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Phenylpropenamide derivatives: Anti-hepatitis B virus activity of the *Z* isomer, SAR and the search for novel analogs

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ABSTRACT

Phenylpropenamides have been reported to be a class of non-nucleoside inhibitors of the hepatitis B virus (HBV). This class of compounds was explored with the objective of developing potent anti-HBV agents, with a novel mechanism of action, that could be combined with nucleos(t)ide analogs currently used to treat HBV infection. To accomplish this objective a series of substituted arylpropenamide derivatives were prepared and the *E* and *Z* geometrical isomers were separated. The structural identity of each of the *E* and *Z* isomers was determined by single crystal X-ray crystallography. Contrary to previous reports, the activity of this class of molecules resides in the *Z* isomer. Further structure–activity relationship studies around the active *Z* isomer identified compounds that displayed potent antiviral activity against HBV with EC_{90} value of approximately 0.5 μM in vitro.Attempts to develop ring constrained analogs did not lead to active HBV inhibitors.

Hepatitis B virus (HBV) infection is a major health problem that leads to chronic liver disease, estimated to affect more than 5% of the world's population.[1] HBV can cause both acute and chronic infections. Chronic HBV infection can progress to liver cirrhosis and hepatocellular carcinoma with high mortality. There are approximately 350–400 million chronically infected individuals, resulting in 0.5–1.2 million deaths annually.[2,3]

The current agents approved for the treatment of HBV infection include interferon-α, pegylated interferon α-2a, nucleoside analogues lamivudine, telbivudine,[4] entecavir,[5] adefovir and tenofovir[6] (Fig. 1). Unfortunately, the use of interferon-α is limited because of its low success rate, high cost and serious side effects.[7,8] The nucleoside analogues all inhibit HBV replication by inhibiting the viral polymerase. As is the case for HIV infection, developing combination therapies containing agents with complimentary mechanisms of action for treating HBV infection is highly desirable. However, there are no direct acting antiviral agents that inhibit HBV via a non-polymerase mechanism of action currently in clinical development.

Phenylpropenamides were reported as a non-nucleoside class of inhibitors of HBV replication in cell culture.[9] It was also reported that phenylpropenamides were specific inhibitors of HBV replication and most likely inhibited replication by interfering with the packaging of pregenomic RNA into immature core particles.[10]

We were interested in further investigating arylpropenamides as inhibitors of HBV with the hope of developing combination therapies with current nucleos(t)ide therapies. Earlier reports describing the identification and activity of phenylpropenamides as HBV inhibitors reported the propenamide double bond having

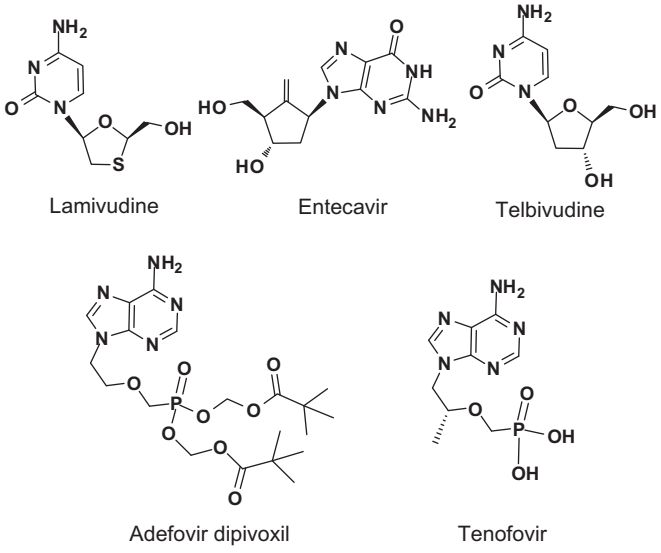

Figure 1. Agents for the treatment of hepatitis B infection.

