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Phenylpropenamide Derivatives as Inhibitors of Hepatitis B Virus Replication

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Abstract—A non-nucleoside class of compounds that inhibits the replication of hepatitis B virus (HBV) in cell culture has been discovered. A series of substituted analogues of phenylpropenamide **6** has been prepared and evaluated in the HepAD38 cellular assay. Structure–activity relationships of this series are discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The treatment of hepatitis B virus infection represents one of the current therapeutic challenges in virology. An estimated 300 million people are chronically infected worldwide with roughly 4 million deaths annually from the resulting cirrhosis and hepatocellular carcinoma.[1] Although interferon-α has been a mainstay of HBV treatment regimens, sustained response rates tend to be low (20–30%) and resistance builds rapidly.[2,3] Other strategies are being pursued including stimulation of TH1 response, antisense oligonucleotides, glycosidase inhibitors,[4] and nucleoside antiviral chemotherapy.[5]

Lamivudine (3TC, a nucleoside, **1**)[6,7] has recently been approved and (−)-FTC (**2**)[8,9] a related compound, is currently undergoing clinical trials. A number of other nucleosidic agents are also being investigated including L-FMAU (**3**), adefovir dipivoxil (**4**), and penciclovir (**5**).[10] These drugs are being studied as single agents as well as in combination therapies.[5] The required prolonged usage of these compounds leads almost invariably to resistance problems. In addition, anti-HBV nucleosides are often burdened with difficult and costly synthetic preparations. There is a clear need for a non-nucleoside therapeutic for hepatitis B virus infection. A potent non-nucleosidic small molecule HBV inhibitor could be subsequently exploited as a single agent or in combination therapy with nucleosides (Fig. 1).

We herein report a series of substituted propenamide derivatives that have been found to possess potent anti-hepatitis B activity. This series was discovered by the random screening in the HepAD38 cellular assay,[11–13]

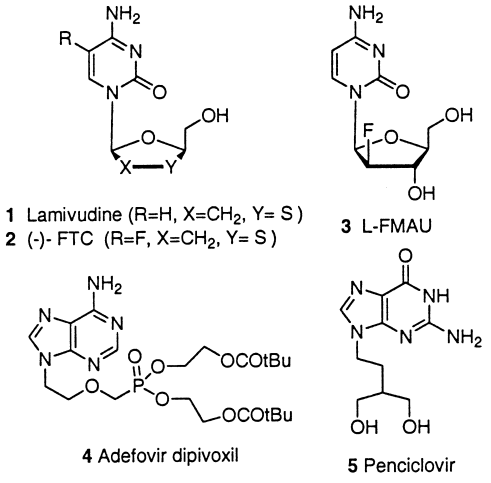

Figure 1.

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of an internal compound collection. The initial hit, **6**, and subsequent active analogues from the existing compound collection led to the preparation and evaluation of derivatives with modified A and B rings. In addition to high levels of potency these compounds generally exhibit low cellular toxicity (Fig. 2).

Chemistry

All of the targets were prepared analogously via an oxazolone intermediate as shown in Scheme 1. Condensation of a benzaldehyde **8** with hippuric acid (*N*-benzoylglycine) or a substituted hippuric acid **7** in acetic anhydride (100 °C) afforded the oxazolones, **9**, in generally good yields.[14,15] Where the required substituted hippuric acids were not commercially available they were prepared by a recently published route.[16] The oxazolones, **9**, were converted by the simple addition of piperidine or other desired amine (0 °C, $CHCl_3$) followed by in situ halogenation with elemental chlorine or bromine in the presence of $CaCO_3$ to afford targets **6** and **10**–**43**.[14,17]

Results and Discussion

Inhibition of hepatitis B virus replication as determined by HBV DNA levels,[11–13] and toxicity data for compounds **6** and **10**–**36** are presented in Table 1. Although this series of compounds does not display an obvious structure–activity relationship, several structural requirements for good antiviral activity are evident. Replacement of the piperidine amide with a variety of secondary and tertiary amides resulted in uniformly inferior activity relative to piperidine amides (Table 2). In general, vinyl bromides were slightly more active and less toxic than the corresponding vinyl chlorides (e.g., **12** vs **19**, **15** vs **16**, and **24** vs **29**). With the possible exception of **6** vs **13**, there were no examples of chlorinated derivatives being more potent than brominated compounds. Non-halogenated derivatives of compounds **6**, **12**, and **14** (Scheme 1, where X = H) displayed poor activity ($IC_{50} > 50\,\mu M$). Substitution at the 4-position of the A-ring (**10**, **12**, **17**, **18**, and **19**) is uniformly detrimental to activity and appears primarily due to a steric effect although fluorinated examples (**11** and **14**) are also less active than **6**. Substitution of the 2-position of the A-ring (with the

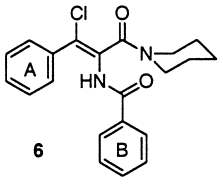
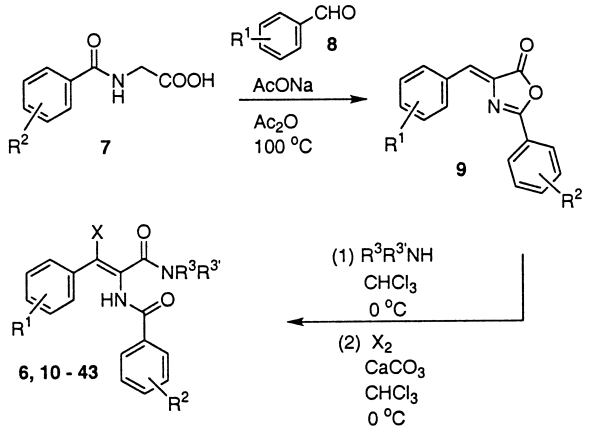
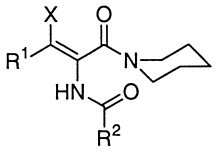


Figure 2.



