleoside analogues targeting the viral DNA polymerase result in development of drug-resistant virus after long-term treatment [8–10]. Therefore, the current therapies remain unsatisfactory and development of novel classes of anti-HBV agents with new structures and mechanisms of action is urgently needed.

As a part of our continuous search for active anti-HBV leads from natural and synthetic compounds [11–16], a rational screening suggested that 4-aryl-6-chloroquinolin-2-one (**1**, Fig. 1) possessed moderate activity inhibiting the production of HBsAg with an IC_{50} value of 0.46 mM in HBV-infected HepG 2.2.15 cells. In view of its novel structural template different from those of all reported anti-HBV agents, the preliminary structure–activity relationships of the relevant compounds were studied leading to the discovery of 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one (**2**) with a 5.3-fold increased activity against the secretion of HBsAg (IC_{50} = 0.086 mM vs IC_{50} = 0.46 mM), and with an IC_{50} value of 0.36 mM on the secretion of HBeAg, which revealed that introduction of hydroxyethyl to C-3 of 4-aryl-6-chloro-quinoline-2-one was an important feature in conferring enhanced anti-HBV activity [12].Therefore, it is of interest to synthesize additional analogues for investigation of SARs and the development of more potent anti-HBV agents.

In this paper, we synthesized novel 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one analogues via chemical modifications on *N*-1, C-2, and hydroxyethyl moiety and evaluated their anti-HBV activities. The most active compounds **42**–**49**, **56**, and **57** against the secretion of HBsAg and HBeAg were further assayed on their inhibition of HBV DNA replication. Significantly, compound **44** inhibited not only the secretion of HBsAg (IC_{50} = 0.010 mM, SI > 135), HBeAg (IC_{50} = 0.026 mM, SI > 51) but also HBV DNA replication

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis surface antigen; HBeAg, hepatitis e antigen; HCC, hepatocellular carcinoma; SARs, structure–activity relationships; SI, selectivity index; CC_{50}, 50% cytotoxic concentration; IC_{50}, 50% inhibitory concentration.

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0223-5234/$ – see front matter
doi:10.1016/j.ejmech.2010.11.019

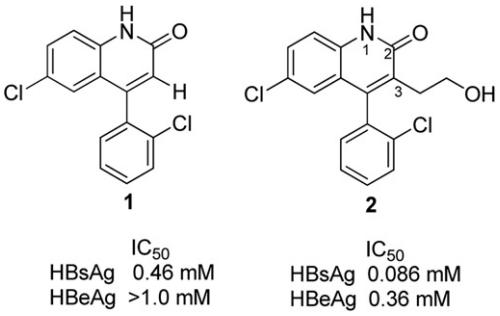


Fig. 1. Structures of compounds 1 and 2.

(IC_{50} = 0.045 mM) in vitro. Preliminarily, the anti-HBV mechanism study proposed that compound **44** could influence the HBV replication cycle by enhancing the transcript activity of HBV ENI (enhancer I), EN-II(enhancer II) on HepG 2 cells.

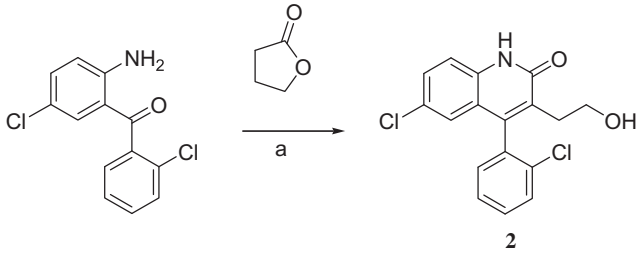
2. Chemistry

As illustrated in Scheme 1, 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one **2** was conveniently synthesized from commercially available 2-amino-benzophenone with γ-valerolaction using lithium bis(trimethylsily)amide (LiHMDS) solution in THF in good yield followed by a tandem amidation/Knoevengel condensation [17].

The modification of compound **2** performed on *N*-1 was summarized in Scheme 2. Treatment of compound **2** with *tert*-butyl diphenylchlorosilane (TBDPSCl) in the presence of *N*-ethyldiisopropylamine (DIPEA) in CH_2Cl_2 afforded compound **3** with protection of hydroxyl group [18], followed by alkylation with appropriate alkyl halides in the existence of potassium carbonate in acetone to produce the *N*-alkylation derivatives **4**–**7** [19]. The remaining *O*-protecting groups were removed in the presence of *tetra*-butylammoniumfluoride (TBAF) in THF to provide the target compounds **8**–**11** [20].

Oxidation of compound **2** with Dess-Martin periodinane (DMP) in CH_2Cl_2 afforded aldehyde **12** which was treated with $NaClO_2$, NaH_2PO_4, and 2-methyl-2-butene in *tert*-butyl alcohol (*t*-BuOH)/H_2O (1.5/1) at room temperature to yield carboxylic acid **13** [21]. The presence of free hydroxyl group allowed us to prepare ester derivatives of compound **2** in order to evaluate the influence of ester side chain on their anti-HBV activities. Acylation of compound **2** with various carboxylic acids, anhydrides, and acyl chlorides afforded products **16**–**53** (Scheme 3).

Bioisosteric replacement is a valuable approach in drug design and can produce compounds with similar biological activity. In order to convert hydroxyethyl group to aminoethyl moiety, reaction of compound **2** with methanesulfonyl chloride in the presence of triethylamine in CH_2Cl_2 did not give methanesulfonylate intermidate but



Scheme 1. Synthesis of compound **2**.

afforded derivative **14**. Compound **2** was refluxed with Lawesson's reagent (LR) in toluene to give compound **15**(Scheme 3) [14].

To optimize chain length between 6-chloro-4-(2-chlorophenyl) quinolin-2(1*H*)-one and ester moieties, compounds **54** and **55** were prepared by reaction of 2-amino-benzophenone with *beta*-propiolatone and *delta*-valerolactone, and then converted to corresponding esters **56** and **57**, respectively (Scheme 4).

3. Results and discussion

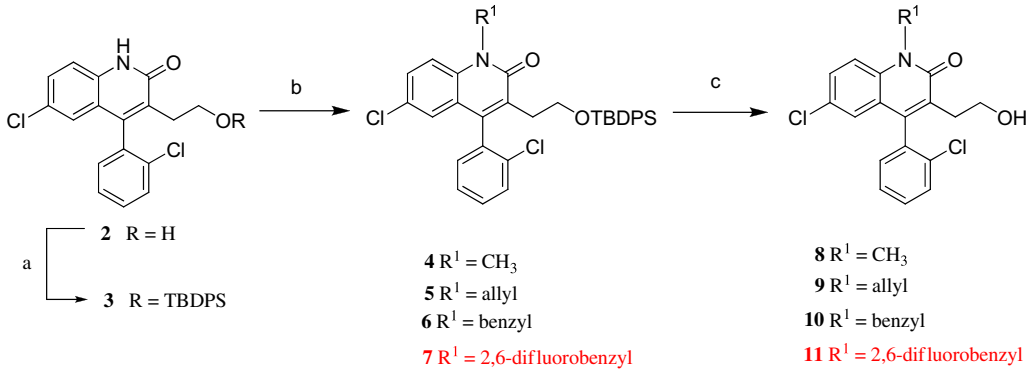
The synthesized 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one analogues (**2**, **8**–**53**, **56**, and **57**) were screened for their anti-HBV activities, namely the abilities to inhibit the secretion of HBsAg and HBeAg in HBV-infected 2.2.15 cells using lamivudine (3TC, an anti-HBV therapeutic agent) as a positive control. The potent active compounds **42**–**49**, **56** and **57** were further evaluated for their activities against HBV DNA replication. The anti-HBV activity of each compound was expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) to the secretion of HBsAg and HBeAg. The cytotoxicity of each compound was expressed as the concentration of compound required to kill 50% (CC_{50}) of the HepG 2.2.15 cells. The selectivity index (SI), a major pharmaceutical parameter that estimates possible future clinical development, was determined as the ratio of CC_{50} to IC_{50}. The results were summarized in Tables 1–3.

Compound **2** showed inhibitory potency to the secretion of HBsAg (IC_{50} = 0.086 mM) and HBeAg (IC_{50} = 0.36 mM), but appeared toxic (CC_{50} = 0.38 mM), which led to relatively low SI values (SI_{HBsAg} = 4.4, SI_{HBeAg} = 1.0). The effects of introducing different patterns of substituents to the *N*-1 position of compound **2** were studied by replacement of *N*-1 position with methyl, alkyl, benzyl, or 2,6-difluorobenzyl groups to yield the derivatives **8**–**11**. As shown in Table 1, compounds **8**–**10** enhanced anti-HBV activities on the secretion of HBsAg and HBeAg, whereas increased the cytotoxicities, resulting in relatively small SI values compared with compound **2**. Introducing 2,6-difluorobenzyl to the *N*-1 position of the 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one core (**11**) retained potency to the secretion of HBsAg and led to the decreased cytotoxicity (CC_{50} > 2.3 mM). However, compound **11** lost suppressant property on the secretion of HBeAg.

As shown in Table 2, compound **2** was oxidated to aldehyde **12** which retained the activity against secretion of HBsAg and lost the suppressant property on the secretion of HBeAg. Further oxidation of aldehyde **12** gave carboxylic acid **13**, leading to decreased inhibition to the secretion of HBsAg (IC_{50} = 0.35 mM) relative to compound **2** (IC_{50} = 0.086 mM). Compounds **14**–**15** lost the activities against the secretion of HBsAg and HBeAg.