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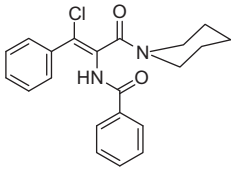
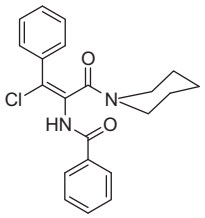


10*E*

Figure 2. Reported active propenamide having the *E* configuration (reported EC_{50} = 0.6–5.7 μM).[9,10]



10*Z*

Figure 3. Active compound **10*Z*** having *Z* configuration.

the *E* configuration (*trans*) (Fig. 2). However, there was no discussion regarding the assignment of the double bond geometry or activity of the molecule having the *Z*-configuration (*cis*).

Here we report the definitive structural assignment of phenylpropenamide derivatives and the structure–activity relationship of the active *Z* isomers.

In our effort to explore more completely propenamides as inhibitors of HBV, we chose to study the SAR around propenamide templates **10*Z*** (*Z* isomer) and **10*E*** (*E* isomer). As previously reported, the phenylpropenamides were prepared as shown in Scheme 1. Reaction of substituted benzoyl chloride **1** with glycine **2** gave the *N*-benzoylglycine (hippuric acid **3**). Condensation of **3** with a benzaldehyde **4** in acetic anhydride (100 °C) provided the oxazolones **5**. Amination of the oxazolones **5** by reaction of either piperidine or other desired amines afforded *N*-(3-amino-3-oxo-1-phenylprop-1-en-2-yl)benzamide derivatives **7**.

Halogenation of **7** with bromine in the presence of $CaCO_3$ or sulfuryl dichloride afforded the propenamides as a mixture of *Z* and *E* isomers. *E* and *Z* isomers were subsequently separated by either HPLC or SFC chromatography. Based on isolated yields, the ratio of *Z*/*E* isomers was approximately 2.5∼10:1.

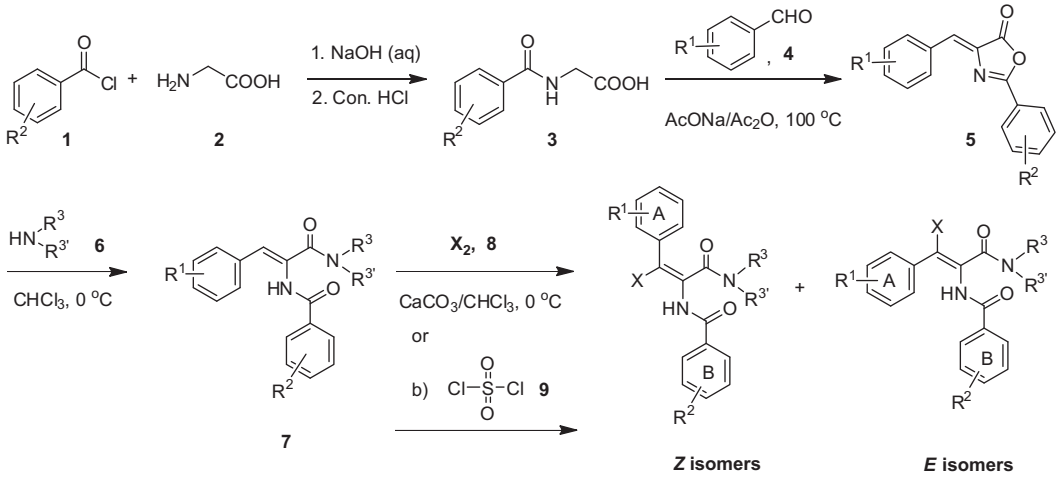
The final products were characterized by ^{1}H NMR, NOE studies and MS.[12] Since the double bond geometries could not be unequivocally assigned using NMR spectral data, the stereochemistry of the double bond moiety, was determined by obtaining single crystal X-ray structures. Thus, the major isomer was determined to have the *Z* configuration, (*Z*)-*N*-(1-chloro-3-oxo-1-phenyl-3-(piperidin-1-yl)prop-1-en-2-yl)benzamide (**10*Z***) and the minor isomer the *E*-configuration, **10*E*** (Fig. 4).

