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Article

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Discovery of RG7834: The First-in-Class Selective and Orally Available Small Molecule Hepatitis B Virus Expression Inhibitor with Novel Mechanism of Action

Xingchun Han, [†] Chengang Zhou, [†] Min Jiang, [†] Yongguang Wang, [†] Jianhua Wang, [†] Zhanling Cheng, [†] Min Wang, [†] Yongqiang Liu, [†] Chungen Liang, [†] Jianping Wang, [†] Zhanguo Wang, [†] Robert Weikert, [‡] Wenzhe Lv, [&] Jianxun Xie, [&] Xin Yu, [ǂ] Xue Zhou, [ǂ] Souphalone Luangsay, [§] Hong C. Shen, [†] Alexander V. Mayweg, [†] Hassan Javanbakht, [§] Song Yang*, [†]

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Department of [‡]Medicinal Chemistry, [§]Discovery Virology, Roche Innovation Center Basel, Roche Pharma Research and Early Development, Grenzacherstrasse 124, CH-4070 Basel, Switzerland

Abstract: Chronic hepatitis B virus (HBV) infection is a serious public health burden and current therapies cannot achieve satisfactory cure rate. There are high unmet medical needs of novel therapeutic agents with differentiated mechanism of action (MOA) from the current standard of care. RG7834, a compound from the dihydroquinolizinone (DHQ) chemical series, is a first-in-class highly selective and orally bioavailable HBV inhibitor which can reduce both viral antigens and viral DNA with a novel mechanism of action. Here we report the discovery of RG7834 from a phenotypic screening and the structure-activity relationship (SAR) of the DHQ chemical series. RG7834 can selectively inhibit HBV but not other DNA or RNA viruses in a virus panel screening. Both in *vitro*

and *in vivo* profiles of RG7834 are described herein, and the data support further development of this compound as a chronic HBV therapy.

Keywords: RG7834, chronic HBV, inhibition of HBsAg expression, inhibition of HBV DNA production, dihydroquinolizinone (DHQ), viral gene expression, novel mechanism of action, humanized mice, SAR

1. Introduction

Hepatitis B virus (HBV) infection is a serious public health burden worldwide with over 250 million people chronically infected, defined as hepatitis B surface antigen (HBsAg) positive. About one quarter of the patients are likely to develop serious liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). It was reported that hepatitis B resulted in 887 000 deaths in 2015, mostly from complications (including cirrhosis and HCC). [1]

In clinics, a high level of HBsAg is considered as one of the hallmarks of chronic HBV infection. HBsAg may contribute to exhaustion of antiviral CD8+ T-cell[2-5] and it negatively regulates HBV-specific immune responses.[6-8] Persistence of HBsAg is the principal marker of risk for developing chronic liver diseases and HCC later in life.[1] HBsAg loss, which is defined as HBV "functional cure" and subsequently sero-conversion with detectable anti-HBs antibody levels are considered an ideal end point for HBV therapy.[9, 10] As such, agents that reduce HBsAg levels will provide potential therapies for chronic HBV infection and reduce the risk of developing severe liver diseases.

The current therapies for HBV infection include nucleos(t)ide analogues (lamivudine, tenofovir or entecavir (ETV) etc.) and interferons (IFNs). Although these two types of therapies can reduce viral load (HBV DNA), neither can reduce HBsAg levels effectively.[11] Moreover, the nucleos(t)ides therapies require a lifelong treatment in most patients and the interferon based therapy is poorly tolerated. Thus, there are huge unmet medical needs to develop novel medicines with differentiated mechanism of action (MOA) to improve the HBV cure rate.

In a previous report,[12] we described the biological profile of RG7834, a novel, orally bioavailable small molecule from dihydroquinolizinone (DHQ) chemical series which selectively reduced HBV

antigens (both HBsAg and HBeAg) as well as HBV DNA both *in vitro* and *in vivo*. To the best of our knowledge, this is the first small molecule which displayed significant HBsAg reduction in the HBV-infected human liver chimeric uPA-SCID mice model. RG7834 is highly selective for HBV, which was demonstrated in an anti-viral assay against a panel of 15 DNA and RNA viruses including human immunodeficiency virus(HIV) and hepatitis C virus (HCV). The mechanism is clearly different from the current therapies like nucleos(t)ides, which have no significant effect on HBV antigens reduction. Moreover, the MOA of RG7834 is different from the reported HBsAg inhibitors in recent years. For example, HB-0259 could inhibit HBsAg secretion but have no effect on HBV DNA or HBeAg secretion.[13, 14] A benzimidazole derivative, BM601, was another reported HBsAg inhibitor which inhibited HBsAg secretion but not production. In addition, BM601 had no effect on HBV DNA replication and HBeAg secretion.[15] A series of fluorine substituent nucleoside analogues (FNCs) [16-18] were reported as inhibitors of HBsAg, HBV DNA and HBeAg, but they were also active against HIV and HCV, and not specific HBV inhibitors as RG7834.

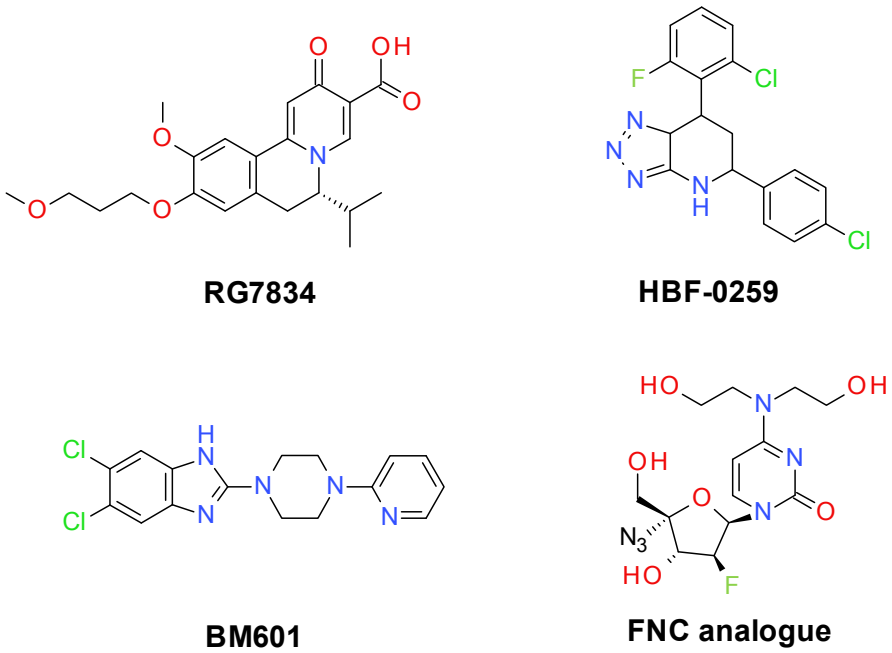


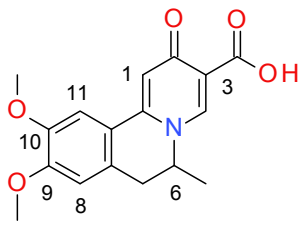
Figure 1. Structures of RG7834 and reported HBsAg inhibitors

In this article, we report the discovery of RG7834 from a phenotypic screening and the preclinical data supporting its progression for further development.

2. Results and Discussion

2.1. Primary SAR of Dihydroquinolizinones (DHQs)

The research for novel HBsAg inhibitors started by performing a phenotypic high-throughput screening (HTS) of a library containing approximately one million compounds in a cell-based assay detecting the extracellular HBsAg level. From this screening, compound **1** with dihydroquinolizinone (DHQ) scaffold was identified as a singleton hit. It reduced the level of secreted HBsAg and HBV DNA with IC_{50} values of 0.5 μM and 0.2 μM, respectively, in HBV-expressing HepG2.215 cells. To confirm the antiviral activity, compound **1** was then evaluated in differentiated HepaRG (dHepaRG) cells and showed inhibition of HBsAg expression with IC_{50} value of 0.88 μM. Hence compound **1** was considered as a valid starting point.



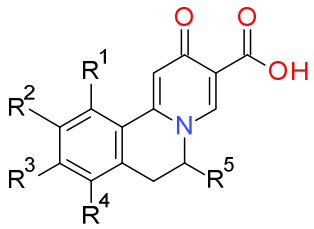
1

We subsequently explore the structure-activity relationship (SAR) with hit expansion utilizing the analogues of compound **1** in the Roche collection. This effort led to the identification of compound **2–4** (Table 1), which showed that the substituents at C6, C9 and C10 had a significant impact on the potency. Without the C6 methyl, compound **2** showed a ~5-fold potency drop. Removal of the C10 methoxy resulted in a ~10-fold potency reduction (**3** vs **1**), and further removal of the C9 methoxy (**4**) resulted in a complete loss of anti-HBV activity.

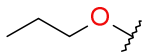
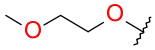
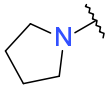
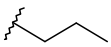
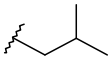
With these observations we first synthesized several analogues with structure variations in the left part (C8–C10 positions) and evaluated their anti-HBV activities. The results are summarized in Table 1. It turned out that the activity was sensitive to the substituents at C9 and C10 positions. Small groups like methyl and chloro at C10 position gave improved potency (**5**, **6** compared to **1**), but with elongation or size growth of the substituents, compounds **7-10** showed gradually reduced potency. At the C9 position, demethylation (**11**) or removal of the methoxy (**12**) led to a dramatic potency loss, while

larger groups ethoxy and benzyloxy brought a 5-fold enhancement of potency (**13**, **14** vs. **1**). These observations indicated that some large groups could be accommodated at the C9 position or may even help to improve anti-HBV activity. In addition, a bromo at the C9 position was well tolerated (**15** vs **3**). Further derivatization of the bromo of **15** gave compounds **16**, **17** and **18**, in which the pyrrolidinyl analogue **17** showed much improved potency with an IC_{50} of 0.08 μM. Methylamino analogue **18** containing a -N-H at the C9 position showed lower potency compared to that of **3**, suggesting that a hydrogen bond donor is not favored. Compared to **13**, analogue **19** with a hydroxy at the C10 positon showed much reduced anti-HBV activity. Combined with the C10 SAR findings mentioned above, the observations suggested that a small hydrophobic group was preferred at the C10 position. We also explored the C8 and C11 positions, and it seemed that these two positions were less sensitive to substituents compared to C9 and C10, as the C8 chloro analogue **20** and C11 methoxy analogue **21** afforded similar potency as that of **3**.

Table 1. Effects on HBsAg Reduction of Analogs 1-24



ID	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)[a]	CC_{50} (μM)[b]
1	-H	-OMe	-OMe	-H	-Me	0.87	>50
2	-H	-OMe	-OMe	-H	-H	2.64	>50
3	-H	-H	-OMe	-H	-Me	4.97	>50
4	-H	-H	-H	-H	-Me	52	>50
5	-H	-Me	-OMe	-H	-Me	0.24	>50
6	-H	-Cl	-OMe	-H	-Me	0.29	>50
7	-H	-OEt	-OMe	-H	-Me	1.2	>50

8	-H		-OMe	-H	-Me	1.98	>50
9	-H	-OBn	-OMe	-H	-Me	29.	>50
10	-H		-OMe	-H	-Me	>50	>50
11	-H	-OMe	-OH	-H	-Me	7.24	>50
12	-H	-OMe	-H	-H	-Me	7.67	>50
13	-H	-OMe	-OEt	-H	-Me	0.097	>50
14	-H	-OMe	-OBn	-H	-Me	0.10	>50
15	-H	-H	-Br	-H	-Me	4.75	>50
16	-H	-H		-H	-Me	0.58	>50
17	-H	-H		-H	-Me	0.081	>50
18	-H	-H		-H	-Me	8.99	>50
19	-H	-OH	-OEt	-H	-Me	1.86	>50
20	-H	-H	-OMe	-Cl	-Me	5.03	>50
21	-OMe	-H	-OMe	-H	-Me	4.56	>50
22	-H	-OMe	-OMe	-H		0.11	>50
***(R)*-22**	-H	-OMe	-OMe	-H		0.049	>50
***(S)*-22**	-H	-OMe	-OMe	-H		10.9	>50
23	-H	-OMe	-OMe	-H		0.068	>50
24	-H	-OMe	-OMe	-H		0.052	>50

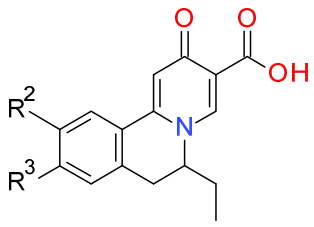
[a] IC_{50} is the geometric mean value for reduction of HBsAg by 50% in HepG.2.2.15 cells. [b]CC_{50} is the geometric mean value for cytotoxicity measured by CCK-8 in HepG.2.2.15 cells.