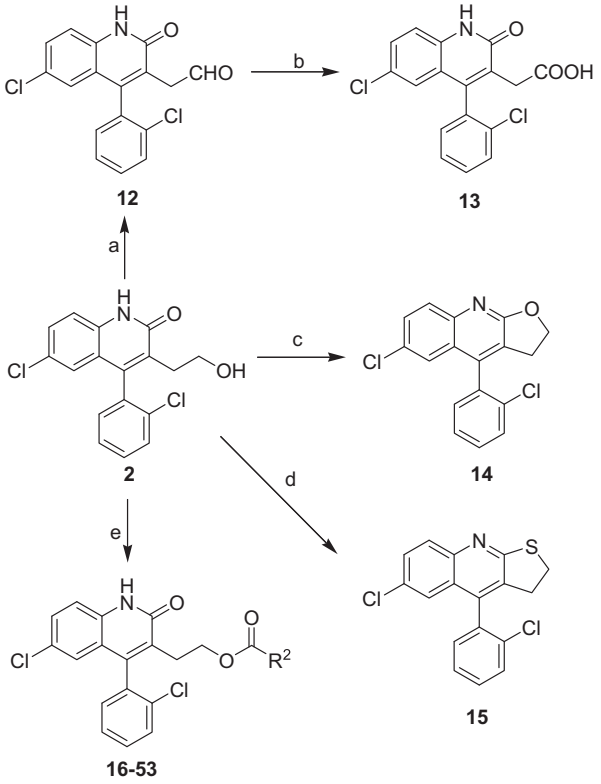
The subseries of derivatives **16**–**41** has different patterns of substitution on hydroxyl group. These compounds generally exhibited good potency to the secretion of HBsAg, except that compounds **31**, **34**, and **36** lost suppressant potency to the secretion of HBsAg. This result indicated that a range of acyls with different lipophilic, electronic, and steric characters was tolerated at hydroxyethyl moiety for their suppressant potency to the secretion of HBsAg. Compounds **17**, **26**, **28**, **29**, **35**, **37**, **40**, and **41** also showed activity to the secretion of HBeAg. It is worth noting that compound **41** exhibited 2.9-fold and 6.4-fold increased activities against the secretion of HBsAg (IC_{50} = 0.030 mM vs IC_{50} = 0.086 mM) and HBeAg (IC_{50} = 0.056 mM vs IC_{50} = 0.36 mM) comparing with compound **2**, which suggested that cinnamylation of hydroxyl seemed important for its high potency against the secretion of HBsAg and HBeAg. Thus, compound **41** was selected as the benchmark compound for subsequent optimization.

To extend this result, different patterns of substitutions were introduced to the phenyl ring of ester moiety. As shown in Table 3,



Scheme 2. Synthesis of compounds **3**–**11**. Reagents and conditions: (a) TBDPSCl, DIPEA, CH_2Cl_2, r.t.; (b)MeI or R^1Br, K_2CO_3, acetone, r.t.; (c) TBAF, THF, r.t.

compounds **42**–**49** demonstrated potent activities against the secretion of HBsAg and HBeAg, and low cytotoxicities, which indicated that different types of substituents could be present at positions 2(OCH_3), 3(OCH_3, F, CF_3), 4(OCH_3, CF_3), and 3,4(methylenedioxyl, dimethoxyl) without substantially affecting potency to inhibit the secretion of HBsAg and HBeAg. These changes were typified by the 3′-methoxyl substituted compound **44** with high potency against the secretion of HBsAg (IC_{50} = 0.010 mM), HBeAg (IC_{50} = 0.026 mM), low cytotoxicity (CC_{50} > 1.3 mM), resulting in remarkable SI values (SI_{HBsAg} > 135, SI_{HBsAg} > 51). It was observed that the addition of a nitrogen to 3′-position of the phenyl ring, i.e., on moving from compound **41** to pyridine **50**, retained suppressant potency to the secretion of HBsAg (IC_{50} = 0.034 mM) and led to the loss of activity against the secretion of HBeAg. However, it also appeared toxic (CC_{50} = 0.039 mM), resulting in a relatively low SI value (SI_{HBsAg} = 1.1). Replacement of phenyl moiety with methyl group afforded compound **55**, which showed high potency to the secretion of HBsAg (IC_{50} = 0.025 mM) and HBeAg (IC_{50} = 0.022 mM) but high cytotoxicity (CC_{50} = 0.030 mM). Addition of a methyl group to 2′-position of compound **51** resulted in compound **53**, which demonstrated low cytotoxicity (CC_{50} = 0.35 mM) and slightly decreased activities against the secretion of HBsAg (IC_{50} = 0.058 mM) and HBeAg (IC_{50} = 0.062 mM) comparing with compound **51**. The increase of the number of C=C bond compound **52** showed similar activity and lower cytotoxicity (CC_{50} = 0.44 mM) relative to compound **51**.



Scheme 3. Synthesis of compounds **12**–**53**. Reagents and conditions: (a) DMP, CH_2Cl_2, r.t.; (b) $NaClO_2$,NaH_2PO_4, ^tBuOH, H_2O, r.t.; (c) methanesulfonyl chloride, triethylamine, CH_2Cl_2, 0°C; (d) LR, toluene,reflux; (e) R^2COOH, DMAP, DCC, CH_2Cl_2; or $(R^2CO)_2O$, DMAP (cat) , pyridine, 95°C; or R^2COCl,pyridine, r.t.

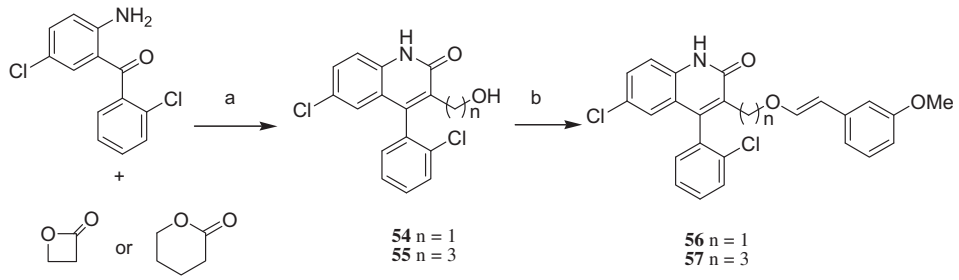
To identify the optimal chain length between the 6-chloro-4-(2-chlorophenyl)quinolin-2(1*H*)-one and ester moieties, different alkyl linkers of variable length were introduced between the 6-chloro-4-(2-chlorophenyl) quinolin-2(1*H*)-one and ester, producing the three derivatives **44**, **56**, and **57**. Compounds **44**, **56**, and **57** showed similar activities against the secretion of HBsAg (IC_{50} = 0.010–0.020 mM) and HBeAg (IC_{50} = 0.018–0.028 mM) and cytotoxicities (CC_{50} > 1.1 mM), which suggested that alkyl linkers of length (n = 1, 2, 3) had little effect on activities against the secretion of HBsAg and HBeAg.

Importantly, the most active compounds **42**–**49**, **56**, and **57** with high activities against HBsAg and HBeAg were selected to investigate inhibition of HBV DNA replication using lamivudine as the reference drug. Compounds **42**, **44**, and **46** exhibited anti-HBV activity with their IC_{50} values against HBV DNA replication of 0.11, 0.045, 0.41 mM, respectively. This result suggested that mono-substituted at 3′-position phenyl ring was an important feature in conferring potent activity against HBV DNA replication. For different chain length between 6-chloro-4-(2-chlorophenyl)quinolin-2(1*H*)-one and ester moieties compounds **44**, **56**, and **57**, the ethylene analogue **44** (IC_{50} = 0.045 mM) showed about four times more potent than that of the corresponding methane analogue **56** (IC_{50} = 0.16 mM), whereas propylene analogue **57** lost inhibitory activity. These findings proposed that ethylene linker between 6-chloro-4-(2-chlorophenyl) quinolin-2(1*H*)-one and ester moieties was preferred for potent anti-HBV DNA activity.

The activity for compound **44** (0.02 mM) in the luciferase reporter assay [22–24] is shown in Fig. 2 (Cells viability were 95.25% and 80.40% by MTT assay at 48 and 72 h, respectively). Compound **44** could enhance the transcript activity of HBV enhancers ENI up to 12-fold and ENII up to 14-fold, as well as core/pregenomic, X, preS1 promoter, approximately 4-fold more potent compared with mock-treated control, however, slightly inhibit preS2 promoter activity.

4. Conclusions

In summary, a series of novel 6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1*H*)-one analogues based on compound **2** were synthesized and tested for their in vitro anti-HBV activity. Most



Scheme 4. Synthesis of compounds **54**–**57**. Reagents and conditions: (a) LiHMDS, THF, 0°C; (b) 3-Methoxycinnanic acid, DCC, DMAP, CH_2Cl_2, r.t.

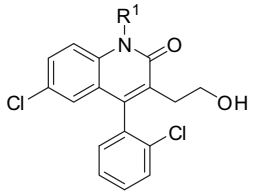
of the compounds exhibited suppressant property on the secretion of HBsAg. Interestingly, compounds **42**, **44**, **46**, **48**, and **56** displayed optimal profiles inhibiting not only HBsAg and HBeAg secretion but also HBV DNA replication, however, 3TC showed significantly activity against HBV DNA replication. Preliminary mechanism study suggested that compound **44** mainly promote the transcript activity of HBV enhancers ENI and ENII to exert anti-HBV action.

5. Experimental

5.1. Chemistry

MS spectra were run on a VG Auto Spec-3000 spectrometer (VG, Manchester, England); NMR spectra were recorded on Bruker AM 400 ($^1H/^{13}C$, 400 MHz/100 MHz) or DRX-500 ($^1H/^{13}C$, 500 MHz/125 MHz) spectrometer (Bruker, Bremerhaven, Germany) and chemical shifts were given in δ with TMS as internal reference; column chromatography (CC): silica gel (200–300 mesh; Qingdao Makall Group Co., Ltd; Qingdao; China); All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates.

Table 1
Anti-HBV activity, cytotoxicity, and selectivity index of analogues (**2**, **8**–**11**) in vitro.[a]



Compd	R^1	CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
			IC_{50}[e](mM)	SI[f]	IC_{50} (mM)	SI
2	H	0.38	0.086	4.4	0.36	1.0
8	CH_3	0.034	0.022	1.6	0.034	1.0
9		0.023	0.010	2.3	0.012	1.9
10		0.026	0.012	2.2	0.012	2.2
11		>2.3	0.087	>2.6	>2.3	–
3TC[g]	–	30	27	1.1	30	1.0

[a] All values are the mean of two independent experiments, standard deviation values <10% in all cases.
[b] CC_{50}: 50% cytotoxic concentration.
[c] HBsAg: HBV surface antigen.
[d] HBeAg: HBV e antigen.
[e] IC_{50}: 50% inhibitory concentration.
[f] SI (selectivity index) = CC_{50}/IC_{50}.
[g] 3TC: Lamivudine, an antiviral agent used as positive control.