The anti-HBV activity for both *E* and *Z* isomers was evaluated in the Hep AD38 cellular assay.[13] Contrary to previous reports,[9] it was determined that the anti-HBV activity of this class of molecules resides predominantly in the *Z* isomer. The *E* isomers were shown to be weakly active or not active against HBV (data not shown). For example, the *Z* isomer **10*Z*** (Fig. 3) exhibited anti-HBV activity with an EC_{90} of 5.51 μM, whereas the *E* isomer, compound **10*E*** (Fig. 2), which was reported to have an EC_{50} of 1.2 μM and EC_{90} of 13 μM,[9] in fact was shown to be inactive up to 100 μM, the highest concentration we tested.

SAR investigation of A-ring modification of the *Z* propenamides is summarized in Table 1. Compounds **11**, **12**, **13**, and **10*Z*** showed good activity with EC_{90}'s of 1.71, 1.80, 1.81, and 5.51 μM, respectively. The *ortho*-substitution on the A-ring provided improved anti-HBV activity, such as seen for compounds **11**, **12**, and **13**. Benzo[1,3]dioxo-4-yl derivative **14** was less potent than compounds containing an *o*-substituted or unsubstituted phenyl group. *Para*-substitution on the A-ring, such as *p*-OMe, *p*-Me, and *p*-Cl, decreased the antiviral activity.

Further SAR assessment focused on the substitutions on the B-ring (Table 2). Among the B-ring modifications, the *p*-NO_2 analog **18**, *p*-chloro analog **19**, and *p*-fluoro analog **21** displayed activity. Propenamides with a *p*-methyl (**20**), *p*-bromo (**22**), or a 2-bromo-4-nitro (**25**) substituent on the B-ring also showed good activity. However, some *para*-substitutions on the B-ring, such as *p*-OMe **23** and 4-$MeSO_2$ **24** resulted in significant loss in antiviral activity. Similarly, several 2,4-disubstituted B-ring compounds, such as **26**, **27**, and **28**, did not show anti-HBV activity up to 10 μM.

In Table 3 we summarize the SAR for the amide moiety. The non-substituted piperidine analog **10*Z*** exhibited good activity with an EC_{90} value of 5.51 μM. 3-Methyl-piperidin-1-yl derivative **29** was found to have similar activity with an EC_{90} of 6.43 μM. Other substituted piperidine amides, such as **30** having the 3,3-di-fluoro-piperidine, provided reduced activity or no activity (up to 10 μM). 4-Methyl-piperazine **31** and morpholine **32** were also less potent (EC_{90} > 10 μM). Piperidine amide derivative **33**, with an *o*-OMe on the A-ring and a *p*-NO_2 on the B-ring, exhibited the most potent activity, with an EC_{90} value of 0.56 μM. The



Scheme 1. Synthesis of arylpropenamides.