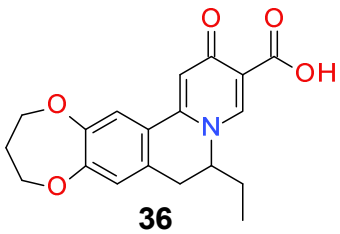
In parallel to the SAR exploration in the left part, we also synthesized compounds **22-24** (Table 1) to explore the tolerability of larger alkyl groups at the C6 position. It was encouraging to see that compound **22** showed a ~5-fold increase in potency compared to **1**. Then, in order to further understand the anti-HBV activity of this new scaffold, we conducted chiral separation of **22** by supercritical fluid chromatography (SFC), and the two enantiomers showed marked difference in anti-HBV activities. The absolute configuration of (*S*)-**22** was confirmed by single crystal X-ray studies (see the Supporting Information), and optical rotation was measured. In the crystal structure, an intramolecular hydrogen bond is formed between the carboxyl group with the carbonyl at the C2 position. The enantiomer (*R*)-(+)-**22** was active in HepG2.2.15 with an IC_{50} value of 0.049 μM, while the enantiomer (*S*)-(-)-**22** was much less active with an IC_{50} value of 10.9 μM. Propyl and isobutyl substituents (**23**, **24**) showed slightly better potency than **22**.

2.2. Further SAR exploration at the C9 and C10 positions

Having identified the favorable substituents in both the left part and the C6 position, and to quickly improve the anti-HBV activity in this series, we decided to prepare compounds with combination of these substituents. The results are shown in Table 2. To our delight, the combination the C9 benzyloxy and C6 ethyl (**25**) led to an IC_{50} value of 0.039 μM, ~2 fold more potent than that of **14** and **22**. Then, consistent with the SAR finding, compound **26** also showed a ~2-fold increase in potency compared to that of **13** and **22**.

Table 2. Further SAR at the C9 and C10 Positions: Effects on HBsAg Reduction and Physicochemical Properties of 25-52





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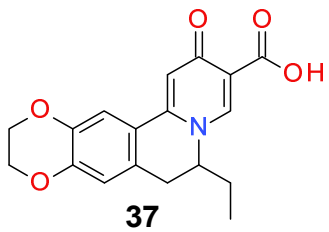

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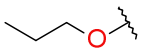
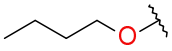
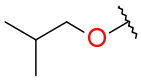
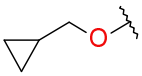
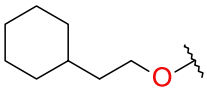
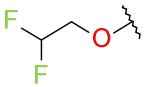
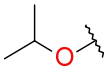
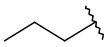
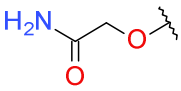
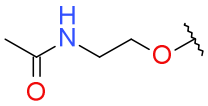
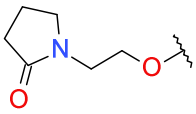
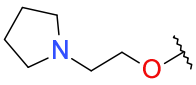
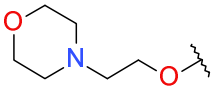
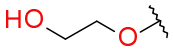
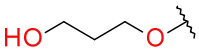
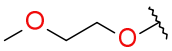
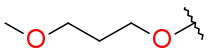
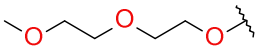
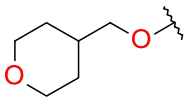
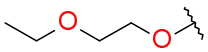
ID	R^2	R^3		IC_{50}	CC_{50}	LYSA	MLM	HLM

			(μM)[a]	(μM)[a]	(μg/mL)[b]	(μL/min/mg)[c]	(μL/min/mg)[d]
25	-OMe	-OBn	0.039	>50	<1.0	3.6	1.4
26	-OMe	-OEt	0.043	>50	25.0	5.3	1.3
27	-OMe		0.020	>50	36.0	2.3	0.33
28	-OMe		0.018	>50	15.0	5.7	0
29	-OMe		0.013	>50	3.0	2.5	1.3
30	-OMe		0.015	>50	3.0	1.6	4.8
31	-OMe		0.043	20.7	<1.0	67.9	10.7
32	-OMe		0.059	>50	12	3.8	0.23
33	-OMe		0.090	>50	36	0.63	1.7
34	-OMe		0.37	>50	11.0	16.7	4.2
35	-OMe	-CN	0.33	>50	17.0	1.9	6.7
36	-	-	1.94	>50			
37	-	-	0.67	>50			
38	-Et	-OMe	0.024	>50	10.0	22.2	2.5
39		-OMe	0.013	>50	9.0	23.6	1.5

40	-OMe		26.1	>50	-	-	-
41	-OMe		3.9	>50	>470	1.9	0
42	-OMe		5	>50	36	3.4	0
43	-OMe		0.18	>50	>470	2.3	3.3
44	-OMe		3.1	>50	>510	6.5	3.2
45	-OMe		0.16	>50	>535	3.1	3.0
46	-OMe		0.56	>50	172	3.5	8.7
47	-OMe		0.2	>50	101	9.5	1.8
48	-OMe		0.064	>50	225	0	2.4
49	-OMe		0.019	>50	63.5	4.0	2.5
50	-OMe		0.22	>50	208	4.6	0.096
51	-OMe		0.028	>50	71	2.0	0
52	-OMe		0.056	>50	97	0.97	0.72

[a]Definitions are the same as those described in Table 1. [b]Lyophilization solubility assay (LYSA) (μg/mL).

[c]Mouse liver microsome (MLM) (mL/min/kg). [d]Human liver microsome (HLM).

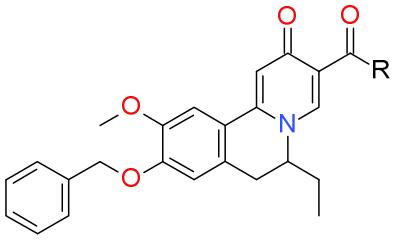
As C9 and C10 substituents showed marked impact on anti-HBV activity, especially C9 substituent was indicated as an important moiety which potentially could help to improve the activity, we then extensively explored the SAR at these two positions, with fixed ethyl group at C6. Based on **26**, elongation of the ethoxy to propoxy resulted in 2-fold increase in potency (**27** vs **26**), but further elongation to butoxy did not give appreciable improvement (**28** vs **27**). Interestingly, still with four carbon at C9, isobutoxy (**29**) and cyclopropylmethoxy (**30**) afforded improved potency with IC50 values of 0.013 μM and 0.015 μM. Larger substituent (**31**) or difluoroethoxy (**32**) were also well tolerated, but isopropoxy group resulted in 2-fold potency drop (**33** vs **26**), suggesting that steric bulk close to C9 oxygen was not favored. Replacement of the C9 ethoxy with propyl or cyano group, or cyclization of C9 and C10 substituents led to significant potency drop (**34-37** vs **26**). At C10 position, considering the SAR observations mentioned above, we replaced the methoxy with ethyl (**38**) and cyclopropyl (**39**). The two compounds were very active against HBsAg in HepG2.2.15 cells with IC50 values of 0.024 μM and 0.013 μM, respectively.

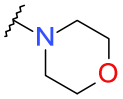
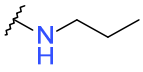
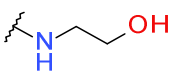
The above-discussed SAR study indicated that appropriate hydrophobic groups at C6, C9 and C10 positions helped to dramatically improve the anti-HBV activity in HepG2.2.15 cells. However, combination of these hydrophobic groups also led to unfavorable physiochemical properties. For example, although analogue **29** and **30** demonstrated >2-fold increase in potency compared to that of **26**, they also showed significantly reduced aqueous solubility in lyophilization solubility assay (LYSA). Moreover, the potent analogue **39** showed not only reduced aqueous solubility, but also reduced microsomal stability compared to that of **26**. Therefore, in an attempt to improve the physiochemical properties, we explored the tolerability of solubilizing groups at C9 position. The results showed that amide (**40**), reversed amide (**41**) and methanesulfonamide (**42**) at terminal of the C9 side chain were not tolerated, but without –N-H, lactam analogue gave better activity (**43** vs **41**). Pyrroline (**44**) brought very high aqueous solubility (LYSA > 510 μg/mL), but also led to dramatic potency drop. Compared to **44**, morpholine analogue **45** with lower basicity showed higher anti-HBV

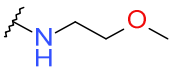
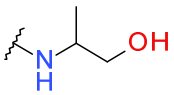
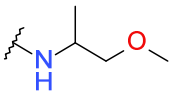
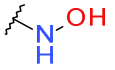
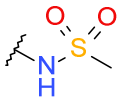
activity while keeping high solubility. With a terminal hydroxy group, analogue **46** and **47** showed good solubility but moderate anti-HBV activity. Notably, ether side chain analogues **48**–**52** appeared to give balanced properties. Among these modifications, methoxypropoxy analogue **49** demonstrated better anti-HBV activity and higher aqueous solubility than that of **26**, which increased the potential for further optimization.

2.3. SAR study at the C3 position

Table 3. SAR study at C3 position: Effects on HBsAg Reduction of Compounds 53-63, compared with Compound 25



ID	R	IC_{50} (μM)[a]	CC_{50} (μM)[a]
25	-OH	0.039	>50
53	$-NH_2$	>50	>50
54		19	>50
55		>50	>50
56		>50	>50
57		6.4	>50
58		43	>50

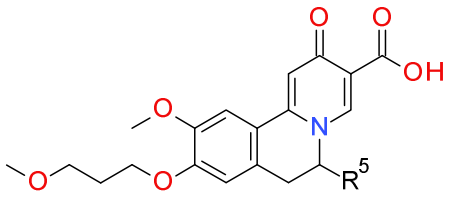
59		13	>50
60		6.6	>50
61		0.52	>50
62		19	48.7
63		0.98	>50

[a]Definitions are the same as those described in Table 1.

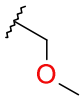
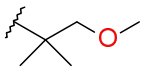
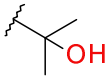
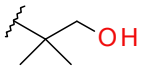
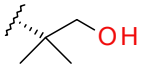
As part of our SAR study, we also synthesized a number of compounds to replace the C3 carboxylic acid. The results in Table 3 showed that replacement of the carboxylic acid with primary amide (**53**) or tertiary amides (**55**, **56**) led to a complete loss of activity. Secondary amides **57**–**59** afforded weak activity in HepG2.2.15 cells. A methyl group adjacent to the amide nitrogen was beneficial to potency (**60** vs **58**, **61** vs **59**). Meanwhile, bioisostere replacement of the C3 carboxylic acid was explored as well. Hydroxamic acid analogue **62** was only weakly active with an IC_{50} of 19 μM, while the acylsulfonamide analogue **63** maintained a moderate IC_{50} of 0.98 μM.

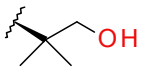
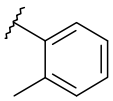
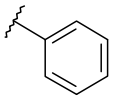
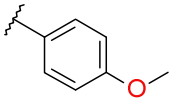
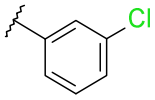
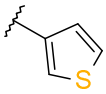
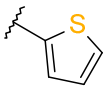
2.4. Further SAR at the C6 position

Table 4. Further SAR at the C6 position: Effects on HBsAg Reduction and Physicochemical Properties of Compounds 64-77, Compared with Compound 49



ID	R5	IC50	CC50	LYSA	MLM (mL/min/kg)[a]	HLM (mL/min/kg)[a]

		(μM)[a]	(μM)[a]	(μg/mL)[a]		
49		0.019	>50	63.5	9.4	1.9
64		0.009	>50	71	5.0	1.1
(*S*)-64 (RG7834)		0.003	>50	82	13	3.7
(*R*)-64		3.15	>50	85	9.3	0.77
65		0.001	>50	5.0	0	0.63
66		0.002	>50	10	14.	0.03
67		0.039	>50	119	0.92	0.76
68		0.074	>50	192	8.4	4.6
69		0.005	>50	137	6.2	0
70		0.13	>50	195	6.3	0.91
71		0.006	>50	51	8.6	0.75
(*S*)-71		0.002	>50	>515	0	0

(*R*)-71		0.088	>50	>550	0	0
72		0.010	>50	-	-	-
73		0.064	>50	7.0	5.5	0
74		0.80	>50	6.0	-	-
75		0.092	>50	<1.0	20	6.2
76		0.010	>50	1.0	10	0
77		0.003	>50	5.0	10	1.1

[a]Definitions are the same as those described in Table 2.