5.1.1. 6-Chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl)quinolin-2(1H)-one (**2**) [12]

A 100 mL, 2-neck flask was charged with 2-amino-2′,5-dichlorobenzo-phenone (3.76 mmol, 1.0 g) and THF (20 mL). The resulting solution was cooled to 0 °C and LiHMDS 28.2 mL (1 M in THF) was added over 15 min. The internal temperature was controlled 0 °C for 10 min, then γ-valerolactone (16.9 mmol, 1.7 mg) was added over 10 min. The reaction solution was allowed to warm up to room temperature and stirred at room temperature for 2 h. Then water (10.2 mL) was added over 10 min, and the reaction mixture was stirred at room temperature for 24 h. The aqueous layer was extracted with ethyl acetate (100 mL) and the organic solution was washed with water (80 mL) and brine (3 × 50 mL) and dried anhydrous Na_2SO_4. Removal of the solvents gave a residue which was purified by column chromatography on silica gel with $CHCl_3/CH_3OH$ (98:2) to give white amorphous powder **2**, yield 86%; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.16 (1H, br.s), 7.68 (1H, d, J = 1.6, 5.2 Hz), 7.59–7.49 (3H, m), 7.41–7.36 (2H, m), 6.56 (1H, s), 4.61 (1H, br.s), 3.43 (2H, t, J = 7.2 Hz), 2.56–2.48 (1H, m), 2.31–2.29 (1H, m); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.6, 144.5, 136.4, 134.1, 131.9, 131.2, 130.7, 130.5, 129.8, 129.7, 127.9, 125.9, 124.3, 120.4, 117.4, 58.8, 32.3; ESIMS: m/z 334 $[M + H]^+$, HRESIMS: calc for $C_{17}H_{14}NO_2Cl_2$ $[M + H]^+$ 334.0401, found 334.0409.

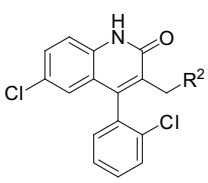
5.1.2. 3-[2-(tert-Butyldiphenylsilyloxy)ethyl]-6-chloro-4-(2-chlorophenyl)quinolin-2(1H)-one (**3**) [18]

Compound **2** (909 mg, 2.7 mmol) was dissolved in 15 mL of DMF, stirred and cooled to 0 °C in an ice bath. DIPEA and TBDPSCl (1.5 g, 5.4 mmol) were added to the solution and the resulting mixture was stirred for 12 h at room temperature. The mixture was quenched with water. The mixture was extracted with EtOAc. The organic layer was washed with 5% HCl, brine, dried over $MgSO_4$, filtered and concentrated. Purification by chromatography on silica gel (elution with $CHCl_3$/MeOH, 70:1) afforded the white amorphous power, yield 86%; ^1H NMR ($CDCl_3$, 400 MHz) δ 13.1 (1H, br.s), 7.57 (5H, m), 7.50–7.18 (11H, m), 6.85 (1H, s), 4.10–4.04 (1H, m), 3.98–3.92 (1H, m), 3.07–3.00 (1H, m), 2.79–2.72 (1H, m), 1.04 (9H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.1, 146.6, 135.9, 135.42(2C), 135.40 (2C), 134.4, 133.9 (2C), 133.8, 132.9, 131.1, 130.0, 129.99 (2C), 129.96 (2C), 129.4, 127.8, 127.7, 127.51(2C), 127.49 (2C), 127.1, 125.3, 117.5, 62.1, 32.0, 27.0 (3C), 19.3; ESIMS: m/z 572 $[M + H]^+$, HRESIMS: calc for $C_{33}H_{32}NO_2SiCl_2$ $[M + H]^+$ 572.1579, found 572.1585.

5.1.3. General procedure for the preparation of intermediates **4**–**7** [19]

To a solution of the compound **2** in acetone were added K_2CO_3 (1.5 equiv mol), and alkyl halides (1.5 equiv mol) at room

Table 2
Anti-HBV activity, cytotoxicity, and selectivity index of analogues (**12**–**41**) in vitro.[a]



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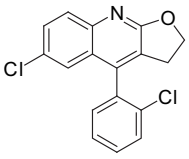

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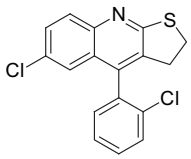

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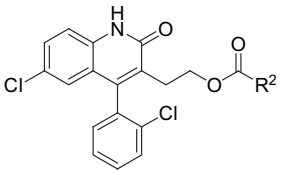

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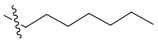
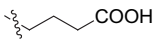
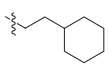
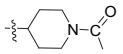
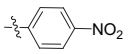
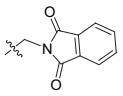
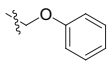
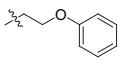
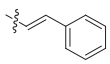
Compd	R^2	CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
			IC_{50}[e](mM)	SI[f]	IC_{50} (mM)	SI
2	–	0.38	0.086	4.4	0.36	1.0
12	CHO	0.12	0.076	1.6	>0.12	<1
13	COOH	0.95	0.35	2.8	>0.95	<1
14	–	>0.28	>0.28	–	>0.28	–
15	–	>2.4	>2.4	–	>2.4	–
16	CH_3	0.027	0.074	<1	>0.027	<1
17		0.095	0.059	1.6	0.062	1.5
18		0.062	0.037	1.7	>0.062	<1
19		0.052	0.035	1.5	>0.052	<1
20		0.20	0.082	2.4	>0.20	<1
21		0.078	0.040	1.9	>0.078	<1
22		0.11	0.15	<1	>0.11	<1
23		0.10	0.075	1.3	>0.10	<1
24		0.074	0.032	1.4	>0.074	<1
25		0.13	0.095	1.4	>0.13	<1
26		0.10	0.057	1.8	0.071	1.4
27		0.11	0.074	1.4	>0.11	<1
28		0.046	0.046	1.0	0.046	1.0
29		0.038	0.027	1.4	0.034	1.1
30		0.16	0.085	1.9	0.11	1.5

(*continued on next page*)

Table 2 (*continued*)

Compd	R^2	CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
			IC_{50}[e](mM)	SI[f]	IC_{50} (mM)	SI
31		>1.8	1.5	>1.2	>1.8	–
32		0.082	0.051	1.6	>0.082	<1
33		0.043	0.032	1.42	>0.043	<1
34		1.6	1.2	1.4	1.6	1.0
35		0. 17	0.055	3.0	0.055	3.0
36		>1.7	>1.7	–	>1.7	–
37		0.098	0.051	1.9	0.058	1.6
38		0.96	0.17	5.7	>0.96	<1
39		0.081	0.056	1.5	>0.081	<1
40		0.046	0.033	1.4	0.035	1.3
41		0.23	0.030	7.6	0.056	4.0
3TC	–	30	27	1.1	30	1.0

[a] All values are the mean of two independent experiments, standard deviation values <10% in all cases.
[b] CC_{50}: 50% cytotoxic concentration.
[c] HBsAg: HBV surface antigen.
[d] HBeAg: HBV e antigen.
[e] IC_{50}: 50% inhibitory concentration.
[f] SI (selectivity index) = CC_{50}/IC_{50}.

temperature. The resulting mixture was stirred at room temperature until the starting material disappeared on the TLC. The reaction mixture was filtered, concentrated under reduced pressure to obtain the intermediates (**4**–**7**), which were used in the next step without further purification.

5.1.4. General procedure for the preparation of derivatives **8**–**11** [20]

To a solution of compounds (**4**–**7**) in THF at room temperature was added TBAF (2 equiv mol) in one portion. After stirring at room temperature for 12 h, the solvent was evaporated under reduced pressure and the residue was purified by chromatography to give the products (**8**–**11**).

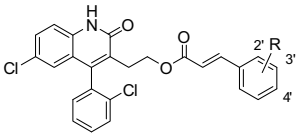
5.1.4.1. 6-Chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl)-1-methylquinolin-2(1H)-one (**8**).

White amorphous power, two step yield 78% (after chromatography with petroleum ether/acetone, 90:10); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.56 (1H, d, J = 9.0 Hz), 7.57 (5H, m), 6.87 (1H, d, J = 2.0 Hz), 3.82–3.76 (3H, m), 3.69–3.67 (2H, m), 2.82–2.80 (1H, m), 2.61–2.57 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.6, 144.6, 137.2, 134.2, 131.5, 131.0, 130.6, 130.3, 130.2, 130.1, 128.2, 127.4, 126.5, 122.0, 115.7, 62.4, 32.8, 30.4; ESIMS: m/z 348 $[M + H]^+$, HRESIMS: calc for $C_{18}H_{16}NO_2Cl_2$ $[M + H]^+$ 348.0558, found 348.0561.

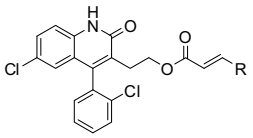
5.1.4.2. 1-Allyl-6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl)quinolin-2(1H)-one (**9**).

White amorphous power, two step yield 61%

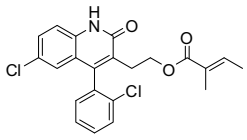
Table 3
Anti-HBV activity, cytotoxicity, and selectivity index of analogues (**41**–**53**, **56** and **57**) in vitro.[a]



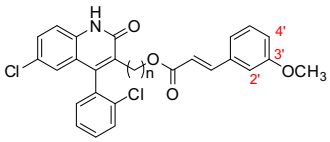
41-49



50-52



53



56, 57

Compd	n	$R^{2'}$	$R^{3'}$	$R^{4'}$	R	CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]		HBV DNA
							IC_{50}[e](mM)	SI[f]	IC_{50} (mM)	SI	IC_{50}[g] (mM)
41	–	H	H	H	–	0.23	0.030	7.6	0.056	4.0	ND[h]
42	–	H	F	H	–	>1.6	0.033	>48	0.027	>58	0.11
43	–	OCH_3	H	H	–	0.77	0.061	13	0.024	32	NA[i]
44	–	H	OCH_3	H	–	>1.3	0.010	>135	0.026	>51	0.045
45	–	H	H	OCH_3	–	>1.2	0.49	>2.5	0.048	>25	NA
46	–	H	CF_3	H	–	>1.4	0.058	>24	0.022	>63	0.41
47	–	H	H	CF_3	–	>1.4	0. 23	>6.1	0.019	>73	NA
48	–	H	OCH_2O		–	–	0.15	>8.1	0.22	>5.4	0.16
49	–	H	OCH_3	OCH_3	–	>1.2	0.021	>58	0.057	>21	NA
50	–	–	–	–		0.039	0.034	1.1	2.2	<1	ND
51	–	–	–	–	CH_3	0.030	0.025	1.2	0.022	1.3	ND
52	–	–	–	–		0.44	0.023	19	0.026	17	ND
53	–	–	–	–	–	0.35	0.058	6.1	0.062	5.6	ND
56	1	–	–	–	–	>1.8	0.011	>170	0.018	>100	0.16
57	3	–	–	–	–	>1.1	0.020	>55	0.028	>39	NA
3TC						30	27	1.1	30	1.0	0.0012

[a] All values are the mean of two independent experiments, standard deviation values <10% in all cases.
[b] CC_{50}: 50% cytotoxic concentration.
[c] HBsAg: HBV surface antigen.
[d] HBeAg: HBV e antigen.
[e] IC_{50}: 50% inhibitory concentration.
[f] SI (selectivity index) = CC_{50}/IC_{50}.
[g] Concentrations of compounds achieving 50% inhibition of cytoplasmic HBV DNA replication.
[h] Not determined.
[i] Not active.