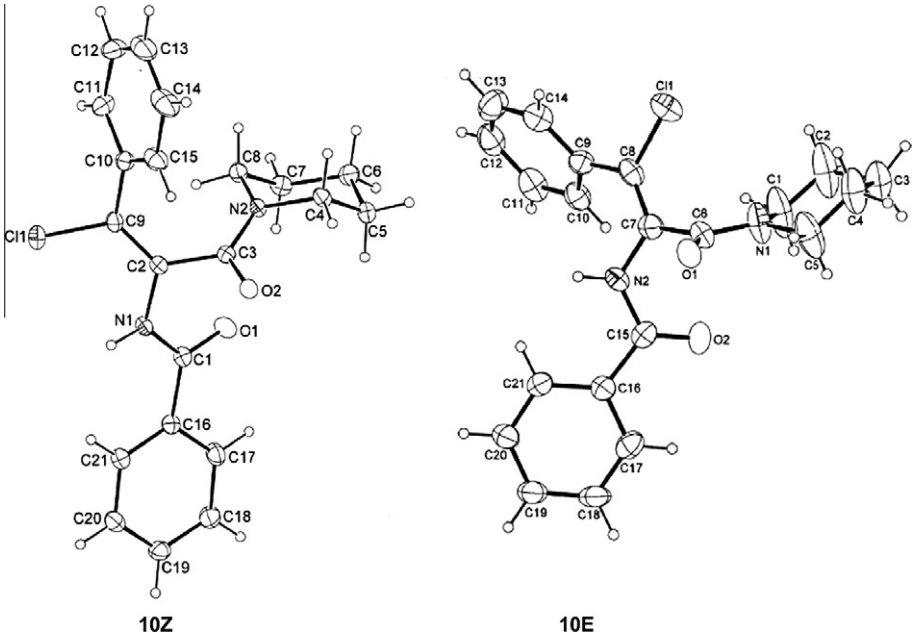
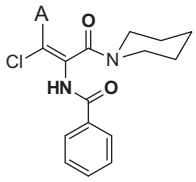


Figure 4. ORTEP drawing of compound **10*Z*** (*Z* isomer) and **10*E*** (*E* isomer).[11]

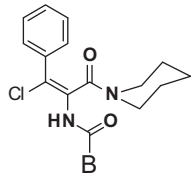
Table 1
Modification on the A-ring



Compd	A (A-ring)	EC_{90} (μM)	CC_{90} (μM)
10*Z*	Ph	5.51	>10
11	2-MeO-Ph	1.71	>10
12	2-Me-Ph	1.80	>10
13	2-F-Ph	1.81	>10
14		9.9	>10
15	4-Cl-Ph	>10	>10
16	4-Me-Ph	>10	>10
17	2-MeO-Ph	>10	>10

Anti-HBV activity was evaluated in the Hep AD38 cellular assay.[13]
EC_{90} = concentration of the compound (in μM) which inhibits the synthesis of viral DNA by 90%. CC_{90} = concentration of drug (in μM) which reduces cell viability by 90%.

Table 2
Modification on the B-ring



Compd	B (B-ring)	EC_{90} (μM)	CC_{90} (μM)
10*Z*	Ph	5.51	>10
18	4-NO_2-Ph	1.67	>10
19	4-Cl-Ph	1.25	>10
20	4-Me-Ph	2.90	>10
21	4-F-Ph	2.17	>10
22	4-Br-Ph	6.04	>10
23	4-MeO-Ph	>10	>10
24	4-$MeSO_2$-Ph	>10	>10
25	2-Br-4-NO_2-Ph	4.06	>10
26	2-MeO-4-4NO_2-Ph	>10	>10
27	2-CF_3-4-NO_2-Ph	>10	>10
28	2,4-Di-NO_2-Ph	>10	>10

Anti-HBV activity was evaluated in the Hep AD38 cellular assay.[13]
EC_{90} = concentration of the compound (in μM) which inhibits the synthesis of viral DNA by 90%. CC_{90} = concentration of drug (in μM) which reduces cell viability by 90%.

corresponding pyrrolidine derivative **34** was less potent. These results revealed that unsubstituted piperidine and pyrrolidine amides are preferred.

The effect on HBV activity of chlorine *vs* bromine substitution on the double bond of the phenylpropenamides is presented in Table 4. In general, most vinyl bromide derivatives were more potent than the corresponding vinyl chloride derivatives, such as **41** versus **34** (~4-fold). Compounds (**34** and **41**) having the *Z* configuration and the substitutions X = Cl or Br, R^1 = *o*-OMe, R^2 = *p*-NO_2 and Y = pyrrolidinyl showed excellent anti-HBV activity with the EC_{90}'s of 1.69 and 0.39 μM, respectively.