Finally, we carried out further SAR exploration at the C6 position with fixed methoxypropoxy at C9 as shown in Table 4. Growth of the C6 substituent into isopropyl led to > 2-fold increase in potency (**64** vs **49**) with an IC_{50} value of 0.009 μM. It was also notable that compound **64** showed comparable solubility with that of **49**. Racemic compound **64** was then separated by SFC, and the absolute configuration was confirmed by the X-ray study. Consistent with the enantioselective anti-HBV activity observed on the two enantiomers of **22**, the enantiomer (*S*)-(+)-**64** (RG7834, Figure 2) had higher potency with an IC_{50} value of 0.003 μM. Bulkier substituent analogues **65** and **66** were the most potent ones with an IC_{50} values of 0.001 μM and 0.002 μM, respectively, without chiral separation. However, unfortunately, the increased hydrophobicity also led to solubility reduction.

Trifluoroethyl (**67**) and alkoxy (**68**) were tolerated, and with gem-dimethyl adjacent to the C6 position, alkoxy compound **69** showed much improved potency and solubility compared to compound **49**. Addition of a hydroxyl group on the C6 substituent of **49** resulted in potency drop, but extended hydroxyl analogue **71** had similar potency as compound **49**. Moreover, the enantiomer (*S*)-(+)-**71** showed very high aqueous solubility (LYSA > 515 μg/mL). C6 aromatic substituents (**71-76**) were also explored, and thienyl **70** and **71** showed a high potency. Not surprisingly, the solubility of the C6 aromatic analogues was generally low.

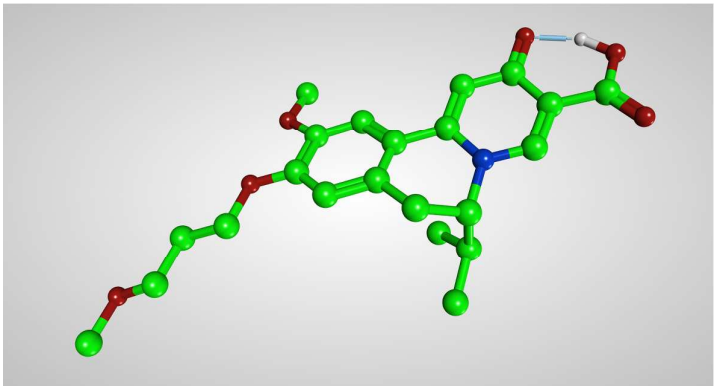


Figure 2. X-ray structure of RG7834

2.5. *In vitro* anti-HBV profile and selectivity of RG7834 and molecular targets for DHQ series

Besides the potent activity in inhibition of HBsAg expression, RG7834 also displayed a very potent activity in inhibition of HBV DNA production in HepaG2.2.15 cells with an IC_{50} < 0.13 nM while the IC_{50} of the nucleoside agent lamivudine (3-TC) was around 25 nM.

In HBV-infected dHepaRG cells, RG7834 inhibited HBsAg, HBeAg and HBV DNA at a single digital nM concentration, while (*R*)-**64**, the enantiomer of RG7834, did not show significant inhibition against all the three viral markers at concentration up to 1 μM (Table 5). The nucleoside drug ETV showed very potent activity in inhibition of HBV DNA production with an IC_{50}=0.06 nM but no activity against HBsAg and HBeAg.[12] The different antiviral profile between RG7834 and ETV indicated a differentiated MOA of RG7834 compare with nucleoside drugs.

Table 5. Antiviral activity of RG7834, (*R*)-64 and ETV in HBV-infected dHepaRG cells

Compound	IC_{50} (nM)		
	HBsAg	HBeAg	HBV DNA
RG7834	2.8	2.6	3.2
(*R*)-64	>1000	>1000	>1000
ETV	>1	>1	0.06

To evaluate the antiviral selectivity of RG7834, the compound was tested in a panel of 15 different DNA and RNA viruses. The IC_{50} of RG7834 against all these viruses was higher than 10 μM which demonstrated the high specificity of RG7834 against HBV.[12]

To get better understanding of the MOA, we tried different approaches to identify the molecular target(s) for DHQ chemical series. Finally, the non-canonical poly(A) RNA polymerases, PAP-associated domain-containing protein 5 and 7 (PAPD5 and PAPD7) were identified as protein targets for DHQ compounds. These two proteins had not been reported previously as targets for HBV therapy. The detailed information about the targets will be reported separately.

2.6. DMPK, Early Safety and in vivo Efficacy Assessment of RG7834

RG7834 was selected for further physicochemical and ADME characterization based on its favorable anti-HBV activity, cytotoxicity, solubility and liver microsome stability. With favorable measured log*D* value of 1.28 and pKa value of 5.79 (acidic), RG7834 showed very good permeability with a P_{app}(A-B) of 12.8 × 10^{-6} cm s^{-1} and P_{app} ratio of 1.3 in Caco-2 assay. The unbound fractions of RG7834 in human and mouse plasma were determined to be 32.8% and 35.2%, respectively. The mouse single dose pharmacokinetics (SDPK) profile of RG7834 was evaluated in male BALB/C mice following intravenous (i.v.) and oral (p.o.) administration. The results are summarized in Table 6. RG7834 had moderate plasma clearance (Cl) (41.9 mL min^{-1} kg^{-1}) and good oral bioavailability (*F*) (62%) in mice. RG7834 also demonstrated satisfactory oral exposure, and particularly, good liver exposure which was 4-fold higher than that in plasma. Although we did not have free liver drug concentration, the reasonably high total concentration of RG7834 in liver was considered as a desirable attribute for anti-HBV drugs because liver is the target organ in chronic HBV infections.

Table 6. Single-Dose Pharmacokinetics of RG7834 in mice[a]

	dose (mg/kg)	CL (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	Plasma C_{max} (ng/mL)	Plasma $AUC_{(0-\infty)}$ (h·ng/mL)	F (%)	Plasma $AUC_{(0-t)}$ (h·ng/mL)	Liver C_{max} (ng/mL)	Liver $AUC_{(0-t)}$ (h·ng/mL)	$AUC_{(0-t)}$ ratio (Liver/Plasma)
i.v.	1	41.9	2.07	0.71	466	398					
p.o.	2			1.48	322	490	62				
po	14.5				2037			4366	10987	17265	4.0

[a] The single-dose pharmacokinetics (SDPK) study of RG7834 was carried out in male BALB/C mice according to standard procedures. Major parameters, including plasma clearance (CL), volume of distribution at steady state (V_{ss}), $t_{1/2}$, maximal concentration (C_{max}), area under the curve (AUC), and oral bioavailability (F) are reported.

The cynomolgus monkey SDPK study of RG7834 was conducted subsequently, and it showed low plasma clearance (4.6 mL min^{-1} kg^{-1}) following intravenous administration. RG7834 also showed good oral bioavailability (57%) with a half-life of 4.9 hours following oral administration.

Simultaneously we also assessed the *in vitro* safety profile of RG7834. In the *in vitro* drug-drug interaction (DDI) assessment, RG7834 showed IC_{50} values of >50 μM against CYP3A4, CYP2D6 and CYP2C9. It had no time dependent inhibition (TDI) issue and no CYP induction issue. Moreover, RG7834 demonstrated an excellent *in vitro* toxicology profile, showing no inhibition of hERG channel, no flag for glutathione adduction (GSH), and clean in Ames assay (mutagenicity) and micronucleus test (MNT, clastogenicity).

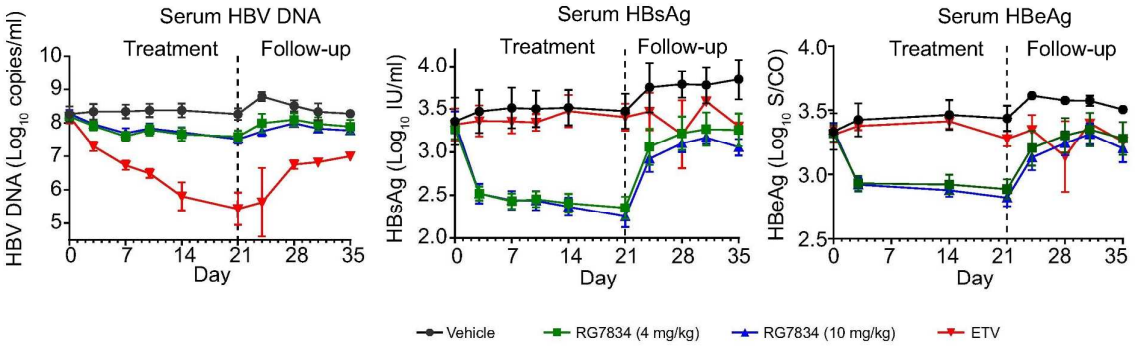


Figure 3. Effect of RG7834 and ETV on HBV viral markers in HBV-infected Human liver chimeric uPA-SCID mice. HBV DNA, HBsAg and HBeAg were determined in serum from HBV-infected uPA/SCID mice during

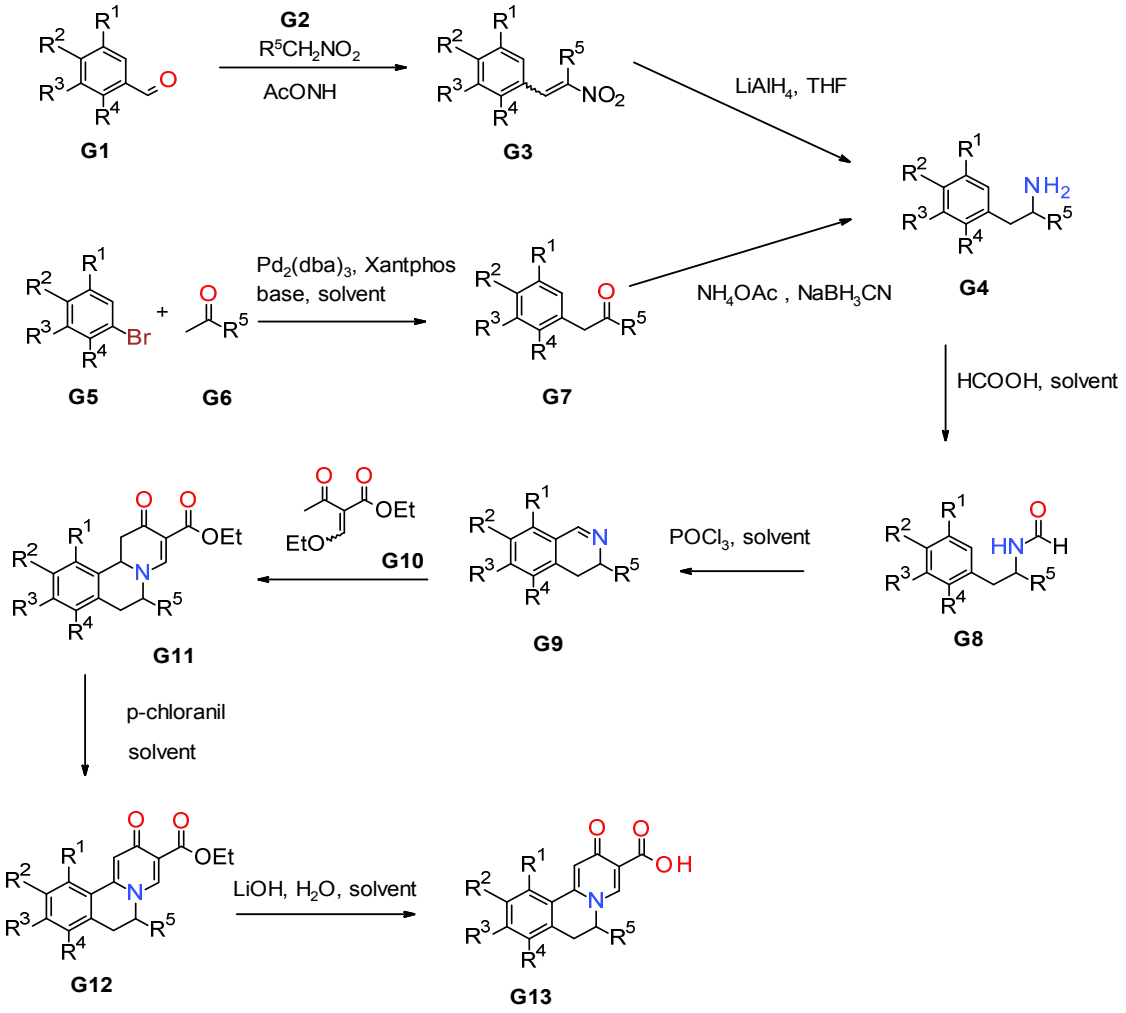
the 21 days' treatment and follow-up period up to 35 days. (figure reprinted from Journal of Hepatology[12] with permission)