(after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56 (1H, dd, J = 7.6, 2.0 Hz), 7.47–7.31 (4H, m), 7.26–7.20 (1H, m), 6.87 (1H, d, J = 2.4 Hz), 6.03–5.94 (1H, m), 5.29–4.99 (4H, m), 3.79–3.73 (1H, m), 3.68–3.63 (1H, m), 2.84–2.78 (1H, m), 2.61–2.55 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 144.9, 136.4, 134.2, 132.9, 131.3, 131.2, 130.5, 130.3, 130.1, 130.0, 128.1, 127.3, 126.3, 122.0, 117.6, 116.3, 62.2, 45.6, 32.8; ESIMS: m/z 396 $[M + Na]^+$, HRESIMS: calc for $C_{20}H_{18}NO_2Cl_2$ $[M + H]^+$ 374.0714, found 374.0705.

*5.1.4.3. 1-Benzyl-6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl) quinolin-2(1H)-one (**10**).* White amorphous power, two step yield 72% (after chromatography with $CHCl_3$/MeOH, 97:3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.59 (1H, dd, J = 7.2, 2.0 Hz), 7.50–7.41 (2H, m), 7.38–7.23 (8H, m), 6.88 (1H, d, J = 2.4 Hz), 5.70 (1H, d, J = 15.6 Hz), 5.56 (1H, d, J = 15.6 Hz), 3.85–3.89 (1H, m), 3.74–3.68 (1H, m), 2.91–2.85 (1H, m), 2.70–2.61 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 145.1, 136.5, 135.6, 134.2, 132.9, 131.4, 130.5, 130.3, 130.2 (2C), 128.9 (2C), 128.2, 127.5, 127.4, 126.4 (2C), 126.3, 122.2, 116.5, 62.4, 47.0, 32.8; ESIMS: m/z 424 $[M + H]^+$, HRESIMS: calc for $C_{24}H_{20}NO_2Cl_2$ $[M + H]^+$ 424.0871, found 424.0876.

*5.1.4.4. 1-(2,6-Difluorobenzyl)-6-chloro-4-(2-chlorophenyl)-3-(2-hydroxyethyl)quinolin-2(1H)-one (**11**).* White amorphous power, two step yield 75% (after chromatography with $CHCl_3$/MeOH, 97:3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (1H, dd, J = 8.8, 1.6 Hz), 7.49–7.23 (6H, m), 6.91–6.86 (3H, m), 5.85 (1H, d, J = 16.0 Hz), 5.72 (1H, d, J = 16.0 Hz), 3.70 (2H, m), 2.89–2.83 (1H, m), 2.65–2.59 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.5, 162.4, 160.0, 144.9, 136.1, 134.2, 132.9, 131.2, 130.5, 130.2, 130.1, 130.0, 129.6, 128.1, 127.3, 126.4, 122.3, 115.5, 111.9, 111.8, 111.6, 62.2, 36.0, 32.8; ESIMS: m/z 482 $[M + Na]^+$, HRESIMS: calc for $C_{24}H_{18}NO_2F_2Cl_2$ $[M + H]^+$ 460.0682, found 460.0679.

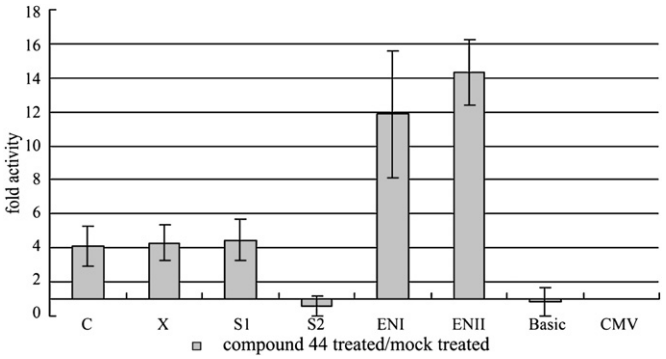


Fig. 2. Normalized fold change in HBV promoter activity. Normalized fold change in HBV four promoters C: the core promoter, X: X promote, S1: S1 promoter and S2: S2 promoter, two enhancer elements (En I: enhancer I, En II: enhancer II) activity. HepG 2 cells were transfected with a constant amount of pGL3 Vector (expressing firefly luciferase) as Basic Control, phRL-CMV vector (expressing Renilla luciferase) as internal control. Firefly and Renilla luciferase activities were assayed using the Dual-Luciferase® Reporter Assay System. Data represent the average ± standard deviation of triplicate samples. Normalized fold change in activity between test groups: Fold activity = compound 44 (0.02 mM) treated group promoter activity/mock-treated group promoter activity.

*5.1.5. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]acetaldehyde (**12**)*

To a solution of compound **2** (50 mg, 0.15 mmol) in dry CH_2Cl_2 (10 mL) was added Dess-Martin periodinane (DMP, 0.3 mmol, 15 wt % in CH_2Cl_2) at room temperature. After 2.5 h, the reaction mixture was quenched with a 1:1 mixture of saturated $Na_2S_2O_3$ (50 mL) and $NaHCO_3$ (50 mL). The resulting mixture was extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, and chromatographed on silica gel eluting with petroleum ether/acetone (4:1) to give 60% yield of compound **12** (30 mg). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.73 (1H, s), 7.59 (1H, d, J = 8.0 Hz), 7.51–7.41 (4H, m), 7.22 (1H, dd, J = 7.4, 1.5 Hz), 6.92 (1H, d, J = 2.1 Hz), 3.70 (1H, d, J = 17.2 Hz), 3.32 (1H, d, J = 17.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.0, 163.4, 148.2, 136.1, 133.7, 132.6, 130.9, 130.7, 130.4, 130.2, 128.4, 127.5, 125.70, 125.66, 121.0, 117.8, 43.0; ESIMS: m/z 332 $[M + H]^+$, HRESIMS: calc for $C_{17}H_{12}NO_2Cl_2$ $[M + H]^+$ 332.0245, found 332.0253.

*5.1.6. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]acetic acid (**13**)*

Compound 13 was prepared according to the literature [18] as white amorphous power, yield 72%. ^{1}H NMR (C_5D_5N, 400 MHz) δ 8.71 (1H, s), 7.74–7.62 (5H, m), 7.09 (1H, d, J = 2.2 Hz), 4.17 (1H, d, J = 16.3 Hz), 3.65 (1H, d, J = 16.3 Hz); ^{13}C NMR (C_5D_5N, 100 MHz) δ 173.7, 163.2, 146.3, 138.0, 135.1, 133.3, 131.7, 131.4, 130.82, 130.78, 129.8, 128.5, 127.7, 126.0, 121.7, 118.4, 35.7; EIMS: m/z 347 $[M]^+$, HRESIMS: calc for $C_{17}H_{12}NO_3Cl_2$ $[M + H]^+$ 348.0194, found 348.0195.

*5.1.7. 6-Chloro-4-(2-chlorophenyl)-2,3-dihydrofuro[2,3-b] quinoline (**14**)*

Methanesulfonyl chloride (372 mg, 3.3 mmol) was added dropwise to a solution of compound **2** (300 mg, 0.9 mmol) and Et_3N (109 mg, 1.1 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction was warmed to room temperature and stirred 4 h. The reaction was then quenched with 5% HCl and extracted with EtOAc. The combined extracts were washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure. Chromatography of residue on silica gel (petroleum ether/acetone, 20:1) give 124 mg (44%), yellow amorphous power, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (1H, d, J = 8.8 Hz), 7.47 (1H, dd, J = 5.6, 1.6 Hz), 7.40–7.31 (3H, m), 7.72 (1H, dd, J = 7.2, 2.4 Hz), 7.11 (1H, d, J = 2.0 Hz), 4.58 (2H, m), 3.11 (1H, m), 2.95 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2, 145.6, 141.6, 133.8, 132.7, 130.4, 130.2, 130.1, 129.6, 129.1, 127.2, 125.1, 124.0, 122.1, 120.7, 69.2, 27.3; ESIMS: m/z 316 $[M + H]^+$, HRESIMS: calc for $C_{17}H_{12}NOCl_2$ $[M + H]^+$ 316.0295, found 316.0304.

*5.1.8. 6-Chloro-4-(2-chlorophenyl)-2,3-dihydrothieno[2,3-b] quinoline (**15**) [14]*

A solution of compound **2** (50 mg, 0.15 mmol), Lawesson's reagent (121 mg, 0.3 mmol) in toluene (5 mL) was heated at refluxed temperature for 2 h. The reaction mixture was concentrated under reduced pressure to give a residue which was purified by silica gel column chromatography with petroleum ether/acetone (100:1) to afford compound **15** (39 mg), yield 81%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.90 (1H, d, J = 8.8 Hz), 7.59–7.42 (4H, m), 7.23 (1H, dd, J = 7.3, 1.8 Hz), 7.17 (1H, d, J = 2.2 Hz), 3.48–3.40 (2H, m), 3.25–3.19 (1H, m), 3.13–3.06 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ168.1, 146.0, 138.9, 134.5, 133.3, 132.9, 131.3, 130.4, 130.3, 130.2, 130.0, 128.9, 127.3, 125.5, 124.2, 32.5, 30.6; ESIMS: m/z 332 $[M + H]^+$, HRESIMS: calc for $C_{17}H_{12}NSCl_2$ $[M + H]^+$ 332.0067, found 332.0060.

*5.1.9. General procedure for the preparation of derivatives **16**, **18**, **22** and **26***

To a solution of compound **2**, 4-dimethylaminopyridine (DMAP, 0.2 equiv mol), and an appropriate anhydride (1.2 equiv mol) in anhydrous pyridine (5–10 mL), was heated overnight at 95 °C until the starting material was not observed by TLC. The reaction mixture was diluted with 50 mL of EtOAc and washed three times with 50 mL of 5% HCl and and saturated $NaHCO_3$ (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed using a silica gel column to afford the products.