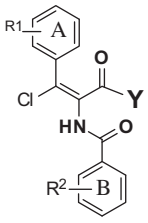
The SAR of the propenamides having the *Z* double bond geometry is consistent with previously reported SAR for compounds assigned as having an *E* configuration, indicating that the compounds originally reported were likely to have had predominately *Z* geometry.

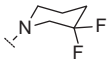
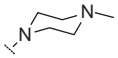
With the establishment of the correct double bond configuration, we turned our attention to identifying analogs that replace the vinyl halide with a more pharmacologically attractive system.

First, we explored whether the vinyl halide could be replaced with an alkyl substituted alkene. A methyl group is considered to be isosteric with Cl and Br.[14] This substitution was also supported by superposition of the modeled conformers of **10*Z*** and **42**, the methyl substituted derivative, for which the lowest energy conformer of each is depicted in Figure 5a.[15] Therefore we synthesized 1-methyl substituted propenamide **42** and **43** from the corresponding 1-bromopropenamides via vinyllithiation and methyl iodide treatment.[16]

Compounds **42** and **43** exhibited comparable anti-HBV activity to their corresponding vinyl halide analogs **19** and **22** with EC_{90} values of 1.92 and 3.92 μM, respectively.

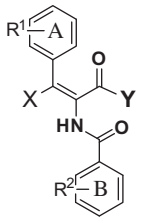
Table 3
Amide variants



Compd	R^1 (A-ring)	R^2 (B-ring)	Y	EC_{90} (μM)	CC_{90} (μM)
10*Z*	H	H		5.51	>10
29	H	H		6.43	>10
30	H	H		>10	>10
31	H	H		>10	>10
32	H	H		>10	>10
33	2-MeO	4-NO_2		0.56	>10
34	2-MeO	4-NO_2		1.69	>10

Anti-HBV activity was evaluated in the Hep AD38 cellular assay.[13]
EC_{90} = concentration of the compound (in μM) which inhibits the synthesis of viral DNA by 90%. CC_{90} = concentration of drug (in μM) which reduces cell viability by 90%.

Table 4
Comparison of vinyl chloride *vs* bromide derivatives



Compd	X	R^1 (A-ring)	R^2 (B-ring)	Y	EC_{90} (μM)	CC_{90} (μM)
10*Z*	Cl	H	H		5.51	>10
35	Br	H	H		5.90	>10
19	Cl	H	4-Cl		1.25	>10
36	Br	H	4-Cl		0.91	>10
37	Cl	2,3-Dimethyli-denedioxy–	4-NO_2		4.66	>10
38	Br	2,3-Dimethyli-denedioxy–	4-NO_2		1.25	>10
39	Cl	2-MeO	H		4.72	>10
40	Br	2-MeO	H		2.78	>10
34	Cl	2-MeO	4-NO_2		1.69	>10
41	Br	2-MeO	4-NO_2		0.39	>10
42	CH_3	H	4-Cl		1.92	>10
43	CH_3	H	4-Br		3.92	>10

Anti-HBV activity was evaluated in the Hep AD38 cellular assay.[13]
EC_{90} = concentration of the compound (in μM) which inhibits the synthesis of viral DNA by 90%. CC_{90} = concentration of drug (in μM) which reduces cell viability by 90%.

Knowing that the preferred double bond geometry is *Z* and that methyl is tolerated in place of the halogen on the double bond, we proceeded to introduce ring systems that would link the amide group and the far end of the double bond. We hypothesized that these would maintain the overall conformation of the molecule while providing additional opportunities to explore other potential binding interactions. Therefore, we designed the novel ring constrained propenamide type derivatives by incorporating the dihydropyrrole (**A**), pyrrolidine (**B**) and pyrrole (**C**) skeletons into the structure of the propenamides (Table 5).