The *in vivo* anti-HBV efficacy was evaluated in HBV-infected human liver chimeric uPA-SCID mice with ETV as a control. After 21 days of treatment with 4 mg/kg twice daily dosing, RG7834 reduced both HBsAg and HBeAg significantly while ETV did not show clear efficacy on the reduction of the two antigens. RG7834 also reduced serum HBV DNA by 0.6 $\log_{10}$ with 4 mg/kg treatment and ETV showed more pronounced HBV-DNA reduction (Figure 3). The *in vivo* results are consistent with the *in vitro* observation, and the detailed biological results were reported recently.[12]

3. Chemistry.

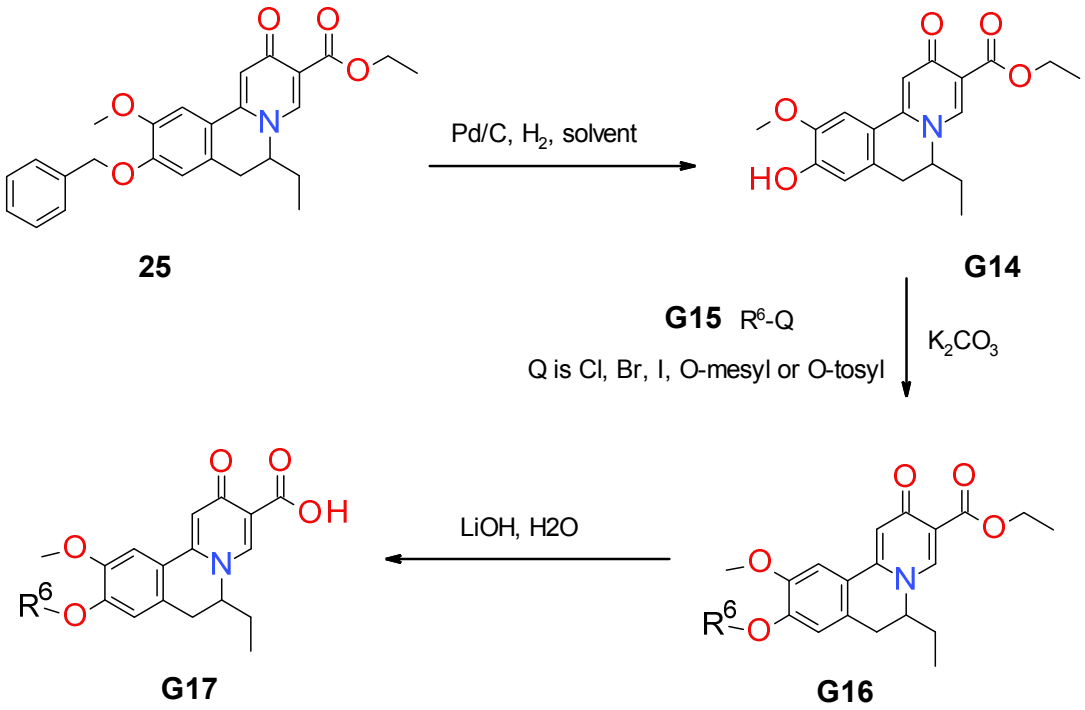
The synthesis of these molecules was carried out as shown in the various schemes. A general synthetic route for the C3 carboxylic acid DHQs is shown in Scheme 1. The Henry reaction of aldehyde **G1** and nitroalkane **G2** afforded nitroalkene **G3**. Reduction of **G3** by $LiAlH_4$ gave a key intermediate amine **G4**. This intermediate could also be prepared by another route, namely, the coupling reaction of bromide **G5** with ketone **G6** to afford **G7**, which then underwent reductive amination to afford amine **G4**. Subsequently, the treatment of **G4** with formic acid generated formamide **G8**, which then underwent Bischler-Napieralski cyclization to produce 3,4-dihydroisoquinoline **G9**. The further cyclization employing ethyl 2-(ethoxymethylene)-3-oxo-butanoate **G10** yielded diastereoisomers **G11**. Ester **G12** was obtained by oxidation of **G11** mixture with *p*-chloranil. Finally, basic hydrolysis afforded desired acid **G13**. Although this synthetic route was employed for the synthesis of most of the analogues, other routes for specific analogues will be given in the Support Information.

Scheme 1



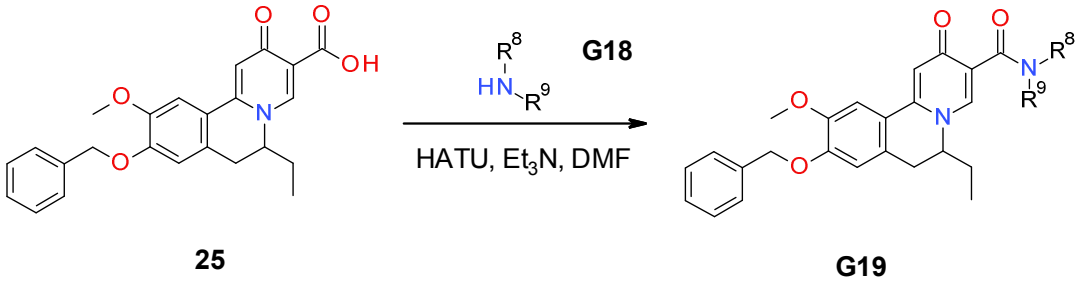
During exploration of the C9 position, another synthetic route was used to quickly introduce various substituents (Scheme 2). De-protection of **25** afforded phenol **G14**, which underwent substitution reaction to give ester **G16**. Lithium hydroxide hydrolysis produced desired acid **G17**.

Scheme 2



In addition, analogues **53-63** were prepared using analogue **25** as starting material as shown in Scheme 3. Coupling reaction of **25** with amine **G18** in the presence of HATU afforded desired amide **G19**.

Scheme 3



4. Conclusion

In summary, we have discovered DHQ chemical series from a phenotypic screening as a novel class of HBV inhibitors with differentiated MOA from current therapies. PAPD5 and PAPD7 were identified as molecular targets for this chemical series. The SAR and structure-property relationship (SPR) of DHQ series were explored systematically. Of the two enantiomers, one is much more potent

than the other and the absolute configuration of two compounds (RG7834 and (*S*)-**22**) was established by X-ray crystal structures.

The representative lead compound RG7834 displayed potent (single digital nM IC_{50}) inhibitory activity against HBsAg, HBeAg and HBV DNA in HBV-infected hepatocytes while no cytotoxicity was observed up to 50 μM. Moreover, RG7834 is highly specific for HBV showing no inhibitory activity against other 15 viruses in a panel screening. In addition to favourable physico-chemical and *in vitro/in vivo* DMPK properties, RG7834 also displayed a favourable *in vitro* safety profile. This compound was well tolerated in preclinical *in vivo* toxicology studies including a 4-day *in vivo* toxicology study in rats, and 4-week GLP studies in rat and monkey (data not shown). Furthermore, RG7834 demonstrated unprecedented PD efficacy in the human liver chimeric uPA-SCID mouse model. All the data together supported moving RG7834 forward into clinical investigation.

EXPERIMENTAL SECTION

Synthetic Chemistry General Comments. All of the intermediates were purified by silica gel chromatography using either a Biotage or an ISCO CombiFlash chromatography instrument. All of the final compounds were purified by preparative HPLC (prep-HPLC) on a reversed-phase column using a Waters XBridge OBD Phenyl (30 mm × 100 mm, 5 μm) or OBD RP18 (30 mm × 100 mm, 5 μm) column under acidic conditions (A, 0.1% formic acid in H_2O; B, 0.1% formic acid in acetonitrile) or basic conditions (A, 0.1% ammonia in H_2O; B, acetonitrile). For SFC chiral separation, the intermediates were separated using a chiral column (Daicel Chiralpak IC, 30 mm × 250 mm, 5 μm) on a Mettler Teledo SFC-Multigram system (solvent system of 95% CO_2 and 5% IPA (0.5% TEA in IPA), backpressure of 100 bar, UV detection at 254 nm). NMR spectra were obtained using Bruker AVANCE 400 MHz spectrometer, operating at 400.13 MHz (^1H) and 100.62 MHz (^{13}C). LC−MS spectra were obtained using a MicroMass Platform LC (Waters Alliance 2795-ZQ2000). Optical rotation was measured using a Rudolph Autopol V automatic polarimeter at a wavelength of 589 nm. High-resolution mass spectra were obtained on Xevo G2-XS-QTOF mass spectrometer (Waters,

Manchester, U.K.) equipped with an electrospray ionization source. All of the starting materials were obtained commercially. All of the final compounds had purities greater than 95% based upon HPLC, LC−MS, and 1H NMR analyses. All of the reported yields are for isolated products and are not optimized.

General Procedure for the Preparation of G4. *Method A***.** A mixture of aldehyde **G1**(12.4 mmol) and ammonium acetate (12.4 mmol) in toluene (40 mL) was refluxed with a Dean-Stark trap for 2 hours. Then **G2** (62 mmol) was added and the resultant mixture was refluxed for additional 36 hours. The mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (100 mL). The resultant solution was washed with water (60 mL), and then dried over anhydrous Na_2SO_4 and then concentrated. The residue was purified by column chromatography to give **G3**.

To a mixture of $LiAlH_4$ (30 mmol) in THF (15 mL) was added a solution of **G3** (10 mmol) in THF (20 mL) dropwise in an ice-water bath. The mixture was refluxed for 6 hours and then stirred at room temperature for additional 16 hours. Then water (1.1 g) was added dropwise at 0 ^oC, and then followed by addition of 15% NaOH aqueous solution (1.1 mL) and water (3.3 mL). The resultant mixture was filtered, and the filtrate was concentrated to afford crude **G4** which was used in the next step without further purification.

Method B. To a mixture of **G5** (80 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.8 mmol), 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene (1.6 mmol) and t-BuONa (264 mmol) in THF (200 mL) was added **G6** (240 mmol). The resultant mixture was heated to 50 ^oC for 3 hours under argon atmosphere, and then cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column to afford **G7** as brown oil which was directly used for the next step without further purification.

To a mixture of **G7** (57.4 mmol) and ammonium acetate (861 mmol) in CH_3OH (160 mL) was added $NaBH_3CN$ (7.23 g, 114.8 mmol) in portions at 0 ^oC. The resultant mixture was stirred from 0 ^oC to room temperature overnight and then basified by 2 M NaOH aqueous solution to pH 12-14. The mixture was extracted with CH_2Cl_2 (300 mL x 3), and the combined organic layers were washed with

brine, and then dried over anhydrous Na_2SO_4 and then concentrated to give **G4** as yellow oil which was directly used for the next step without purification.

General Procedure for the Preparation of G8. A solution of **G4** (54.4 mmol) and formic acid (1 mL) in ethyl formate (100 mL) was heated at 90 ^oC overnight, and then concentrated to give **G8** as yellow oil which was directly used for the next step without purification.