*5.1.9.1. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylacetate (**16**).* White amorphous power, yield 95% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.13 (1H, br.s), 7.59 (1H, dd, J = 9.5, 2.0 Hz), 7.46 (4H, m), 7.25 (1H, m), 6.86 (1H, d, J = 1.8 Hz), 4.30 (2H, t, J = 6.8 Hz), 2.95 (1H, m), 2.67 (1H, m), 1.97 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8, 164.0, 147.1, 136.0, 134.2, 133.0, 130.7, 130.4, 130.3, 130.1, 129.1, 128.1, 127.2, 125.5, 121.1, 117.6, 62.3, 28.1, 21.1; FABMS: m/z 376 $[M + H]^+$, HRESIMS: calc for $C_{19}H_{16}NO_3Cl_2$ $[M + H]^+$ 376.0507, found 376.0497.

*5.1.9.2. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylisobutyrate (**18**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (1H, d, J = 7.6 Hz), 7.51–7.43 (4H, m), 7.25 (1H, dd, J = 1.7, 6.4 Hz), 6.86 (1H, d, J = 2.7 Hz), 4.31 (2H, t, J = 7.2 Hz), 2.99–2.94 (1H, m), 2.71–2.65 (1H, m), 2.49–2.44 (1H, m), 1.83 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.9, 163.9, 147.0, 136.0, 134.2, 132.9, 130.7, 130.4, 130.3, 130.1, 129.1, 128.0, 127.2, 125.4, 121.1, 117.7, 62.1, 33.8, 28.2, 18.9 (2C); ESIMS: m/z 426 $[M + Na]^+$, HRESIMS: calc for $C_{21}H_{19}NO_3Cl_2Na$ $[M + Na]^+$ 426.0627, found 426.0639.

*5.1.9.3. 4-{2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethoxy}-4-oxobutanoic acid (**22**).* White amorphous power, yield 68% (after chromatography with $CHCl_3$/MeOH, 98:2), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58 (1H, d, J = 7.6 Hz), 7.57–7.45 (4H, m), 7.23 (1H, d, J = 6.8 Hz), 6.86 (1H, s), 4.22 (1H, t, J = 6.0 Hz), 2.92–2.88 (1H, m), 2.66–2.63 (1H, m), 2.40–2.33 (4H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.0, 172.9, 163.9, 147.6, 135.4, 133.9, 132.8, 130.6, 130.6, 130.5, 130.2, 128.9, 128.5, 127.3, 125.5, 121.2, 117.7, 62.3, 33.4, 33.2, 27.8, 18.9; ESIMS: m/z 470 $[M + Na]^+$, HRESIMS: calc for $C_{22}H_{20}NO_5Cl_2$ $[M + H]^+$ 448.0718, found 448.0731.

*5.1.9.4. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-2-acetoxyacetate (**26**).* White amorphous power, yield 69% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.00 (1H, br.s), 7.61 (1H, d, J = 8.0 Hz), 7.51–7.46 (4H, m), 7.25 (1H, dd, J = 7.0, 2.0 Hz), 6.87 (1H, s), 4.54 (2H, s), 4.40 (2H, t, J = 7.0 Hz), 2.99–2.94 (1H, m), 2.76–2.70 (1H, m), 2.11 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 167.6, 163.7, 147.5, 135.9, 134.0, 132.9, 130.7, 130.5, 130.5, 130.2, 128.5, 128.3, 127.3, 125.6, 121.1, 117.7, 63.1, 60.6, 28.0, 20.4; ESIMS: m/z 434 $[M + H]^+$, HRESIMS: calc for $C_{21}H_{18}NO_5Cl_2$ $[M + H]^+$ 434.0562, found 434.0557.

*5.1.10. General procedure for the preparation of derivatives **17**, **19**–**21**, **23**–**25**, **27**–**53***

To a solution of compound **2** in acetone, an appropriate carboxylic acid (1.2 equiv mol), and 4-dimethylaminopyridine (DMAP, 0.2 equiv mol) in anhydrous CH_2Cl_2 was added *N*′,*N*′-dicyclohexylcarbodiimide (DCC, 1.2 equiv mol) at 0 °C. The resulting mixture was stirred at room temperature overnight until the starting material disappeared on the TLC. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (3 × 50 mL). The CH_2Cl_2 solution was washed with saturated $NaHCO_3$ (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and chromatographed over silica gel to afford the products.

*5.1.10.1. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylpropionate (**17**).* White amorphous power, yield 90% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58 (1H, dd, J = 7.6, 1.6 Hz), 7.48 (4H, m), 7.26 (1H, dd, J = 7.2, 2.0 Hz), 6.86 (1H, d, J = 2.1 Hz), 4.32 (2H, t, J = 6.8 Hz), 2.97 (1H, m), 2.69 (1H, m), 2.45 (2H, m), 1.07 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2, 164.0, 147.0, 136.0, 134.1, 133.0, 130.7, 130.4, 130.3, 130.1, 129.1, 128.0, 127.1, 125.4, 121.0, 117.6, 62.1, 28.2, 27.4, 9.0; FABMS: m/z 390 $[M + H]^+$, HRESIMS: calc for $C_{20}H_{18}NO_3Cl_2$ $[M + H]^+$ 390.0663, found 390.0657.

*5.1.10.2. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylbutyrate (**19**).* White amorphous power, yield 78% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (1H, d, J = 7.6 Hz), 7.48 (4H, m), 7.26 (1H, d, J = 7.2 Hz), 6.86 (1H, s), 4.27 (2H, t, J = 6.8 Hz), 2.92 (1H, m,), 2.69 (1H, m), 2.22 (2H, t, J = 7.4 Hz), 1.57 (2H, t, J = 7.4 Hz), 0.88 (3H, t, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 163.2, 147.0, 135.8, 134.1, 132.8, 130.6, 130.4, 130.3, 130.1, 129.1, 128.0, 127.2, 125.5, 121.0, 117.3, 62.0, 36.1, 28.1, 18.2, 13.5; FABMS: m/z 404 $[M + H]^+$, HRESIMS: calc for $C_{21}H_{20}NO_3Cl_2$ $[M + H]^+$ 404.0820, found 404.0826.

*5.1.10.3. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylpentanoate (**20**).* White amorphous power, yield 82% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.21 (1H, br.s), 7.60 (1H, d, J = 7.6 Hz), 7.51–7.44 (4H, m), 7.27 (1H, dd, J = 7.2, 2.0 Hz), 6.87 (1H, d, J = 2.0 Hz), 4.31 (2H, t, J = 6.8 Hz), 2.97 (1H, m), 2.67 (1H, m), 2.23 (2H, t, J = 7.6 Hz), 1.53 (2H, m), 1.28 (2H, m), 0.85 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6, 164.0, 147.1, 136.0, 134.2, 133.0, 130.7, 130.4, 130.3, 130.1, 129.1, 128.1, 127.2, 125.5, 121.1, 117.7, 62.0, 34.0, 28.2, 26.9, 22.2, 13.7; ESIMS: m/z 440 $[M + Na]^+$, HRESIMS: calc for $C_{22}H_{22}NO_3Cl_2$ $[M + H]^+$ 418.0976, found 418.0972.

*5.1.10.4. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyloctanoate (**21**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 97:3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.24 (1H, br.s), 7.60 (1H, d, J = 8.0 Hz), 7.51–7.43 (4H, m), 7.27 (1H, d, J = 7.2 Hz), 6.87 (1H, d, J = 2.0 Hz), 4.32 (2H, t, J = 6.8 Hz), 2.97 (1H, m), 2.69 (1H, m), 2.22 (2H, t, J = 7.6 Hz), 1.55 (2H, t, J = 6.8 Hz), 1.27–1.23 (8H, m), 0.85 (3H, t, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6, 164.0, 147.1, 136.0, 134.2, 133.0, 130.7, 130.4, 130.2, 130.1, 129.1, 128.1, 127.2, 125.5, 121.1, 117.7, 62.0, 34.3, 31.6, 29.1, 28.9, 28.2, 24.8, 22.5, 14.0; ESIMS: m/z 460 $[M + H]^+$, HRESIMS: calc for $C_{25}H_{28}NO_3Cl_2$ $[M + H]^+$ 460.1446, found 460.1447.

*5.1.10.5. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylcyanoformate (**23**).* White amorphous power, yield 59% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, J = 6.8 Hz), 7.53–7.47 (4H, m), 7.28 (1H, m), 6.89 (1H, s), 4.45 (2H, m), 3.40 (2H, s), 2.98 (1H, m), 2.78 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 162.8, 147.6, 135.9, 133.9, 132.8, 130.8, 130.6, 130.5, 130.3, 128.4, 128.2, 127.5, 125.6, 121.0, 117.6, 64.7, 27.8, 24.8; ESIMS: m/z 423 $[M + Na]^+$, HRESIMS: calc for $C_{20}H_{15}N_2O_3Cl_2$ $[M + H]^+$ 401.0459, found 401.0464.

*5.1.10.6. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl 2-methoxyacetate (**24**).* White amorphous power, yield 90% (after chromatography with $CHCl_3$/MeOH, 100:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58 (1H, dd, J = 7.6, 1.6 Hz), 7.48 (4H, m), 7.26 (1H, dd, J = 7.2, 2.0 Hz), 6.86 (1H, d, J = 2.1 Hz), 4.32 (2H, t, J = 6.8 Hz), 2.97 (1H, m), 2.69 (1H, m), 2.45 (2H, m), 1.07 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2, 164.0, 147.0, 136.0, 134.1, 133.0, 130.7, 130.4, 130.3, 130.1, 129.1, 128.0, 127.1, 125.4, 121.0, 117.6, 62.1, 28.2, 28.2, 9.0; ESIMS: m/z 428 $[M + Na]^+$, HRESIMS: calc for $C_{20}H_{18}NO_4Cl_2$ $[M + H]^+$ 406.0612, found 406.0616.

*5.1.10.7. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl 2-ethoxyacetate (**25**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 100:1), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.11 (1H, br.s), 7.60 (1H, d, J = 7.4 Hz), 7.48 (4H, m), 7.28 (1H, dd, J = 7.8, 2.0 Hz), 6.87 (1H, s), 4.41 (2H, t, J = 6.6 Hz), 4.01 (2H, s), 3.53 (2H, m), 2.86 (1H, m), 2.71 (1H, m), 1.20 (3H, t, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 163.9, 147.2, 136.0, 134.1, 132.9, 130.7, 130.5, 130.4, 130.2, 128.7, 128.1, 127.3, 125.5, 121.0, 117.6, 68.0, 67.1 62.5, 28.1, 14.9; FABMS: m/z 420 $[M + H]^+$; HRESIMS: calc for $C_{21}H_{19}NO_4Cl_2Na$ $[M + Na]^+$ 442.0588, found 442.0594.