Two consequences of the propenamide cyclized constructs are the conversion of the conformationally restricted secondary amide into a flexible tertiary amide and concomitant loss of the H-bond donor ability of the amide. Of the three classes of analogues, **A** showed the greatest propensity to maintain the *s-trans* shape of the secondary amide by ~1 kcal/mol. All four diastereomers of **B** were least capable of aligning well with the parent propenamide; those conformers which did were ~9 kcal/mol above the lowest energy conformers found. The alignment of the lowest energy conformers of **10*Z*** and the corresponding dihydropyrrole, **53**, are depicted in Figure 5b.

Conformationally constrained analogues of the propenamides were prepared as shown in Scheme 2. Diethyl 2-aminomalonate (**44**) was reacted with benzoic acid **45** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). The resulting diethyl 2-benzamidomalonate **46** was subjected to condensation with the α,β-unsaturated aldehyde **47**, followed by a deoxygenation reaction using triethylsilane to afford **49**. Saponification of the diesters **49** with 1 equiv of sodium hydroxide followed by oxidative decarboxylation gave the dihydropyrrole derivative **51**.[17] The desired dihydropyrroles **53–55** were obtained via a saponification of the ester **51** with aqueous sodium hydroxide followed by coupling of intermediate **52** with piperidine in the presence of HATU and DIPEA. Hydrogenation using palladium hydroxide on carbon gave pyrrolidine derivative **56** and **57**. For the pyrrole-carboxamide target, acylation followed by elimination of

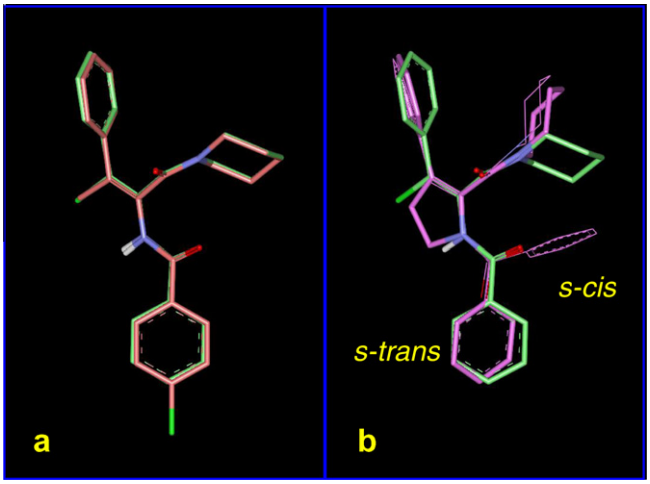
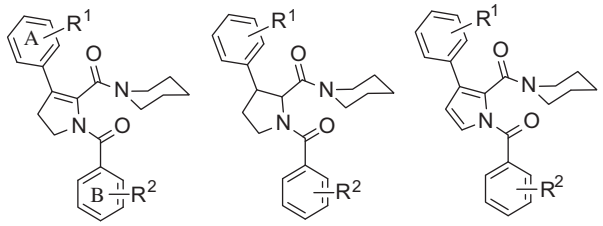


Figure 5. (a) Superposition of the modeled lowest energy conformers for **10*Z*** (green carbons) and **42** (pink carbons) and (b) **10*Z*** and **53** (purple carbons). The higher energy *s-cis* conformer of **53** is displayed in wire.

5-hydroxy pyrrolidine **48** gave the dihydropyrrole diester **58**. Saponification of **58** with one equivalent of sodium hydroxide, oxidative decarboxylation and further saponification afforded **59**.