General Procedure for the Preparation of G9. To a solution of **G8** (52 mmol) in acetonitrile (150 mL) was added $POCl_3$ (62.4 mmol). The resultant mixture was heated at 90 ^oC for 2 hours and then concentrated. The residue was dissolved in acetonitrile (50 mL), and then cooled to 0 ^oC. Ammonium hydroxide was added dropwise at 0 ^oC to basify the mixture. The mixture was extracted with CH_2Cl_2 (200 mL x 5), and the combined organic layers were washed with brine, and then dried over anhydrous Na_2SO_4 and then concentrated to give crude **G9** as dark-green oil which was directly used for the next step without purification.

General Procedure for the Preparation of G11. A mixture of **G9** (46 mmol) and ethyl 2-(ethoxymethylene)-3-oxo-butanoate **G10** (138 mmol) in EtOH (150 mL) was heated to 100 ^oC overnight. The mixture was concentrated, and the residue was purified by flash column chromatography to afford **G11** as red oil which was directly used in the next step without further purification.

General Procedure for the Preparation of G12. A mixture of **G11** (25 mmol) and p-chloranil (25 mmol) in DME (100 mL) was heated to 70 ^oC for 3 hours under argon atmosphere. Removed the solvent and the residue was dissolved in CH_2Cl_2 (150 mL) and then washed with saturated $NaHCO_3$ aqueous solution (100 mL x 5). The separated organic layer was washed with water and brine, and then dried over anhydrous Na_2SO_4, and then concentrated. The residue was purified by flash column chromatography to afford **G12** as brown oil.

General Procedure for the Preparation of G13. To a mixture of **G12** (4.8 mmol) in CH_3OH (32 mL) and H_2O (8 mL) was added lithium hydroxide monohydrate (19.2 mmol). The resultant reaction mixture was stirred at room temperature for 3 hours. The mixture was diluted with CH_2Cl_2, and then acidified by 1 M hydrochloric acid to pH 2-3, and then extracted with CH_2Cl_2 (100 mL x 3). The

combined organic layers were washed with brine, and then dried over anhydrous Na_2SO_4 and then concentrated. The residue was washed with EtOH/Et_2O to afford **G13** as a white or yellow solid.

General Procedure for the Preparation of G14. A mixture of ethyl 9-benzyloxy-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylate (**25**, 5.2 g) and 10% palladium on carbon (300 mg) in THF/MeOH (1/1, 40 mL) was stirred under hydrogen atmosphere for 12 hours. The mixture was filtered through celite pad, and filtrate was concentrated under reduced pressure to afford **G14** as yellow solid (4.2 g).

General Procedure for the Preparation of G16. To a solution of **G14** (0.29 mmol) in DMF was added **G15** (0.87 mmol) and K_2CO_3 (0.58 mmol). The mixture was stirred for 2 hours at 80 ^oC, and then cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* to give crude **G16** which was used for the next step without further purification.

General Procedure for the Preparation of G17. To a solution of crude **G16** (0.25 mmol) in MeOH (9 mL) and water (3 mL) was added $LiOH{\cdot}H_2O$ (36.7 mg). The mixture was stirred for 2 hours and then concentrated under reduced pressure. The residue was dissolved in water (5 mL), and then acidified with 6 M hydrochloric acid and filtered. The filter cake was dried *in vacuo* to give **G17**.

General Procedure for the Preparation of G19. To a solution of 9-benzyloxy-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (**25** 0.2 mmol) in DMF (10 mL) was added HATU (0.3 mmol), triethylamine (100 μL) and amine **G18** (2.2 mmol). The resultant solution was stirred at room temperature for 3h and then purified by preparative HPLC to afford **G19**.

For the Following Compounds, Except Described Specifically, All the Analogues Are Prepared in Analogy to General Procedure from the Commercially Available Building Blocks.

9,10-Dimethoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.54 (s, 1H), 7.47 (s, 1H), 7.02 (s, 1H), 4.95 (m, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.40-3.28 (m, 1H), 2.91(dd, 1H), 1.20 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 316.

9-Methoxy-6,10-dimethyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (5). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.91 (s, 1H), 7.30 (s, 1H), 6.99 (s, 1H), 4.96 (m, 1H), 3.89 (s, 3H), 3.39 (dd, 1H), 2.95 (d, 1H), 2.21 (s, 3H), 1.20 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 300.

10-Chloro-9-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (6). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.29 (br. s, 1H), 7.51 (s, 1H), 6.75 (s, 1H), 6.61 (br. s, 1H), 4.25 - 4.18 (m, 1H), 3.55 (s, 3H), 3.07 - 2.97 (m, 1H), 2.62 - 2.50 (m, 1H), 0.90 (br. s, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 320.

10-Ethoxy-9-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (7). ^{1}H NMR (400 MHz, CD_3OD): δ 8.80 (s, 1H), 7.45 (s, 1H), 7.29 (s, 1H), 7.02 (s, 1H), 4.18 (m, 2H), 3.95 (s, 3H), 3.43 (m, 2H), 2.90 (m, 1H), 1.47 (t, 3H), 1.35 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 330.

9-Methoxy-6-methyl-2-oxo-10-propoxy-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (8). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H), 7.22 (s,1H), 7.10 (s, 1H), 6.77 (s, 1H), 4.56 (m, 1H), 4.05 (t, 2H), 3.97 (s, 3H), 3.43 (m ,1H), 2.85 (m, 1H), 1.94 (m, 2H), 1.40 (d, 3H), 1.11 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 344.

10-Benzyloxy-9-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (9). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H), 7.44 (m, 5H), 7.25 (s, 1H), 6.95 (s, 1H), 6.79 (s, 1H), 5.22 (s, 2H), 4.55 (m, 1H), 3.99 (s, 3H), 3.43 (dd ,1H), 2.85(d, 1H), 1.38 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 392.

9-Methoxy-10-(2-methoxyethoxy)-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 7.52 (m, 1H), 7.25 (s, 1H), 6.95 (s, 1H), 4.71 (q, 1H), 4.24 - 4.12 (m, 2H), 3.89 (s, 3H), 3.70 (t, 2H), 3.4 (s, 3H), 3.33 (m, 1H), 2.99 (d, 1H), 1.38 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 360.

9-Hydroxy-10-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (11). ^{1}H NMR (400 MHz, CD_3OD): δ 8.80 (s, 1H), 7.47 (s, 1H), 7.33 (s, 1H), 6.83 (s,1H), 4.91-4.83 (m, 1H), 3.99 (s, 3H), 3.45-3.39 (m, 1H), 2.91-2.87 (m, 1H), 1.35 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 302.

10-Methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (12). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 7.26 (d, 2H), 7.21 (s, 1H), 7.09 (dd, 1H), 4.58 (m, 1H), 3.90 (s, 3H), 3.43 (dd, 1H), 2.90 (d, 1H), 1.38 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 286.

9-Ethoxy-10-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (13). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.82 (s, 1H), 7.52 (s, 1H), 7.46 (s, 1H), 7.00 (s, 1H), 4.97-4.91 (m, 1H), 4.11 (q, 2H), 3.88 (s, 3H), 3.34-3.30 (m, 1H), 2.91-2.87 (m, 1H), 1.37 (t, 3H), 1.19 (d, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 330.

9-Benzyloxy-10-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (14). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (s, 1H), 7.56 (s, 1H), 7.48-7.35 (m, 6H), 7.15 (s, 1H), 5.18 (s, 2H), 5.01-4.91 (m, 1H), 3.89 (s, 3H), 3.41-3.37 (m, 1H), 2.91-2.87 (m, 1H), 1.20 (d, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 392.

9-Bromo-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (15). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.59 (s, 1H), 7.71 - 7.58 (m, 2H), 7.54 (s, 1H), 7.19 (s, 1H), 4.65 - 4.54 (m, 1H), 3.55 - 3.43 (m, 1H), 2.95 (dd, 1H), 1.39 (d, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 334.

9-Ethoxy-10-hydroxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (19). ^{1}H NMR (400MHz, DMSO-d_6): δ 9.26 (br. s, 1H), 8.81 (s, 1H), 7.36 (s, 1H), 7.04 (s, 1H), 6.96 (s, 1H), 4.92 (t, 1H), 4.13 (q, 2H), 3.32 (dd, 1H), 2.89 - 2.81 (m, 1H), 1.38 (t, 3H), 1.19 (d, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 316.

8-Chloro-9-methoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (20). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 7.74 (d, 1H), 7.07 (s, 1H), 7.04 (d, 1H), 4.61 (m, 1H), 4.06 (s, 3H), 3.46 (m, 1H), 3.27 (m, 1H), 1.38 (d, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 320.

9,11-Dimethoxy-6-methyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (21). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.54 (s, 1H), 6.69 (d, 1H), 6.64 (d, 1H), 4.91-4.88 (m, 1H), 3.95 (s, 3H), 3.88(s, 3H), 3.34-3.30 (m, 1H), 2.93-2.89 (m, 1H), 1.17 (d, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 316.

6-Ethyl-9,10-dimethoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (22). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.53 (s, 1H), 7.47 (s, 1H), 7.04 (s, 1H), 4.72 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.36 (dd, 1H), 3.02 (d, 1H), 1.52-1.44 (m, 2H), 0.81 (t, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 330. HRMS: calcd (MH$^+$) 330.1342, exp (MH$^+$) 330.1341.

(6*R*)-(+)-6-Ethyl-9,10-dimethoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid ((*R*)-

22). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.53 (s, 1H), 7.47 (s, 1H), 7.04 (s, 1H), 4.72 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.36 (dd, 1H), 3.02 (d, 1H), 1.52-1.44 (m, 2H), 0.81 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 330. $[\alpha]_D^{20}$ = +121.21 (0.165%, CH_3CN), the absolute stereochemistry was determined by the X-ray diffraction study of its (6*S*)-enantiomer (*S*)-**22**.

(6*S*)-(-)-6-Ethyl-9,10-dimethoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid ((*S*)-22). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.53 (s, 1H), 7.47 (s, 1H), 7.04 (s, 1H), 4.72 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.36 (dd, 1H), 3.02 (d, 1H), 1.52-1.44 (m, 2H), 0.81 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 330, the absolute stereochemistry was determined by X-ray diffraction study.

,10-Dimethoxy-2-oxo-6-propyl-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (23). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.21 (s, 1H), 7.11 (s, 1H), 6.77 (s, 1H), 4.40 - 4.26 (m, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.44 (dd, 1H), 2.94 (d, 1H), 1.70 - 1.51 (m, 2H), 1.40 - 1.19 (m, 2H), 0.90 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 344.

6-Isobutyl-9,10-dimethoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (24). ^{1}H NMR (400MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.53 (s, 1H), 7.48 (s, 1H), 7.05 (s, 1H), 4.88 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.31 (d, 1H), 2.99 (d, 1H), 1.46 (qd, 1H), 1.34 (t, 2H), 0.88 (d, 3H), 0.83 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 358.

9-Benzyloxy-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (25). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.46-7.30 (m, 5H), 7.21 (s, 1H), 7.10 (s, 1H), 6.78 (s, 1H), 5.22 (d, 2H), 4.24-4.19 (m, 1H), 3.96 (s, 3H), 3.37 (d, 1H), 2.88 (d, 1H), 1.86-1.59 (m, 2H), 0.90 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 406. HRMS: calcd (MH^+) 406.1655, exp (MH^+) 406.1652.

9-Ethoxy-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (26). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 6.76 (s, 1H), 4.31 - 4.15 (m, 3H), 3.96 (s, 3H), 3.42 (dd, 1H), 2.94 (d, 1H), 1.66-1.62 (m, 2H), 1.54 (t, 3H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 344. HRMS: calcd (MH^+) 344.1498, exp (MH^+) 344.1499.

6-Ethyl-10-methoxy-2-oxo-9-propoxy-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (27). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.52 (s, 1H), 7.46 (s, 1H), 7.04 (s, 1H), 4.70 (m, 1H),

4.04-3.98 (m, 2H), 3.88(s, 3H), 3.33 (d, 1H), 3.00 (d, 1H), 1.77 (m, 2H), 1.55-1.35 (m, 2H), 0.99 (t, 3H), 0.87 (m, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 358.