*5.1.10.8. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-2-acetamidoacetate (**27**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 97:3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.63 (1H, br.s), 7.61–7.23 (6H, m), 6.85 (1H, s), 6.48 (1H, s), 4.37 (2H, m), 3.99 (2H, d, J = 4.0 Hz), 2.92 (1H, m), 2.72 (1H, m), 2.0 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 170.3, 163.6, 147.3, 135.8, 134.0, 132.8, 130.6, 130.5, 130.5, 130.2, 128.8, 128.3, 127.4, 125.5, 121.1, 117.5, 63.5, 43.4, 28.0, 22.8; ESIMS: m/z 455 $[M + Na]^+$, HRESIMS: calc for $C_{21}H_{19}N_2O_4Cl_2$ $[M + H]^+$ 433.0721, found 433.0721.

*5.1.10.9. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylcyclopentanecarboxylate (**28**).* White amorphous power, yield 77% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.10 (1H, br.s), 7.60 (1H, dd, J = 7.6, 1.6 Hz), 7.51–7.43 (4H, m), 7.27 (1H, m), 6.86 (1H, d, J = 2.0 Hz), 4.30 (2H, t, J = 7.6 Hz) 3.01–2.94 (1H, m), 2.71–2.64 (1H, m), 1.83–1.50 (9H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.6, 163.9, 147.0, 136.0, 134.2, 132.9, 130.7, 130.4, 130.3, 130.1, 129.2, 128.1, 127.2, 125.5, 121.1, 117.7, 62.1, 43.7, 30.0 (2C), 28.2, 25.8 (2C); ESIMS: m/z 452 $[M + Na]^+$, HRESIMS: calc for $C_{23}H_{22}NO_3Cl_2$ $[M + H]^+$ 430.0976, found 430.0973.

*5.1.10.10. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl cyclohexanecarboxylate (**29**).* White amorphous power, yield 78% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.12 (1H, br.s), 7.60 (1H, d,

J = 8.0 Hz), 7.50–7.44 (4H, m), 7.27 (1H, m), 6.86 (1H, d, J = 2.0 Hz), 4.28 (2H, t, J = 6.8 Hz), 2.97 (1H, m), 2.67 (1H, m), 2.21 (1H, m) 1.83–1.70 (5H, m), 1.39–1.12 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.9, 163.9, 146.0, 136.0, 134.2, 132.9, 130.7, 130.4, 130.2, 130.1, 129.2, 128.0, 127.2, 125.5, 121.1, 117.6, 62.0, 43.1, 28.9 (2C), 28.1, 25.7, 25.4 (2C); ESIMS: m/z 466 $[M + Na]^+$, HRESIMS: calc for $C_{24}H_{24}NO_3Cl_2$ $[M + H]^+$ 444.1133, found 444.1135.

*5.1.10.11. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-cyclohexylpropanoate (**30**).* White amorphous power, yield 80% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.60 (1H, d, J = 8.0 Hz), 7.52–7.42 (4H, m), 7.24 (1H, m), 6.89 (1H, s), 4.23 (2H, t, J = 6.9 Hz), 2.91 (1H, m), 2.64 (1H, m), 2.39 (2H, t, J = 7.8 Hz), 2.21 (2H, t, J = 7.8 Hz), 1.74–1.55 (9H, m), 1.43 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.7, 169.1, 158.9, 143.0, 130.6, 129.1, 128.1, 125.9, 125.7, 125.4, 124.1, 123.9, 122.5, 120.9, 116.6, 112.8, 57.2, 32.4, 28.2, 28.1, 27.4, 27.0, 23.2, 21.7, 21.42, 21.40; ESIMS: m/z 494 $[M + Na]^+$, HRESIMS: calc for $C_{26}H_{27}NO_3Cl_2Na$ $[M + Na]^+$ 494.1265, found 494.1272.

*5.1.10.12. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carboxylate (**31**).* White amorphous power, yield 65% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.9 (1H, br.s), 7.60–7.35 (6H, m), 6.86 (1H, m), 4.57–4.51 (1H, m), 4.41–4.35 (1H, m), 3.03–2.97 (1H, m), 2.76–2.70 (1H, m), 2.41–2.34 (1H, m), 2.00–1.82 (2H, m), 1.67–1.58 (1H, m), 1.07 (3H, s), 0.97 (3H, s), 0.82 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.2, 167.4, 163.7, 147.5, 135.9, 133.9, 132.6, 131.2, 130.9, 130.5, 130.4, 130.0, 129.9, 128.3, 127.6, 121.1, 117.5, 91.0, 63.4, 54.7, 54.2, 30.5, 28.8, 28.1, 16.60, 16.57, 9.6; ESIMS: m/z 536 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{25}NO_5Cl_2Na$ $[M + Na]^+$ 536.1007, found 536.0997.

*5.1.10.13. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-1-acetylpiperidine-4-carboxylate (**32**).* White amorphous power, yield 67% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.59 (1H, d, J = 7.5 Hz), 7.50–7.43 (4H, m), 7.24 (1H, m), 6.84 (1H, d, J = 2.0 Hz), 4.30 (2H, m), 3.71 (1H, m), 3.05 (1H, t, J = 10.4 Hz), 2.91 (1H, m), 2.71 (2H, m), 2.48–2.42 (1H, m), 2.05 (3H, s), 1.57 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9, 168.9, 163.7, 147.1, 136.0, 134.1, 132.9, 130.5, 130.4, 130.2, 128.9, 128.1, 127.3, 125.5, 121.0, 117.6, 62.5, 45.6, 40.8, 30.9, 28.2, 28.0 (2C), 27.7, 21.4; ESIMS: m/z 487 $[M + H]^+$, HRESIMS: calc for $C_{25}H_{25}N_2O_4Cl_2$ $[M + H]^+$ 487.1191, found 487.1181.

*5.1.10.14. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl benzoate (**33**).* White amorphous power, yield 69% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (2H, m), 7.58 (1H, d, J = 6.4 Hz), 7.49–7.28 (7H, m), 7.18 (1H, d, J = 7.5 Hz), 6.86 (1H, s), 4.57 (2H, t, J = 6.8 Hz), 3.13 (1H, m), 2.83 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.4, 164.0, 147.2, 136.0, 134.1, 132.84, 132.80, 130.7, 130.4, 130.3, 130.1, 129.6, 129.2, 129.2 (2C), 128.2 (2C), 128.1, 127.3, 125.4, 121.2, 117.8, 58.8, 32.3; ESIMS: m/z 460 $[M + Na]^+$, HRESIMS: calc for $C_{24}H_{17}NO_3Cl_2Na$ $[M + Na]^+$ 460.0483, found 460.0482.

*5.1.10.15. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl4-nitrobenzoate (**34**).* White amorphous power, yield 68% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.89 (1H, br.s), 8.12 (4H, m), 7.60 (1H, d, J = 6.4 Hz), 7.51–7.36 (5H, m), 6.85 (1H, s), 4.58 (2H, t, J = 7.2 Hz), 3.14–3.09 (1H, m), 2.90–2.85 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.5, 162.7, 150.3, 147.0, 135.7, 135.5, 133.8, 132.6, 130.6 (2C), 130.4, 130.4, 130.3, 130.1, 128.8, 128.0, 127.2, 125.4, 123.2 (2C), 120.9, 117.0, 63.7, 27.8; ESIMS: m/z 505 $[M + H]^+$, HRESIMS: calc for $C_{24}H_{17}N_2O_5Cl_2$ $[M + H]^+$ 483.0514, found 483.0526.

*5.1.10.16. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylnicotinate (**35**).* White amorphous power, yield 65% (after chromatography with $CHCl_3$/MeOH, 25:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.19 (1H, br.s), 9.12 (1H, d, J = 1.4 Hz) 8.70 (1H, dd, J = 4.8, 1.7 Hz), 8.21 (1H, m), 7.58 (1H, d, J = 7.2 Hz), 7.52–7.40 (3H, m), 7.40–7.25 (2H, m), 7.19 (1H, dd, J = 7.6, 1.5 Hz), 6.8 (1H, s), 4.60 (2H, m), 3.12–3.07 (1H, m), 2.89–2.84 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.5, 162.7, 150.2, 147.0, 135.7, 135.5, 133.8, 132.7, 130.6, 130.39, 130.35, 130.3, 130.1, 128.8, 128.0, 127.2, 125.4, 123.2, 120.8, 118.2, 117.0, 63.6, 27.8; ESIMS: m/z 461 $[M + Na]^+$, HRESIMS: calc for $C_{23}H_{16}N_2O_3Cl_2Na$ $[M + Na]^+$ 461.0435, found 461.0445.

*5.1.10.17. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylthiophene-2-carboxylate (**36**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.11 (1H, br.s), 7.73–7.23 (8H, m), 7.02 (1H, m), 6.85 (1H, s), 4.77 (2H, m), 3.30 (1H, m), 3.00 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.8, 162.0, 147.0, 136.0, 134.1, 133.9, 133.4, 132.8, 132.2, 130.8, 130.4, 130.3, 130.2, 129.1, 128.0, 127.5, 127.3, 125.5, 121.1, 117.5, 63.0, 28.3; ESIMS: m/z 466 $[M + Na]^+$, HRESIMS: calc for $C_{22}H_{15}NO_3Cl_2SNa$ $[M + Na]^+$ 466.0047, found 466.0054.

*5.1.10.18. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylfuran-2-carboxylate (**37**).* White amorphous power, yield 56% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.18 (1H, br.s), 7.58–7.25 (7H, m), 7.02 (1H, m), 7.12 (1H, d, J = 3.6 Hz), 6.86 (1H, d, J = 2.0 Hz), 6.45 (1H, m), 4.58 (2H, m), 3.10 (1H, m), 2.79 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 158.4, 147.2, 146.2, 144.7, 136.1, 134.1, 132.8, 130.7, 130.5, 130.3, 130.1, 128.8, 128.0, 27.1, 125.5, 121.1, 17.9, 117.7, 111.8, 62.7, 28.3; ESIMS: m/z 450 $[M + Na]^+$, HRESIMS: calc for $C_{22}H_{16}NO_4Cl_2$ $[M + H]^+$ 428.0456, found 428.0462.