Table 5
Ring constrained derivatives



A B C

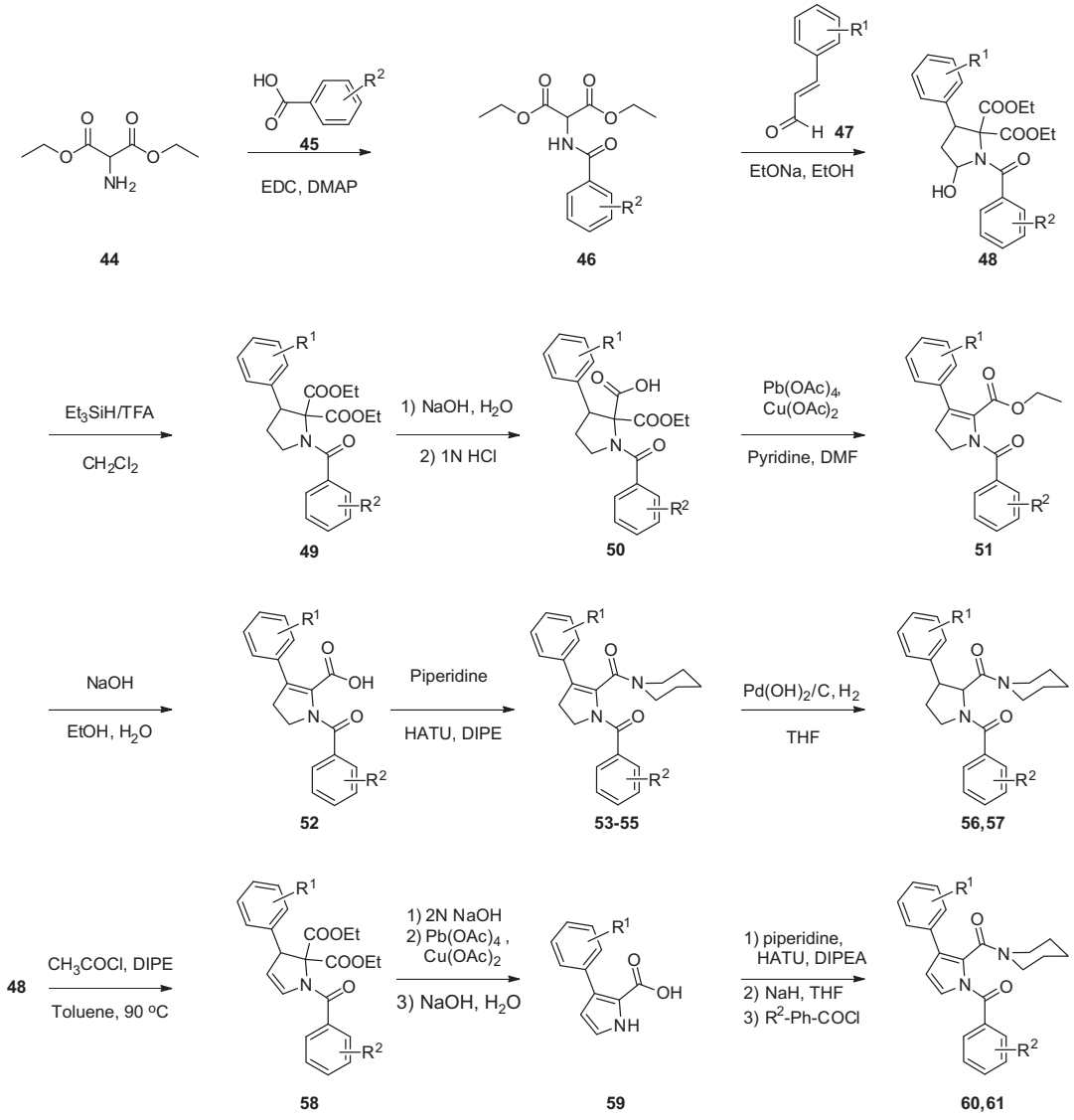
Compd	Type	R^1 (A-ring)	R^2 (B-ring)	EC_{90} (μM)	CC_{90} (μM)
53	**A**	H	H	>10	>10
54	**A**	H	4-Br	>10	>10
55	**A**	2-MeO	H	>10	>10
56	**B**	H	H	>10	>10
57	**B**	H	4-Br	>10	>10
60	**C**	H	H	>10	>10
61	**C**	H	4-Br	>10	>10

Anti-HBV activity was evaluated in the Hep AD38 cellular assay.[13]
EC_{90} = concentration of the compound (in μM) which inhibits the synthesis of viral DNA by 90%. CC_{90} = concentration of drug (in μM) which reduces cell viability by 90%.