9-Butoxy-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (28). ^1H NMR (400 MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.18 (s, 1H), 7.09 (s, 1H), 6.75 (s, 1H), 4.25-4.21 (m, 1H), 4.10 (dt, 2H), 3.94 (s, 3H), 3.41 (d, 1H), 2.93 (d, 1H), 1.92-1.85 (m, 2H), 1.70-1.58 (m, 2H), 1.58-1.49 (m, 2H), 1.01 (t, 3H), 0.93 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 372.

6-Ethyl-9-isobutoxy-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (29). ^1H NMR (400MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.20 (s, 1H), 7.09 (s, 1H), 6.75 (s, 1H), 4.27 - 4.17 (m, 1H), 3.95 (s, 3H), 3.86 (d, 2H), 3.46 - 3.36 (m, 1H), 2.97 - 2.90 (m, 1H), 2.28 - 2.17 (m, 1H), 1.74 - 1.62 (m, 2H), 1.10 (d, 6H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 372.

9-(Cyclopropylmethoxy)-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (30). ^1H NMR (400MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 6.74 (s, 1H), 4.23 (q, 1H), 4.00 - 3.90 (m, 5H), 3.40 (s, 1H), 2.93 (d, 1H), 1.74 - 1.63 (m, 2H), 1.44 - 1.34 (m, 1H), 0.94 (t, 3H), 0.77 - 0.69 (m, 2H), 0.46 - 0.38 (m, 2H). MS obsd. (ESI^+) [$(M+H)^+$]: 370.

9-(2-Cyclohexylethoxy)-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (31). ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.18 (s, 1H), 7.08 (s, 1H), 6.74 (s, 1H), 4.25-4.20 (m, 1H), 4.11 (dt, 2H), 3.93 (s, 3H), 3.41 (dd, 1H), 2.92 (d, 1H), 1.82-1.52 (m, 9H), 1.34-1.17 (m, 4H), 1.05-0.97 (m, 2H), 0.93 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 426.

9-(2,2-Difluoroethoxy)-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (32). ^1H NMR (400MHz, $CDCl_3$): δ 8.53 (s, 1H), 7.25 (s, 1H), 7.12 (s, 1H), 6.83 (s, 1H), 6.32 (t, 0.3H), 6.19 (t, 0.53H), 6.05 (t, 0.32H), 4.41 - 4.17 (m, 3H), 3.96 (s, 3H), 3.43 (d, 1H), 2.96 (d, 1H), 1.84 - 1.65 (m, 2H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 380.

6-Ethyl-9-isopropoxy-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (33). ^1H NMR (400 MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.19 (s, 1H), 7.09 (s, 1H), 6.76 (s, 1H), 4.68-4.65 (m, 1H), 4.25-4.21 (m, 1H), 3.93 (s, 3H), 3.40 (d, 1H), 2.92 (d, 1H), 1.69-1.60 (m, 2H), 1.45 (d, 3H), 1.43 (d, 3H), 0.93 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 358.

6-Ethyl-10-methoxy-2-oxo-9-propyl-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (34). ^{1}H NMR(400 MHz, DMSO-d_6): δ 8.84 (s, 1H), 7.57 (s, 1H), 7.51 (s, 1H), 7.19 (s, 1H), 4.73 (m, 2H), 3.91 (s, 3H), 2.99 (d, 1H), 1.62-1.53 (m, 2H), 1.51-1.39 (m, 5H), 0.94-0.88 (m, 2H), 0.79 (m, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 342.

6-Ethyl-2-oxo-6,7,10,11-tetrahydro-12H-[1,5]benzodioxepine[7,6-a]quinolizine-3-carboxylic acid (36). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 8.75 (s, 1H), 7.59 (s, 1H), 7.24 (s, 1H), 6.99 (s, 1H), 4.59 (br d, 1H), 4.20 - 4.38 (m, 4H), 3.36 - 3.44 (m, 1H), 3.05 (dd, 1H), 2.20 - 2.36 (m, 2H), 1.50 - 1.70 (m, 2H), 0.92 (s, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 342.

6-Ethyl-2-oxo-6,7,10,11-tetrahydro-[1,4]benzodioxino[7,6-a]quinolizine-3-carboxylic acid (37). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 8.49 (s, 1 H), 7.29 (s, 1 H), 7.04 (s, 1 H), 6.81 (s, 1 H), 4.31 - 4.42 (m, 3 H), 4.17 - 4.27 (m, 1 H), 3.31 - 3.40 (m, 1 H), 2.92 (dd, 1 H), 1.55 - 1.74 (m, 3 H), 0.93 (t, 3 H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 328. HRMS: calcd (MH$^+$) 328.1185, exp (MH$^+$) 328.1180.

6,10-Diethyl-9-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (38). ^{1}H NMR (400 MHz, CD_3OD): δ 8.74 (s, 1H), 7.74 (s, 1H), 7.28 (s, 1H), 6.99 (s, 1H), 4.59 (m, 1H), 3.97 (s, 3H), 3.43 (m ,1H), 3.13 (d, 1H), 2.73 (m, 2H), 1.64 (m, 2H), 1.24 (t, 3H), 0.93 (t, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 328. HRMS: calcd (MH$^+$) 328.1549, exp (MH$^+$) 328.1546.

10-Cyclopropyl-6-ethyl-9-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (39). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H), 7.23 (s, 1H), 7.08 (s, 1H), 6.73 (s, 1H), 4.23 (m, 1H), 3.97 (s, 3H), 3.42 (m, 1H), 2.98 (m, 1H), 2.17 (m, 1H), 1.64 (m, 2H), 1.03 (m, 2H), 0.95 (t, 3H), 0.70 (m, 2H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 340.

9-(2-Amino-2-oxo-ethoxy)-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (40). ^{1}H NMR (400 MHz, METHANOL-d_4) δ 8.07 (s, 1H), 7.39 (s, 1H), 7.01 (s, 1H), 6.97 (s, 1H), 4.37-4.44 (m, 1H), 4.17 (m, 2H), 3.95 (s, 3H), 3.54-3.66 (m, 2H), 3.36-3.46 (m, 3H), 3.04 (m, 1H), 1.54-1.71 (m, 2H), 0.93 (t, 3H). MS obsd. (ESI$^+$) [(M+H)$^+$]: 373.

9-(2-Acetamidoethoxy)-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (41). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.21 (s, 1H), 7.10 (s, 1H), 6.81 (s, 1H), 6.24

(br. s, 1H), 4.34 - 4.09 (m, 3H), 3.96 (s, 3H), 3.83 - 3.64 (m, 2H), 3.49 - 3.32 (m, 1H), 2.95 (d, 1H), 2.04 (s, 3H), 1.64-1.53 (m, 2H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 401.

6-Ethyl-9-[2-(methanesulfonamido)ethoxy]-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (42). ^1H NMR (400MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.55 (s, 1H), 7.48 (s, 1H), 7.36 - 7.26 (m, 1H), 7.10 - 7.04 (m, 1H), 4.77 - 4.64 (m, 1H), 4.18 - 4.06 (m, 2H), 3.89 (s, 3H), 3.47 - 3.34 (m, 3H), 3.18 (d, 1H), 3.00 (s, 3H), 1.60 - 1.40 (m, 2H), 0.81 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 437.

6-Ethyl-10-methoxy-2-oxo-9-[2-(2-oxopyrrolidin-1-yl)ethoxy]-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (43). ^1H NMR (400MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 6.79 (s, 1H), 4.27-4.22 (m, 3H), 3.94 (s, 3H), 3.80 - 3.74 (m, 2H), 3.72 - 3.63 (m, 2H), 3.41 (dd, 1H), 2.95 (dd, 1H), 2.42 (t, 2H), 2.13 - 2.02 (m, 2H), 1.74 - 1.63 (m, 2H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 427.

6-Ethyl-10-methoxy-2-oxo-9-(2-pyrrolidin-1-ylethoxy)-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (44). ^1H NMR (400MHz, CD_3OD): δ 8.68 (s, 1H), 7.44 (s, 1H), 7.24 (s, 1H), 7.02 (s, 1H), 4.56 (d, 1H), 4.29 (br. s, 2H), 3.96 (s, 3H), 3.46 - 3.35 (m, 1H), 3.21 - 3.01 (m, 3H), 2.92 (br. s, 4H), 1.93 (br. s, 4H), 1.74 - 1.52 (m, 2H), 0.92 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 413.

6-Ethyl-10-methoxy-9-(2-morpholinoethoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (45). ^1H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.59 (s, 1H), 7.51 (s, 1H), 7.13 (s, 1H), 4.75-4.70 (m, 1H), 4.45 (t, 2H), 3.91 (s, 3H), 3.62-3.50 (m, 10H), 3.36 (dd, 1H), 3.01 (d, 1H), 1.54-1.42 (m, 2H), 0.81 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 429.

6-Ethyl-9-(2-hydroxyethoxy)-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (46). ^1H NMR (400 MHz, CD_3OD): δ 8.72 (s, 1H), 7.47 (s, 1H), 7.30 (s, 1H), 7.03 (s, 1H), 4.60-4.58 (m, 1H), 4.20-4.17 (m, 2H), 3.97 (s, 3H), 3.96-3.93 (m, 1H), 3.42 (dd, 1H), 3.37 (s, 1H), 3.09 (d, 1H), 1.70-1.59 (m, 2H), 0.93 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 360.

6-Ethyl-9-(3-hydroxypropoxy)-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (47). ^1H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.52 (s, 1H), 7.46 (s, 1H), 7.05 (s, 1H), 4.73-4.68 (m, 1H), 4.57 (t, 1H), 4.15-4.09 (m, 2H), 3.88 (s, 3H), 3.59-3.55 (m, 2H), 3.35-3.30 (m,

2H), 3.01 (d, 1H), 1.93-1.87 (m, 2H), 1.53-1.43 (m, 2H), 0.80 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 374.

6-Ethyl-10-methoxy-9-(2-methoxyethoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (48). ^1H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.53 (s, 1H), 7.47 (s, 1H), 7.05 (s, 1H), 4.71 (q, 1H), 4.24 - 4.12 (m, 2H), 3.89 (s, 3H), 3.70 (t, 2H), 3.37 - 3.33 (m, 4H), 2.99 (d, 1H), 1.58 - 1.38 (m, 2H), 0.80 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 374. HRMS: calcd (MH^+) 374.1604, exp (MH^+) 374.1612.

6-Ethyl-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (49). ^1H NMR (400MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.19 (s, 1H), 7.09 (s, 1H), 6.80 (s, 1H), 4.23-4.19 (m, 3H), 3.95 (s, 3H), 3.66-3.56 (m, 2H), 3.46-3.37 (m, 4H), 2.94 (d, 1H), 2.17 (q, 2H), 1.81 - 1.66 (m, 2H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 388.

6-Ethyl-10-methoxy-9-[2-(2-methoxyethoxy)ethoxy]-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (50). ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.18 (s, 1H), 7.08 (s, 1H), 6.81 (s, 1H), 4.29-4.26 (m, 2H), 4.24-4.19 (m, 1H), 3.95-3.92 (m, 2H), 3.93 (s, 3H), 3.76-3.73 (m, 2H), 3.60-3.57 (m, 2H), 3.42-3.37 (m, 1H), 3.40 (s, 3H), 2.92 (dd, 1H), 1.70-1.64 (m, 2H), 0.92 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 418.

6-Ethyl-10-methoxy-2-oxo-9-(tetrahydropyran-4-ylmethoxy)-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (51). ^1H NMR (400MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.20 (s, 1H), 7.11 (s, 1H), 6.75 (s, 1H), 4.25 (br. s., 1H), 4.06 (dd, 2H), 3.99 - 3.88 (m, 5H), 3.58 - 3.34 (m, 3H), 2.94 (d, 1H), 2.29 - 2.15 (m, 1H), 1.89 - 1.79 (m, 2H), 1.71 - 1.58 (m, 2H), 1.57 - 1.43 (m, 2H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 414. HRMS: calcd (MH^+) 414.1917, exp (MH^+) 414.1907.

9-(2-Ethoxyethoxy)-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (52). ^1H NMR (400MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 6.84 (s, 1H), 4.34 - 4.18 (m, 3H), 3.95 (s, 3H), 3.88 (t, 2H), 3.64 (q, 2H), 3.42 (dd, 1H), 2.93 (d, 1H), 1.76 - 1.64 (m, 2H), 1.26 (t, 3H), 0.94 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 388.