*5.1.10.19. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-2-(1,3-dioxoisoindolin-2-yl)acetate (**38**).* White amorphous power, yield 80% (after chromatography with $CHCl_3$/MeOH, 100:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.81 (1H, br.s), 7.79 (2H, m), 7.69 (2H, m), 7.57 (1H, m), 7.47 (2H, m), 7.41–7.32 (2H, m), 7.22–7.19 (1H, m), 6.80 (1H, d, J = 2.6 Hz), 4.35–4.31 (4H, m), 2.92–2.85 (1H, m), 2.68–2.62 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.5, 167.1, 162.9, 147.1, 135.8, 134.2, 134.0, 132.8, 131.8, 130.5, 130.4, 130.2, 128.6, 128.0, 127.4, 125.5, 123.5, 121.0, 117.2, 63.6, 38.8, 27.9; ESIMS: m/z 543 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{18}N_2O_5Cl_2Na$ $[M + Na]^+$ 543.0490, found 543.0490.

*5.1.10.20. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-2-phenoxyacetate (**39**).* White amorphous power, yield 68% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.15 (1H, br.s), 7.60 (1H, d, J = 7.9 Hz), 7.51–7.43 (4H, m), 7.30–7.18 (3H, m), 6.94–6.80 (4H, m), 4.60–4.43 (4H, m), 3.00 (1H, m), 2.74 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.8, 163.9, 157.7, 147.3, 136.0, 134.1, 132.9, 130.7, 130.5, 130.4, 130.2, 129.5 (2C), 128.6, 128.1, 127.3, 125.5, 121.7, 121.0, 117.7, 114.5 (2C), 65.4, 63.1, 28.0; ESIMS: m/z 490 $[M + Na]^+$, HRESIMS: calc for $C_{25}H_{19}NO_4Cl_2Na$ $[M + Na]^+$ 490.0588, found 490.0595.

*5.1.10.21. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl 3-phenoxypropanoate (**40**).* White amorphous power, yield 58% (after chromatography with $CHCl_3$/MeOH, 100:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.16 (1H, br.s), 7.58 (1H, dd, J = 8.8, 2.0 Hz), 7.48–7.41 (4H, m), 7.27–7.20 (3H, m), 6.93–6.80

(4H, m), 4.44–4.33 (2H, m), 4.17 (2H, t, J = 6.4 Hz), 2.99 (1H, m), 2.73 (3H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.9, 164.0, 158.4, 147.2, 136.0, 134.2, 132.9, 130.7, 130.5, 130.4, 130.2, 129.6 (2C), 129.0, 128.1, 127.3, 125.5, 121.1, 120.98, 117.7, 115.4 (2C), 63.2, 62.6, 34.6, 28.1; ESIMS: m/z 504 $[M + Na]^+$, HRESIMS: calc for $C_{26}H_{21}NO_4Cl_2Na$ $[M + Na]^+$ 504.0745, found 504.0758.

*5.1.10.22. 2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethylcinnamate (**41**).* White amorphous power, yield 50% (after chromatography with petroleum ether/acetone, 90:10); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63–7.26 (12H, m), 6.86 (1H, d, J = 2.0 H z), 6.36 (1H, d, J = 16 Hz), 4.46 (2H, t, J = 6.4 Hz), 3.04 (1H, m), 2.77 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.7, 163.8, 147.1, 144.7, 135.9, 134.3, 134.1, 132.9, 130.8, 130.5, 130.3, 130.2, 130.17, 129.1, 128.8 (2C), 128.1, 127.9 (2C), 127.2, 125.5, 121.4, 118.0, 117.6, 62.3, 28.2; FABMS: m/z 464 $[M + H]^+$, HRESIMS: calc for $C_{26}H_{19}NO_3Cl_2Na$ $[M + Na]^+$ 486.0639, found 486.0636.

*5.1.10.23. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(3-fluorophenyl) acrylate (**42**).* White amorphous power, yield 76% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.23 (1H, br.s), 7.60–7.44 (6H, m), 7.33–7.03 (5H, m), 6.86 (1H, s), 6.50 (1H, d, J = 16.0 Hz), 4.46 (2H, t, J = 6.9 Hz), 3.07–3.02 (1H, m), 2.81–2.76 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3, 164.0, 162.0, 147.1, 143.2, 136.6, 136.0, 134.2, 133.0, 130.7, 130.4, 130.4, 130.2, 129.2, 128.1, 127.2, 125.5, 123.9, 121.1, 119.5, 117.6, 117.2, 117.0, 114.2, 62.6, 28.2; ESIMS: m/z 504 $[M + Na]^+$, HRESIMS: calc for $C_{26}H_{19}NO_3Cl_2F$ $[M + H]^+$ 482.0726, found 482.0716.

*5.1.10.24. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(2-methoxyphenyl) acrylate (**43**).* White amorphous power, yield 60% (after chromatography with $CHCl_3$/MeOH, 100:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.16 (1H, br.s), 7.91 (1H, d, J = 16.0 Hz),7.60–7.26 (8H, m), 6.94–6.87 (3H, m), 6.46 (1H, d, J = 16.0 Hz), 4.47 (2H, m), 3.84 (3H, s), 3.08–3.03 (1H, m), 2.78–2.78 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2, 163.9, 158.2, 147.2, 140.1, 135.9, 134.2, 132.9, 131.4, 130.8, 130.4, 130.3, 130.1, 129.2, 128.8, 128.1, 127.2, 125.4, 123.3, 121.2, 120.6, 118.6, 117.8, 111.0, 62.2, 55.4, 28.3; ESIMS: m/z 516 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{21}NO_4Cl_2Na$ $[M + Na]^+$ 516.0745, found 516.0759.

*5.1.10.25. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(3-methoxyphenyl) acrylate (**44**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 95:5); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.60–7.42 (6H, m), 7.27 (3H, m), 7.05 (1H, d, J = 7.5 Hz), 6.97 (1H, s), 6.90 (1H, dd, J = 7.5, 2.5 Hz), 6.86 (1H, d, J = 2.0 Hz), 6.35 (1H, d, J = 16.0 Hz), 4.50 (2H, t, J = 5.2 Hz), 3.80 (3H, s), 3.03 (1H, m), 2.77 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.6, 163.8, 159.8, 147.2, 144.6, 135.9, 135.7, 134.1, 132.9, 130.7, 130.5, 130.4, 130.2, 129.8, 129.1, 128.2, 127.2, 125.5, 121.2, 120.6, 118.3, 117.7, 116.1, 112.8, 62.4, 55.2, 28.2; ESIMS: m/z 516 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{22}NO_4Cl_2$ $[M + H]^+$ 494.0925, found 494.0932.

*5.1.10.26. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(4-methoxyphenyl) acrylate (**45**).* White amorphous power, yield 60% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.28 (1H, br.s), 7.60–7.38 (8H, m), 7.28 (1H, d, J = 8.0 Hz), 6.86 (3H, m), 6.24 (1H, d, J = 16.0 Hz), 4.45 (2H, m), 3.81 (3H, s), 3.08–3.03 (1H, m), 2.80–2.74 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.1, 164.0, 161.3, 147.0, 144.3, 136.0, 134.2, 132.9, 130.8, 130.4, 130.3, 130.1, 129.6 (2C), 129.3, 128.0, 127.2, 127.0, 125.4, 121.1, 117.7, 115.5, 114.2 (2C), 62.3, 55.3, 28.2; ESIMS: m/z 516 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{21}NO_4Cl_2Na$ $[M + Na]^+$ 516.0745, found 516.0754.

*5.1.10.27. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-[3-(trifluoro methyl) phenyl]acrylate (**46**).* White amorphous power, yield 91% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.19 (1H, br.s), 7.69–7.42 (10H, m), 7.25 (1H, dd, J = 7.2, 1.6 Hz), 6.86 (1H, d, J = 2.0 Hz), 4.42 (1H, d, J = 16.0 Hz), 4.46 (2H, t, J = 6.8 Hz), 3.07–3.00 (1H, m), 2.83–2.76 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.2, 163.9, 147.3, 142.8, 136.0, 135.1, 134.1, 133.0, 130.9, 130.7, 130.5, 130.4, 130.2, 129.4, 129.0, 128.2, 127.3, 126.6, 126.6, 125.5, 124.5, 124.5, 121.1, 120.0, 117.7, 62.6, 28.2; ESIMS: m/z 554 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{19}NO_3Cl_2F_3$ $[M + H]^+$ 532.0694, found 532.0693.

*5.1.10.28. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-[4-(trifluoro methyl) phenyl]acrylate (**47**).* White amorphous power, yield 34% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.03 (1H, br.s), 7.63–7.42 (10H, m), 7.24 (1H, dd, J = 7.5, 1.6 Hz), 6.86 (1H, d, J = 2.0 Hz), 6.42 (1H, d, J = 16.1 Hz), 4.45 (2H, t, J = 6.7 Hz), 3.06–3.00 (1H, m), 2.82–2.75 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.2, 163.8, 147.4, 142.8, 137.7, 135.8, 134.1, 132.9, 130.7, 130.6, 130.4, 130.3, 129.0, 128.4, 128.1 (2C), 127.3, 125.9, 125.8 (2C), 125.7, 125.6, 121.2, 120.6, 117.7, 62.7, 28.1; ESIMS: m/z 554 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{18}NO_3Cl_2F_3Na$ $[M + Na]^+$ 554.0513, found 554.0529.

*5.1.10.29. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(benzo[d][1,3] dioxol-5-yl)acrylate (**48**).* White amorphous power, yield 60% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (2H, m), 7.32–7.23 (5H, m), 7.15 (1H, d, J = 8.8 Hz), 7.07 (1H, d, J = 7.5, 1.5 Hz), 6.63 (2H, m), 5.99 (1H, d, J = 15.9 Hz), 5.82 (2H, s), 3.17 (2H, m), 2.77–2.72 (1H, m), 2.53–2.47 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.7, 163.0, 150.1, 148.7, 147.4, 145.1, 136.2, 134.4, 133.1, 131.1, 130.7, 130.6, 130.4, 129.0, 128.3, 127.6, 125.9, 124.8, 121.4, 117.4, 115.9, 108.8, 106.7, 106.6, 101.9, 62.6, 28.6; ESIMS: m/z 530 $[M + Na]^+$, HRESIMS: calc for $C_{27}H_{19}NO_5Cl_2Na$ $[M + Na]^+$ 530.0537, found 530.0543.

