Novel dihydroquinolizinones for HBV surface antigen (HBsAg) reduction with Liver targeting properties

Nicky Hwang, Liren Sun, Daisy Noe, Tianlun Zhou, Timothy Block, Yanming Du

Baruch S. Blumberg Institute, Doylestown, PA 18902 USA

Background

Chronic hepatitis B (CHB) is characterized by high levels of hepatitis B virus (HBV) surface antigen (HBsAg) in blood circulation. A major goal of CHB interventions is to reduce or eliminate this antigenemia; however, the current standard of care medications with either pegylated interferon alpha or nucleos(t)ide analogues (NUCs) have failed to do this.[1]

A novel family of dihydroquinolizinone (DHQ) has been shown to reduce circulating levels of HBsAg in animals, representing a first for a small molecule with reliable and promising potential. Reductions of HBsAg were a result of the compound's effect on HBsAg mRNA levels.[2-3]

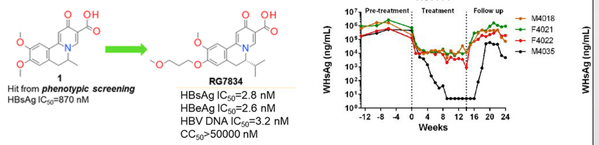


Fig 1. RG-7834 and its surface antigen reduction in a woodchuck model.

Roche's RG7834 was developed for **systemic use**. It has favorable anti-HBV activity, cytotoxicity, and liver microsome stability. RG7834 showed very **good permeability** with a $P_{app(A-B)}$ of 12.8 × 10^{-6} cm s^{-1} and efflux ratio of 1.3 in Caco-2 assay. The mouse single dose pharmacokinetics profile of RG7834 demonstrated that RG7834 had moderate plasma clearance (Cl) (41.9 mL min^{-1} kg^{-1}), **good distribution and satisfactory oral exposure (oral bioavailability F = 62%),** and a marginal liver/plasma ratio.

LYSA (μg/mL)	MLM/HLM (ml/min/kg)	PPB M/H	PK in mouse						log D	PKa	$P_{app(A-B)}$ (10^{-6} cm/s)	Efflux
			Dose (mg/kg)	Cl (ml/min/kg)	Vss (L/Kg)	T1/2 (h)	F (%)	Liver/plasma				
82 (200 μM)	13/3.7	64.8/67.2	1/iv	41.9	2.07	0.71			1.28	5.79	12.8	1.3
			2/po			1.48	62					
			12.5/po					4.0				

Table 1. RG-7834 ADME and PK results showed potential for orally systemic use.

However, the CNS liability observed for RG-7834 raised safety concerns for this compound.[4] Commercial development of RG-7834 by Roche was stopped due to undisclosed toxicity issues.

Method

Our goal, contrary to the regular medicinal practice of pursuing a highly systemically bioavailable lead compound, is to convert the systemic RG-7834 to liver-selective new DHQs that have low to moderate bioavailabilities in plasma but high liver exposure and liver/plasma ratios. We believe that having drugs that are more selective for liver hepatocytes, which are the cells targeted by HBV, is one way to minimize or eliminate unnecessary side effects resulting from the inappropriate distribution of RG7834 to other tissues. Therefore, hepatoselective DHQ compounds should have great potential to improve the safety of this novel family of anti-HBV compounds.

Several distinct medicinal chemistry approaches have emerged to exploit the specific receptors or binding sites on the surface of liver cells, which can facilitate liver-targeted delivery of small molecules, in other disease fields; for example, Transporter-mediated active uptake (**2**, Fig 2), Prodrug strategy (**3**), and Address-and-message approach (**4**, Fig 2).[5]

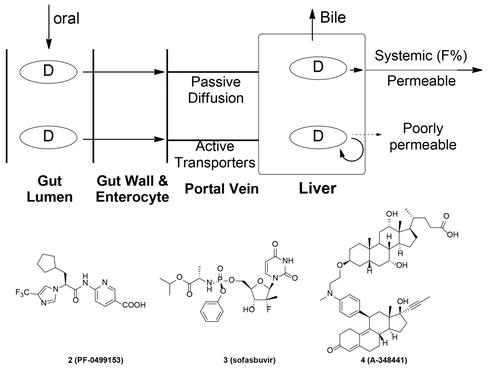
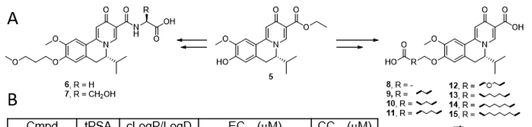


Fig 2. General absorption scheme for oral drugs and representative compounds for three hepatoselective strategies

Results



B

Cmpd	tPSA	cLogP/LogD	EC_{50} (μM)	CC_{50} (μM)
6	114.4	1.83	> 10	> 10
7	134.6	0.95	> 10	> 10
8	113.4	2.2	> 10	> 10
9	113.4	2.9	1.01 +/- 0.35	> 10
10	113.4	3.1	0.38 +/- 0.10	> 10
11	113.4	3.7	0.087 +/- 0.022	> 10
12	122.6	2.9	2.78 +/- 0.71	> 10
13	113.4	4.2/-0.65	0.023 +/- 0.011	> 10
14	113.4	4.7/0.07	0.0076 +/- 0.00083	> 10
15	113.4	5.2/0.70	0.0046 +/- 0.00074	> 10
RG-7834	85.3	3.2/1.39	0.0018 +/- 0.00016	> 10

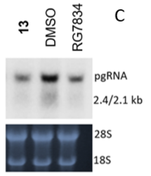


Fig 3. A, Synthesis of DHQ derivatives; B, Activities of new DHQ derivatives for reducing HBsAg (EC_{50}); C, Northern blot analysis of bis-acid **13** for HBV mRNA reduction (at 1 μM after 5-day treatment).