9-Benzyloxy-6-ethyl-10-methoxy-3-(4-methylpiperazine-1-carbonyl)-6,7-dihydrobenzo[a]quinolizin-2-one (54). ^1HNMR (400 MHz, CD_3OD): δ 8.60 (s, 1H), 7.41 (m, 6H),

7.04 (s, 2H), 5.22 (s, 2H), 4.48 (m, 1H), 3.96 (s, 3H), 3.34 (m, 1H), 3.03 (m,2H), 2.98 (s, 3H), 2.61 (m, 2H), 1.63 (m, 2H), 0.90 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 419.

9-Benzyloxy-6-ethyl-10-methoxy-2-oxo-N-propyl-6,7-dihydrobenzo[a]quinolizine-3-carboxamide (57). ^{1}HNMR (400 MHz, CD_3OD): δ 8.59 (s, 1H), 7.40 (m, 6H), 7.03 (s, 2H), 5.21 (s, 2H), 4.47 (m, 2H), 3.95 (s, 3H), 3.75 (m, 2H), 3.54 (m, 2H), 3.33 (m, 1H), 3.01 (m,2H), 2.99 (d, 1H), 1.57 (m, 2H), 0.89 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 447.

9-Benzyloxy-6-ethyl-N-(2-hydroxyethyl)-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxamide (58). ^{1}HNMR (400 MHz, CD_3OD): δ 8.59 (s, 1H), 7.41 (m, 6H), 7.05 (s, 2H), 5.22 (s, 2H), 4.48 (m, 2H), 3.96 (s, 3H), 3.40 (m, 2H), 3.01 (m,2H), 2.99 (d, 1H), 1.65 (m, 2H), 0.87 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 449.

9-Benzyloxy-6-ethyl-10-methoxy-N-(2-methoxy-ethyl)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxamide (59). ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H), 7.26 (m, 6H), 7.22 (s, 1H), 6.96 (s, 1H), 6.76 (s, 1H), 5.21 (s, 2H), 4.19 (m, 1H), 3.96 (s, 3H), 3.68 (m, 4H), 3.43(s, 3H), 3.30 (m, 1H), 2.81 (d, 1H), 1.56 (m,1H), 0.89 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 463. HRMS: calcd (MH^+) 463.2233, exp (MH^+) 463.2244.

9-Benzyloxy-6-ethyl-N-(2-hydroxy-1-methyl-ethyl)-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxamide (60). ^{1}HNMR (400 MHz, CD_3OD): δ 8.67 (s, 1H), 7.50 (m, 6H), 7.14 (s,1H), 7.06 (s, 1H), 5.21 (s,2H), 4.47 (m, 1H), 4.18 (m,1H), 3.98 (s, 3H), 3.61 (m, 1H), 3.45 (m, 2H), 3.33 (m, 1H), 3.02 (d, 1H), 1.62 (m, 3H), 1.29 (m, 3H), 0.92 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 463.

9-Benzyloxy-6-ethyl-10-methoxy-N-(2-methoxy-1-methyl-ethyl)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxamide (61). ^{1}H NMR (400 MHz, CD_3OD): δ 8.57 (s, 1H), 7.40 (m, 6H), 7.03 (s,2H), 5.20 (s, 2H), 4.48 (m, 2H), 4.27 (m, 2H), 3.95 (s, 3H), 3.49 (s, 3H), 3.02 (m, 2H), 1.59 (m, 2H), 1.28 (d, 3H), 0.88 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 477.

9-Benzyloxy-6-ethyl-10-methoxy-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carbohydroxamic acid (62). ^{1}HNMR (400 MHz, CD_3OD): δ 8.59 (s, 2H), 7.40 (m, 6H), 7.04 (d, 2H), 5.21 (s, 2H), 4.60 (m,

1H), 4.49 (m, 1H), 3.96 (s, 3H), 3.33(m, 1H), 3.03 (d, 1H), 1.59 (m, 2H), 0.89 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 421.

9-Benzyloxy-6-ethyl-10-methoxy-N-methylsulfonyl-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxamide (63). ^{1}HNMR (400 MHz, CD_3OD): δ 8.63 (s, 1H), 7.42 (m, 6H), 7.05 (s, 2H), 5.20 (s, 2H), 4.47 (m, 1H), 3.95 (s, 3H), 3.33 (m, 1H), 3.01 (m,2H), 2.96 (s, 3H), 2.63 (m, 2H), 1.64 (m, 2H), 0.92 (t, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 483.

10-Methoxy-6-isopropyl-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (64). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.52 (s, 1H), 7.45 (s, 1H), 7.09 (s, 1H), 4.43 (dd, 1H), 4.08 (m, 2H), 3.88 (s, 3H), 3.48 (t, 2H), 3.13-3.17 (m, 2H), 2.01 (m, 2H), 1.61-1.66 (m, 1H), 0.88 (d, 3H), 0.71 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 402.

(6*S*)-(+)-10-Methoxy-6-isopropyl-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid ((*S*)-64). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.52 (s, 1H), 7.45 (s, 1H), 7.09 (s, 1H), 4.43 (dd, 1H), 4.08 (m, 2H), 3.88 (s, 3H), 3.48 (t, 2H), 3.13-3.17 (m, 2H), 2.01 (m, 2H), 1.61-1.66 (m, 1H), 0.88 (d, 3H), 0.71 (d, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.8, 178.7, 178.6, 178.2, 167.1, 167.0, 166.7, 166.5, 166.4, 166.2, 151.8, 148.8, 147.4, 127.4, 118.8, 114.2, 114.1, 113.8, 113.7, 113.6, 113.5, 113.2, 113.0, 112.9, 112.8, 109.7, 68.9, 67.3, 66.1, 58.4, 56.5,29.4. 29.3, 19.5. MS obsd. (ESI^+) [$(M+H)^+$]: 402. HRMS: calcd (MH^+) 402.1917, exp (MH^+) 402.1919. $[\alpha]_D^{20}$ = +71.19° (0.059%, CH_3CN), the absolute stereochemistry was determined by X-ray diffraction study (Figure 2).

(6*R*)-(-)-10-Methoxy-6-isopropyl-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid ((*R*)-64). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.52 (s, 1H), 7.45 (s, 1H), 7.09 (s, 1H), 4.43 (dd, 1H), 4.08 (m, 2H), 3.88 (s, 3H), 3.48 (t, 2H), 3.13-3.17 (m, 2H), 2.01 (m, 2H), 1.61-1.66 (m, 1H), 0.88 (d, 3H), 0.71 (d, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 402. $[\alpha]_D^{20}$ = -68.73° (0.175%, CH_3CN).

6-*tert*-Butyl-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (65). ^{1}H NMR (400 MHz, $CDCl_3$): δ 15.97-16.02 (s, 1H), 8.37-8.53 (s, 1H), 7.14-7.17 (s, 1H), 7.06-7.09 (s, 1H), 6.74-6.78 (s, 1H), 4.15-4.24 (m, 2H), 4.02-4.06 (m, 1H), 3.92-3.96 (s,

3H), 3.58-3.64 (m, 2H), 3.41-3.48 (m, 1H), 3.37-3.40 (s, 3H), 3.15-3.23 (m, 1H), 2.13-2.21 (m, 2H), 0.84 (s, 9H). MS obsd. (ESI^+) [$(M+H)^+$]: 416. HRMS: calcd (MH^+) 416.2073, exp (MH^+) 416.2071.

10-Methoxy-9-(3-methoxypropoxy)-6-(1-methylcyclopropyl)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (66). ^1H NMR (400MHz, DMSO-*d6*): δ 8.80 (s, 1H), 7.51 (s, 1H), 7.49 (s, 1H), 7.07 (s, 1H), 4.17 - 4.04 (m, 3H), 3.88 (s, 3H), 3.48 (t, 2H), 3.42 - 3.35 (m, 1H), 3.26 (s, 3H), 3.16 (dd, 1H), 1.99 (quin, 2H), 0.91 - 0.77 (m, 1H), 0.63 (s, 3H), 0.52 (td, 1H), 0.45 - 0.33 (m, 2H). MS obsd. (ESI^+) [$(M+H)^+$]: 414.

10-Methoxy-9-(3-methoxypropoxy)-2-oxo-6-(2,2,2-trifluoroethyl)-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (67). ^1H NMR (400MHz, DMSO-*d6*): δ 8.75 (s, 1H), 7.56 (s, 1H), 7.49 (s, 1H), 7.04 (s, 1H), 5.28 (m, 1H), 4.10 (t, 2H), 3.90 (s, 3H), 3.48 (t, 2H), 3.42 (dd, 1H), 3.26 (s, 3H), 3.01 (d, 1H), 2.69 - 2.56 (m, 2H), 1.99 (quin, 2H). MS obsd. (ESI^+) [$(M+H)^+$]: 442.

10-Methoxy-6-(methoxymethyl)-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a] quinolizine-3-carboxylic acid (68). ^1H NMR (400 MHz, DMSO-*d6*): δ 8.63 (s, 1H), 7.53 (s, 1H), 7.48 (s, 1H), 7.01 (s, 1H), 4.91-5.01 (m, 1H), 4.10 (m, 2H), 3.89 (s, 3H), 3.48 (m, 6H), 3.25-3.29 (m, 3H), 3.18 (s, 3H), 1.99 (m, 2H). MS obsd. (ESI^+) [$(M+H)^+$]: 404.

10-Methoxy-6-(2-methoxy-1,1-dimethyl-ethyl)-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo [a]quinolizine-3-carboxylic acid (69). ^1H NMR (400 MHz, DMSO-*d6*): δ 8.56 (s, 1H), 7.47 (s, 1H), 7.43 (s, 1H), 7.08 (s, 1H), 4.64 (m, 1H), 4.01-4.19 (m, 2H), 3.87 (s, 3H), 3.48 (m, 2H), 3.26 (s, 3H), 3.18-3.24 (m, 2H), 2.80-3.01 (m, 2H), 1.99 (m, 2H), 0.87 (s, 3H), 0.46 (s, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 446. HRMS: calcd (MH^+) 446.2179, exp (MH^+) 446.2170.

6-(1-Hydroxy-1-methyl-ethyl)-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo [a]quinolizine-3-carboxylic acid (70). ^1H NMR (400 MHz, DMSO-*d6*) δ 8.62 (s, 1H), 7.48 (s, 1H), 7.45 (s, 1H), 7.04 (s, 1H), 5.00 (s, 1H), 4.47 (d, 1H), 4.09 (d, 2H), 3.87 (s, 3H), 3.48 (s, 2H), 3.26 (s, 3H), 3.17 (m, 2H), 1.99 (m, 2H), 1.21 (s, 3H), 0.50 (s, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 418. HRMS: calcd (MH^+) 418.1866, exp (MH^+) 418.1864.

6-(2-Hydroxy-1,1-dimethyl-ethyl)-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a] quinolizine-3-carboxylic acid (71). ^1H NMR (400 MHz, DMSO-*d6*): δ 8.68 (s,

1H), 7.46 (s, 1H), 7.43 (s, 1H), 7.08 (s, 1H), 5.08 (m, 1H), 4.67 (m, 1H), 4.10 (m, 2H), 3.87 (s, 3H), 3.48 (m, 2H), 3.33 (s, 3H), 3.19-3.25 (m, 2H), 2.96-3.11 (m, 2H), 1.99 (m, 2H), 0.76 (s, 3H), 0.40 (s, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 432.

(*6S*)-(+)-6-(2-Hydroxy-1,1-dimethyl-ethyl)-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo [a]quinolizine-3-carboxylic acid ((*S*)-71). ^1H NMR (400 MHz, DMSO-*d6*): δ 8.68 (s, 1H), 7.46 (s, 1H), 7.43 (s, 1H), 7.08 (s, 1H), 5.08 (m, 1H), 4.67 (m, 1H), 4.10 (m, 2H), 3.87 (s, 3H), 3.48 (m, 2H), 3.33 (s, 3H), 3.19-3.25 (m, 2H), 2.96-3.11 (m, 2H), 1.99 (m, 2H), 0.76 (s, 3H), 0.40 (s, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 432. $[\alpha]_D^{20}$ = +90.00° (0.100%, CH_3CN).