*5.1.10.30. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(3,4-dimethoxy phenyl)acrylate (**49**).* White amorphous power, yield 60% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.18 (1H, br.s), 7.59–6.80 (11H, m), 6.24 (1H, d, J = 16.0 Hz), 4.43 (2H, t, J = 6.8 Hz), 3.88 (3H, s), 3.86 (3H, s), 3.05–2.99 (1H, m), 2.80–2.74 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.9, 163.9, 151.0, 149.1, 147.1, 144.8, 144.6, 136.0, 134.1, 132.9, 130.7, 130.4, 130.3, 130.2, 129.2, 128.0, 127.2, 125.4, 122.5, 121.1, 117.7, 115.7, 115.4, 110.9, 62.2, 55.9, 51.6, 28.2; ESIMS: m/z 546 $[M + Na]^+$, HRESIMS: calc for $C_{28}H_{23}NO_5Cl_2Na$ $[M + Na]^+$ 546.0850, found 546.0855.

*5.1.10.31. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-3-(pyridin-3-yl) acrylate (**50**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 25:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.19 (1H, br.s), 8.85 (1H, s), 8.58 (1H, d, J = 4.4 Hz), 7.73 (1H, d, J = 6.8 Hz), 7.60–7.26 (8H, m), 6.85 (1H, d, J = 1.6 Hz), 6.36 (1H, d, J = 16.0 Hz), 4.46 (2H, t, J = 6.7 Hz), 3.03 (1H, m), 2.75 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0, 164.0, 150.9, 149.5, 147.1, 140.9, 136.0, 134.1, 134.1, 132.9, 130.7, 130.4, 130.3, 130.2, 130.1, 129.1, 128.1, 127.2, 125.5, 123.7, 121.0, 120.2, 117.6, 62.8, 28.2; ESIMS: m/z 465 $[M + H]^+$, HRESIMS: calc for $C_{25}H_{19}N_2O_3Cl_2$ $[M + H]^+$ 465.0772, found 465.0767.

*5.1.10.32. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-but-2-enoate (**51**).* White amorphous power, yield 60% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.59 (1H, d, J = 7.9 Hz), 7.51–7.42 (4H, m), 7.25 (1H, dd, J = 9.0, 1.5 Hz), 6.94–6.87 (2H, m), 5.77 (1H, m), 4.38

(2H, m), 3.02–3.97 (1H, m), 2.74–2.68 (1H, m), 1.80 (3H, dd, $J = 7.1$, 1.4 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3, 163.9, 147.1, 144.7, 136.0, 134.1, 132.9, 130.8, 130.4, 130.3, 130.1, 129.1, 128.1, 127.2, 125.5, 122.6, 121.3, 117.7, 62.0, 28.2, 17.9; ESIMS: m/z 402 $[M + H]^+$, HRESIMS: calc for $C_{21}H_{18}NO_3Cl_2$ $[M + H]^+$ 402.0663, found 402.0673.

*5.1.10.33. (2E,4E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-hexa-2,4-dienoate (**52**).* White amorphous power, yield 65% (after chromatography with $CHCl_3$/MeOH, 98:2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.61 (1H, d, $J = 7.5$ Hz), 7.48–7.44 (4H, m), 7.29–7.18 (2H, m), 6.90 (1H, d, $J = 2.0$ Hz), 6.20–6.09 (2H, m), 6.72 (1H, d, $J = 15.5$ Hz), 4.41 (2H, m), 3.05–3.00 (1H, m), 2.76–2.72 (1H, m), 1.85 (3H, d, $J = 6.0$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.0, 163.6, 147.4, 145.0, 139.3, 135.7, 134.0, 132.9, 130.8, 130.5, 130.3, 130.1, 129.7, 129.1, 128.3, 127.2, 125.5, 121.3, 118.8, 117.7, 62.0, 28.3, 18.6; ESIMS: m/z 428 $[M + H]^+$, HRESIMS: calc for $C_{23}H_{20}NO_3Cl_2$ $[M + H]^+$ 428.0820, found 428.0822.

*5.1.10.34. (E)-2-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]ethyl-2-methylbut-2-enoate (**53**).* White amorphous power, yield 80% (after chromatography with $CHCl_3$/MeOH, 70:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.07 (1H, br.s), 7.57 (1H, d, $J = 9.0$ Hz), 7.51–7.39 (4H, m), 7.24 (1H, dd, $J = 9.5$, 2.0 Hz), 6.84 (1H, d, $J = 2.0$ Hz), 6.75 (1H, m), 4.34 (2H, m), 3.48 (3H, d, $J = 9.5$ Hz) 3.00 (1H, m), 2.70 (1H, m), 1.75 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.9, 163.7, 147.1, 137.2, 135.9, 134.1, 132.9, 130.6, 130.4, 130.3, 130.2, 129.2, 128.6, 128.2, 127.2, 125.4, 121.2, 117.7, 62.3, 28.1, 14.1, 12.0; EIMS: m/z 415 $[M]^+$, HRESIMS: calc for $C_{22}H_{20}NO_3Cl_2$ $[M + H]^+$ 416.0820, found 416.0809.

5.1.11. General procedure for the preparation of compounds **54** and **55**

Compounds **54** and **55** were prepared in a similar manner as described for the synthesis of compound **2**. These were used in the next step without further purification.

5.1.12. General procedure for the preparation of compounds **56** and **57**

Compounds **54** and **55** were prepared in a similar manner as described for the synthesis of compound **17**.

*5.1.12.1. (E)-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]methyl-3-(3-methoxyphenyl) acrylate (**56**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 100:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.09 (1H, br.s), 7.58 (2H, m), 7.49–7.38 (4H, m), 7.27 (2H, m), 7.06 (1H, d, $J = 7.5$ Hz), 6.98 (1H, s), 6.94 (2H, m), 6.34 (1H, d, $J = 16.0$ Hz), 5.25 (1H, d, $J = 11.6$ Hz), 4.97 (1H, d, $J = 11.6$ Hz), 3.80 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.4, 163.4, 159.8, 149.7, 144.9, 136.8, 135.6, 133.2, 132.9, 131.5, 130.6, 130.4, 130.1, 129.8, 128.3, 127.2, 126.6, 126.0, 122.5, 120.74, 120.69, 117.9, 116.2, 112.8, 58.8, 55.2; EIMS: m/z 479 $[M]^+$, HRESIMS: calc for $C_{26}H_{20}NO_4Cl_2$ $[M + H]^+$ 480.0769, found 480.0771

*5.1.12.2. (E)-3-[6-Chloro-4-(2-chlorophenyl)-2-oxo-1,2-dihydroquinolin-3-yl]propyl-3-(3-methoxyphenyl)acrylate (**57**).* White amorphous power, yield 70% (after chromatography with $CHCl_3$/MeOH, 95:5); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.1 (1H, br.s), 7.56–7.24 (9H, m), 7.30–6.85 (3H, m), 6.27 (1H, d, $J = 16.0$ Hz), 4.18 (2H, t, $J = 6.0$ Hz), 3.83 (3H, s), 2.69 (1H, m), 2.51 (1H, m), 2.00 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.7, 164.0, 159.9, 145.6, 144.4, 135.8, 135.7, 134.5, 133.0, 132.7, 130.5, 130.13, 130.07, 129.9, 127.9, 127.3, 125.3, 121.2, 120.6, 118.4, 117.5, 116.1, 116.0, 113.0, 64.3, 55.3, 27.3, 25.3; ESIMS: m/z 530 $[M + Na]^+$, HRESIMS: calc for $C_{28}H_{23}NO_4Cl_2Na$ $[M + Na]^+$ 530.0901, found 530.0890.

5.2. Cell cultures [25]

HepG 2.2.15 cells were cultured at 37 °C in a humidified 5% CO_2/air atmosphere (Forma, Series II, Thermo, USA) in DMEM (Dulbecco's modifiedeagle's medium) supplemented with 10% (vol/vol) fetal bovine serum, and 5 mM L-glutamine, 50 U/mL penicillin, 50 μg/mL streptomycin. Cells were subcultured once a week, and fresh medium was added every other day.

5.2.1. Determination of HBV replication [26]

HepG 2.2.15 cells were seeded in twenty-four well culture plates at a density of 5×10^5 cells per well. Every 2 days, medium was changed. After 6 days, compounds were added to the cell cultures, and fresh medium was fed every other day for another 6 days. Cells were collected and total DNA was isolated by using TIANamp Gemomic DNA Kit (TIANGEN, Biotech Co., Ltd, China) following the manufacturer's instructions. For detection of HBV DNA, a real-time PCR assay was used. Briefly, 10 μL of DNA sample was amplified in a 25 μL mixture containing 2×SYBR Green PCR Master Mix (Applied Biosystems, USA) and 2 primers specific for HBV: a forward primer (HBV-t1: 5′ CAA GGA ACC TCT ATG TAT CCC TCC 3′) and reverse primer (HBV-t2:5′ TCC GTC CGA AGG TTT GGT AC 3′) covering the 50–base pair insertion from 541 bp to 591 bp. Amplification and detection were performed in the Mastercycler ep realplex System (eppendorf, Masteraycler ep realplex, Germen) with incubation at 95 °C for 2 min and subsequently, 40 three-step cycles (20 s at 95 °C; 15 s at 58 °C; 20 min at 72 °C) were performed. The standard was prepared on serial dilutions of a known amount of the cloned HBV plasmid pCP10, carrying two head-to-tail copies of the HBV genome as positive control was kindly provided by professor J. Chen (No. 302 PLA Hospital, Beijing, China). The specificity of 2 primers (HBV-t1 and HBV-t2) was confirmed in every PCR run via dissociation curve analysis.

5.2.2. Analysis of secreted HBV antigens [27,28]

Medium from HepG 2.2.15 cells was collected, centrifuged at 6000.0 g to remove cellular debris, transferred to clean tubes. The levels of HBsAg and HBeAg were determined utilizing commercially available quantitative ELISA test kits (Autobio diagnostics Co., Ltd, China.) and measured with a microplate reader (model 680, Bio-Rad, USA).

Acknowledgments

The work was supported by grants from National Natural Science Foundation of China for Distinguished Young Scholars (No. 81025023), Xibu Zhiguang Joint-Scholarship of Chinese Academy of Sciences, the External Cooperation Program of Chinese Academy of Sciences (No. GJHI200818), and CAS–Croucher Fund (CAS-CF07/08.SC03). The authors are grateful to the staff of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, ChineseAcademy of Sciences, for measurements of all spectra.

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