Coupling of **59** with piperidine and amidation with the appropriate benzoic acid provided the desired **60** and **61**. Unfortunately and somewhat surprisingly, these constrained analogues were found to be devoid of anti-HBV activity (Table 5).

To evaluate the potential impact of the loss of the H-bond donor function of the B-ring amide group, the N-methyl analog of compound **36** was prepared via the reaction of compound **36** with methyl iodide in the presence of sodium hydride in THF. Replacement of the amide hydrogen of **36** with a methyl group resulted in complete loss of activity (structure not shown). Molecular modeling showed that this methylation not only removed the capability of the amide to function as an H-bond donor but also increased the number of low energy conformers available beyond that seen with the cyclic analogues. The desired *s-trans* conformer which mapped with the active parent propenamide is energetically disfavored for the N-methyl derivative by ~1.3 kcal/mol over the *s-cis* lowest energy conformer. These results suggested that the lack of activity in the cyclic analogues was due to either the loss of a critical H-bond interaction, increased flexibility, increased steric interactions with the biological target or a combination of these factors.

In conclusion, a series of new 2-substituted-3-arylpropenamide derivatives having the *Z* configuration were prepared and evaluated as inhibitors of HBV replication. The stereochemistry of the



Scheme 2. Synthesis of ring constrained propenamides.

final products was elucidated by X-ray crystallography. We determined that the activity of this class of molecules resides in the *Z* isomer. Structure–activity relationship studies around the active *Z* isomer showed that a number of compounds displayed potent antiviral activity against hepatitis B virus in vitro and that the halogen on the central double bond was not critical for activity. Attempts to translate this SAR into ring constrained analogs did not lead to compounds with anti-HBV activity.

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11. Structure of compound **10E** in Figure 2 is reported as compound **6** in Ref. 9 and as AT-61 in Ref. 10. CCDC 826064 & 826065 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
12. For instance, compound **10Z**: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.23 (m, 3H), 1.28 (m, 3H), 3.52 (m, 2H), 3.11 (m, 2H), 3.22–3.24 (m, 2H), 7.44–7.50 (s, 5H), 7.52–7.59 (s, 2H), 7.61–7.62 (s, 1H), 7.97–7.99 (s, 2H), 10.13 (m, 1H); MS, *m*/*e* 368.9 $(M+1)^+$, 758.9 $(2M+Na)^+$. For compound **10*E***: ^{1}H NMR(400 MHz, DMSO-d_6): δ = 1.52–1.54 (m, 2H), 1.62–1.66 (m, 4H), 3.52 (m, 2H), 3.67 (m, 2H), 7.34–7.45 (s, 5H), 7.52–7.55 (s, 3H), 7.74–7.76 (s, 2H), 10.09 (m, 1H); MS, *m*/*e* 368.9 $(M+1)^+$, 758.9 $(2M+Na)^+$.
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15. Conformers were generated for every compound modeled using torsional sampling (MCMM) (a) followed by energy minimization to a convergence gradient of 0.005 using MMFFs with a distance dependent dielectric model of 2*r*. (b) An RMSD of 0.25 and a 15 kcal/mol energy window were used as criterion to limit the number of conformers output with an upper limit of 1000 being possible. None of the compounds resulted in more than 100 conformers given these limits. (MacroModel, version 9.8, Schrödinger, LLC, New York, NY, 2010.) (a) MCMM–Monte Carlo Multiple Minimum: Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379 and Saunders, M.; Houk, K. N.; Wu, Y.-D.; Still, C. W.; Lipton, M.; Chang, G.; Guida,W. C. *J. Am. Chem. Soc.* **1990**, *112*, 1419; (b) MMFFs: Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720 and Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 730.
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