(*6R*)-(-)-6-(2-Hydroxy-1,1-dimethyl-ethyl)-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo [a]quinolizine-3-carboxylic acid ((*R*)-71).^1H NMR (400 MHz, DMSO-*d6*): δ 8.68 (s, 1H), 7.46 (s, 1H), 7.43 (s, 1H), 7.08 (s, 1H), 5.08 (m, 1H), 4.67 (m, 1H), 4.10 (m, 2H), 3.87 (s, 3H), 3.48 (m, 2H), 3.33 (s, 3H), 3.19-3.25 (m, 2H), 2.96-3.11 (m, 2H), 1.99 (m, 2H), 0.76 (s, 3H), 0.40 (s, 3H). MS obsd. (ESI^+) [$(M+H)^+$]: 432.

10-Methoxy-9-(3-methoxypropoxy)-6-(*o*-tolyl)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (72). ^1H NMR (400 MHz, CD_3OD): δ 1.95 - 2.07 (m, 2 H), 2.53 (s, 3 H), 3.29 (s, 3 H), 3.35 (d, 1 H) ,3.53 (td, 2 H), 3.62 (dd, 1 H), 3.95 (s, 3 H), 4.02 - 4.11 (m, 2 H), 4.61 (s, 1 H), 6.06 (br. s., 1 H), 6.56 (d, 1 H), 6.82 (s, 1 H), 7.00 (t, 1 H), 7.18 (t, 1 H), 7.24 - 7.31 (m, 1 H), 7.42 (s, 1 H), 7.51 (s, 1 H), 8.54 (s, 1 H). MS obsd. (ESI^+) [$(M+H)^+$]: 450.

10-Methoxy-9-(3-methoxypropoxy)-2-oxo-6-phenyl-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (73). ^1H NMR (400 MHz, DMSO-*d6*): δ 1.87 - 1.98 (m, 2 H) 3.21 (s, 3 H) 3.42 (t, 3 H) 3.66 (dd, 1 H) 3.85 (s, 3 H) 4.00 (t, 2 H) 6.08 (br. s., 1 H) 6.93 (s, 1 H) 6.98 (d, 2 H) 7.19 - 7.33 (m, 3 H) 7.51 (s, 1 H) 7.58 (s, 1 H) 8.82 (s, 1 H). MS obsd. (ESI^+) [$(M+H)^+$]: 435.

10-Methoxy-6-(4-methoxyphenyl)-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (74). ^1H NMR (400 MHz, $CDCl_3$): δ: 2.12 (q, 2 H), 3.28 (dd, 1 H), 3.34 (s, 3 H), 3.55 (t, 3 H), 3.60 (d, 1 H), 3.77 (s, 3 H) 3.94 (s, 3 H), 4.13 (t, 2 H), 5.39 (s, 1 H), 6.69 (s, 1 H), 6.79 - 6.86 (m, 2 H), 6.88 - 6.95 (m, 2 H), 7.15 (s, 1 H) ,7.20 (s, 1 H), 8.45 (s, 1 H). MS obsd. (ESI^+) [$(M+H)^+$]: 466.

6-(3-Chlorophenyl)-10-methoxy-9-(3-methoxypropoxy)-2-oxo-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (75). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.85 (s, 1H), 7.58 (s, 1H), 7.52 (s, 1H), 7.23-7.35 (m, 2H), 7.18 (s, 1H), 6.94 (s, 1H), 6.80 (d, 1H), 6.08 (d, 1H), 4.02 (t, 2H), 3.85 (s, 3H), 3.66-3.70 (m, 1H), 3.42-3.50 (m, 3H), 3.21 (s, 3H), 1.94-2.01 (m, 2H). MS obsd. (ESI^+) [$(M+H)^+$]: 470.

10-Methoxy-9-(3-methoxypropoxy)-2-oxo-6-(3-thienyl)-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (76). ^{1}H NMR (400 MHz, DMSO-*d6*): δ 1.97 (q, 2 H) 3.26 (s, 3 H) 3.36 - 3.45 (m, 1 H) 3.49 (t, 2 H) 3.59 (dd, 1 H) 3.87 (s, 3 H) 4.10 (t, 2 H) 5.99 (br. s., 1 H) 6.83 (d, 1 H) 6.99 (s, 1 H) 7.11 (br. s., 1 H) 7.26 - 7.55 (m, 3 H) 8.74 (br. s., 1 H). MS obsd. (ESI^+) [$(M+H)^+$]: 442. HRMS: calcd (MH^+) 442.1234, exp (MH^+) 442.1230.

10-Methoxy-9-(3-methoxypropoxy)-2-oxo-6-(2-thienyl)-6,7-dihydrobenzo[a]quinolizine-3-carboxylic acid (77). ^{1}H NMR (400 MHz, DMSO-*d6*): δ: 8.99 (s, 1H), 7.50-7.53 (m, 2H), 7.33-7.38 (m, 1H), 7.08 (s, 1H), 7.03 (s, 1H), 6.86-7.01 (m, 1H), 6.35 (s, 1H), 4.08 (t, 2H), 3.87 (s, 3H), 3.66-3.70 (m, 1H), 3.46 (t, 2H), 3.43-3.45 (m, 2H), 3.24 (s, 3H), 1.94-2.01 (m, 2H). MS obsd. (ESI^+) [$(M+H)^+$]: 442.

HBsAg Assay. HepG2.2.15 cells seeded in duplicate into white, 96-well plates at 1.5 x 104 cells/well. The cells were treated with a three-fold serial dilution series of the compounds in DMSO. The final DMSO concentration in all wells was 1% and DMSO was used as no drug control. The HBsAg chemiluminescence immunoassay (CLIA) kit (Autobio Diagnostics Co., Zhengzhou, China) was used to measure the levels of secreted HBV antigens semi-quantitatively. For the detection 50uL/well culture supernatant was used and the procedure conducted as directed by manufacturer's instructions. The cytotoxicity was measured using CellTiter-Glo (Promega, Madison, WI, USA, Cat# G7571). Using the E-WorkBook Suite (ID Business Solutions Ltd., Guildford, UK) dose-response curves were generated and the IEC_{50} and CC_{50} values extrapolated. The EC50 and CC50 are defined as the compound concentration (or conditioned media log dilution) at which HBsAg secrection and cytotoxicity, respectively, are reduced by 50% compared to the no drug control.[19]

HBV DNA assay. The assay employs real-time qPCR (TaqMan) to directly measure extracellular HBV DNA copy number. HepG2.2.15 cells were plated in 96-well microtiter plates. Only the interior wells were utilized to reduce "edge effects" observed during cell culture, the exterior wells were filled with complete medium to help minimize sample evaporation. On the following day, the HepG2.2.15 cells were washed and the medium was replaced with complete medium containing various concentrations of a test compound in triplicate. 3TC was used as the positive control, while media alone was added to cells as a negative control (virus control, VC). Three days later, the culture medium was replaced with fresh medium containing the appropriately diluted drug. Six days following the initial administration of the test compound, the cell culture supernatant was collected, treated with pronase and then used in a real-time qPCR/TaqMan assay to determine HBV DNA copy numbers. Antiviral activity was calculated from the reduction in HBV DNA levels (IC50).[19]

LYSA Solubility Assay. The test samples are prepared in duplicate from 10 mM DMSO stock solution. Compounds are dissolved in a 0.05 M phosphate buffer (pH 6.5), after evaporation of DMSO with a centrifugal vacuum evaporator. Then stirred the mixture for one hour and shaken for two hours. Standing over night, filtered the solution with a microtiter filter plate. The filtrate and its 1/10 dilution are analyzed by HPLC-UV. A four-point calibration curve is prepared for the solubility determination. In case the percentage of sample measured in solution after evaporation divided by the calculated maximum of sample amount is bigger than 80%, the solubility data are reported as bigger than this value. The results are in µg/mL.

Microsomal Stability Assay. To determine the microsomal stability, microsomes were pre-incubated with test compound for 10 min at 37 °C in potassium phosphate buffer (100 mM, pH 7.4). The final incubation mixtures consisted of 0.5 mg microsomal protein/mL liver microsomes, 1 mM NADP, 3 mM glucose 6-phosphate, 3 mM $MgCl_2$, and 0.05 mg/mL glucose 6-phosphate dehydrogenase in a total volume of 400 µL potassium phosphate buffer (100 mM, pH 7.4). The reactions were initiated with the addition of NADPH regenerating system. At different time points (0, 3, 6, 9, 15, and 30 min), an aliquot (50 µL) samples was taken, quenched with 150 µL of acetonitrile

containing internal standard. Following precipitation and centrifugation, the supernatants were analyzed by LC-MS/MS.

Caco-2 Assay. Prepare 10 μM input drug solution in pH7.4 HBSS solution. For apical to Baslateral direction, add 200 μL Input drug solution to the apical side and 700 μL pH 7.4 HBSS (1% DMSO) to the baslateral side of the Caco-2 cells. For Baslateral to Apical side direction, add 700 uL Input drug solution to the Baslateral side and 200 μL pH7.4 HBSS (1% DMSO) to the Apical side of the Caco-2 cells. Incubate the plate for one hour in 5% CO_2 at 37 ºC, 95% humidity condition. Determine the amounts of the drugs at apical side and baslateral side and calculate the permeability from A to B and B to A direction.

Plasma Protein Binding Assay. The unbound compound was determined using a 96-well Micro-Equilibrium Dialysis Devices (HTDialysis, Gales Ferry, CT, USA) with molecular weight cutoff membrane of 12−14 kDa (HTDialysis, Gales Ferry, CT, USA). Diazepam was used as positive control. Pooled mouse and human plasma were purchased from Biopredic (Rennes, France). Compounds were measured in cassette of 2−5 with an initial total concentration of 1 μM, and one of the cassette compounds is the positive control. The integrity of membranes was tested by determining the unbound fraction values of the positive control. Equal volumes of blank dialysis buffer (Soerensen buffer at pH 7.4) and matrix samples containing substances were loaded into the acceptor and donor compartment, respectively. The HTD dialysis block was then sealed and kept in an incubator at 37 °C for 5 h under 5% CO_2 environment. Then the drug concentrations were quantified by LC-MS/MS.

Pharmacokinetic (PK) Analysis in Mice. Compound was evaluated in mice at iv 1 mg/kg, po 2 mg/kg and po 14.5 mg/kg. Compound solutions were prepared by dissolving the solid in 5% DMSO, 40% PEG400 and 55% saline for iv dose, and 1% RC-591 in water for oral dose. Blood samples were collected at predetermined times into sodium heparin containing tubes, and plasma was separated via centrifugation (4 °C, 8000 rpm, 6 min) and stored frozen at -80 °C pending bioanalysis. Liver samples in po group were harvested immediately after the collection of blood. The liver samples were then rinsed with saline, dried with filter paper, and stored at -80 ºC until bioanalysis. Compound concentrations in the plasma and liver samples were determined by LC–MS/MS. The data were

analyzed using a non-compartmental module of WinNonlin® Professional 5.2. This PK study was approved by the Institutional Animal Care and Use Committee (IACUC) of Roche Pharma Research and Early Development China.

ASSOCIATED CONTENTS

SUPPORTING INFORMATION

Detail experimental procedures for the preparation of compounds **16**, **17**, **18** and **35**; X-ray Crystal Structure of (*S*)-**22**; X-ray Crystal Structure of RG7834; ^{1}H NMR and ^{13}C NMR Spectrum of RG7834 Molecular-formula strings of the reported compounds

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Notes

The authors declare no competing financial interest.

ABBREVIATION

3-TC, lamivudine; ADME, absorption, distribution, metabolism and excretion; CYP, cytochromes P450; dHepaRG, differentiated HepaRG; DHQ, dihydroquinolizinone; ETV, entecavir; FNCs, fluorine substituent nucleoside analogues; HBV, hepatitis B virus; HBsAg, HBV surface antigen; HBeAg, hepatitis B e antigen; HLM, Human liver microsome; HRMS, high resolution mass spectra; IPA, 2-propanol; LYSA, lyophilization solubility assay; MLM, Mouse liver microsome; PAPD5 and PAPD7, non-canonical poly(A) RNA polymerases, PAP-associated domain-containing protein 5 and 7; SAR, structure-activity relationship; SCID, severe combined immunodeficiency; SDPK, single-dose pharmacokinetics; SFC, supercritical fluid chromatography; SPR, structure-property relationship; TEA, triethylamine; uPA, urokinase-type plasminogen activator.

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