Cmpd	OATP1B1		OATP1B3	
	Uptake Ratio (-/+ inhibitor)	Uptake Ratio ($Transporter_{(-inhibitor)}$/$Mock_{(-inhibitor)}$)	Uptake Ratio (-/+ inhibitor)	Uptake Ratio ($Transporter_{(-inhibitor)}$/$Mock_{(-inhibitor)}$)
13	11.17	18.99	11.57	24.80
RG7834	0.38	0.71	0.42	0.56

Table 2. Substrate determination of 13 on OATP1B1 and OATP1B3, Estradiol 17-β Glucuronide was used as a positive control. A compound is defined as a potential substrate of the corresponding transporter in these assays when both ratios are bigger than 2. **13 is a substrate for OATP1B1 and OATP1B3 but RG7834 is not.**

Cmpd	$P_{app\ (A-B)}$ 10^{-6} cm/s	$P_{app\ (B-A)}$ 10^{-6} cm/s	Efflux Ratio	BBB Penetration Potential
13	0.38	0.55	1.47	**Low** $P_{app\ (A-B)} < 3$
RG7834	9.17	35.77	3.90	**Moderate** $P_{app\ (A-B)} \geq 3$, and 10 > efflux ≥ 3

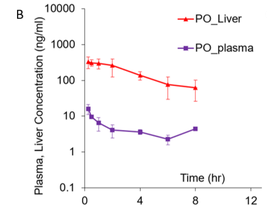
Table 3. BBB penetration potential in MDCK-MDR1 cells. **13 has a low penetration potential to BBB.**

Liver Microsomal Stability				Caco-2			CYPs
Human		Mouse					
Cl (μL/min/mg)	$T_{1/2}$ (min)	Cl (μL/min/mg)	$T_{1/2}$ (min)	P_{app} (A-B) (10^{-6}, cm/s)	P_{app} (B-A) (10^{-6}, cm/s)	Efflux Ratio	IC_{50} against 9 isozymes
0	∞	0.8	1736	0.39	2.06	5.30	> 10 μM

Table 4. ADME evaluation. **13 demonstrates favorable ADME profiles**.

A	Route	Dose (mg/kg)	Cl (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (hr)		C_{max} (ng/mL)		AUC_{last} (h*ng/mL)		F (%)
					Plasm	Liver	Plasm	Liver	Plasm	Liver	In plasma
	IV	3	198	3.26	1.48				255		
	PO	8				3.54	16.1	333	34.7	1307	5.1

B



C

Time (hr)	0.25	0.5	1	2	4	6	8
[C] in liver (ng/mL)	333	309	300	261	138	76	63
[C] in liver (nM)	730	680	660	570	300	170	140
C_{max} ratio (liver/plasma)	20.7						
AUC_{last} ratio (liver/plasma)	37.7						

Fig 4. PK evaluation in mice. **13 displays low exposure in plasma but high liver distribution**.

Conclusions

Based on the structure of RG7834 and the analysis of its ADME and PK profiles, we have incorporated an additional acid group into the side chain of RG7834. Through the increase in tPSA and the modulation of the cLogP/LogD of the new molecules, we have identified a new lead that is potent in HBV mRNA degradation cellular assay. Further evaluation showed that unlike RG7834, the new lead is a substrate of both OATP1B1 and OATP1B3, which may facilitate the absorption into the liver. This in vitro result was translated into an in vivo setting: the new lead demonstrated much better hepatoselective distribution in a mouse PK study than RG7834, with an average liver/plasma ratio of 37.7 over 8 h. More importantly, the new lead demonstrated a low risk for crossing the BBB in comparison to the moderate risk of RG7834.

Bibliography

1. Revill PA, et al. global scientific strategy to cure hepatitis B. Lancet Gastroenterol Hepatol. **2019** Jul;4(7):545-558.
2. Han X, et al. Discovery of RG7834: The First-in-Class Selective and Orally Available Small Molecule Hepatitis B Virus Expression Inhibitor with Novel Mechanism of Action. J Med Chem. **2018** Dec 13;61(23):10619-10634.
3. Menne, S.; et al. Efficacy of an Inhibitor of Hepatitis B Virus Expression in Combination with Entecavir and Interferon-α in Woodchucks Chronically Infected With Woodchuck Hepatitis Virus. Hepatol Commun. 2020, 4 (6), 916–931.
4. Aktoudianakis, E., et al., Compounds for the treatment of hepatitis B virus infection and their preparation, WO**2018**144605 A1.
5. Tu M, et al. Medicinal chemistry design principles for liver targeting through OATP transporters. Curr Top Med Chem. **2013**;13(7):857-66.

printed by MegaPrint Inc. www.postersession.com