Scheme 1. Synthesis of inhibitors.

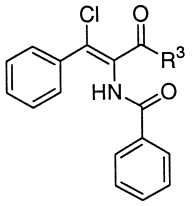
Table 1. A- and B-ring substitutions



Compd[a]	X	R^1 (A-ring)	R^2 (B-ring)	EC_{50}[b,e]	EC_{90}[c,e]	TC_{50}[d,e]
6	Cl	C_6H_5	C_6H_5	1.2	13	>81
10	Cl	4-CH_3O-C_6H_4	C_6H_5	>75		>75
11	Cl	4-F-C_6H_4	C_6H_5	3.1	28	>75
12	Cl	4-Cl-C_6H_4	C_6H_5	Toxic		45
13	Br	C_6H_5	C_6H_5	1.3	21	>72
14	Br	4-F-C_6H_4	C_6H_5	3.9		>70
15	Cl	2-F-C_6H_4	C_6H_5	Toxic		31
16	Br	2-F-C_6H_4	C_6H_5	1.5		70
17	Br	4-CH_3-C_6H_4	C_6H_5	2.8		>70
18	Br	4-CH_3O-C_6H_4	C_6H_5	13		>67
19	Br	4-Cl-C_6H_4	C_6H_5	11		55
20	Br	2-CH_3-C_6H_4	C_6H_5	0.89		>70
21	Br	2-CH_3O-C_6H_4	C_6H_5	2.1		>70
22	Cl	C_6H_5	4-F-C_6H_4	1.3	5.5	>67
23	Br	C_6H_5	4-F-C_6H_4	0.86	4.4	>69
24	Cl	C_6H_5	4-CH_3O-C_6H_4	5.2		>75
25	Br	C_6H_5	4-Cl-C_6H_4	0.54	3.1	>67
26	Br	C_6H_5	4-CH_3-C_6H_4	2.1	12	>70
27	Cl	C_6H_5	4-CF_3-C_6H_4	>69		>69
28	Br	C_6H_5	4-CF_3-C_6H_4	5.0	34	>62
29	Br	C_6H_5	4-CH_3O-C_6H_4	1.9	19	>67
30	Br	C_6H_5	4-Br-C_6H_4	0.53	3.7	>61
31	Br	C_6H_5	4-NO_2-C_6H_4	0.35	3.0	>65
32	Br	2-F-C_6H_4	4-NO_2-C_6H_4	0.31	1.8	42
33	Br	4-F-C_6H_4	4-NO_2-C_6H_4	0.72	11	>63
34	Br	4-Cl-C_6H_4	4-NO_2-C_6H_4	1.6		>60
35	Br	2-CH_3-C_6H_4	4-NO_2-C_6H_4	0.60	4.6	>63
36	Br	2-CH_3O-C_6H_4	4-NO_2-C_6H_4	0.13	0.92	>61
FTC				0.03	0.3	

[a]Proton NMR and mass spectra were consistent with the assigned structures of all compounds.
[b]EC_{50} = concentration of drug (in μM) which inhibits the synthesis of viral DNA by 50%.
[c]EC_{90} = concentration of drug (in μM) which inhibits the synthesis of viral DNA by 90%. Where EC_{90} values are not given, 90% inhibition could not be achieved.
[d]TC_{50} = concentration of drug (in μM) which reduces the number of viable cells by 50%.
[e]The EC_{50}, EC_{90}, and TC_{50} values reflect the mean value of at least three independent experiments in which each compound was tested in quadruplicate. The intra-experimental variation was <20% and the variation between individual experiments was <50%.

Table 2. R^3 Amide variants



Compd	R^3	EC_{50} (μM)
6		1.2
37		5.6
38		>75
39		>75
40		7.3
41		>75
42	$N(CH_2CH_3)_2$	>75
43	$NHCH_2C_6H_5$	>75

B-ring unsubstituted) had little effect on the activity in most cases, although the 2-fluoro derivative **15** was toxic (as defined: $TC_{50}/EC_{50} < 1$). Activity is sustained with a variety of substituents on the B-ring, although substitution in the 2-position did not improve activity. Most examples with substituents in the B-ring 4-position retained strong inhibitory activity ($\leq 10\,\mu M$). Electron-withdrawing substituents at the B-ring 4-position, particularly halogen and nitro, were highly active (**23**, **25**, and **30**–**36**).

Where both rings are substituted, the proper selection of a B-ring substituent can clearly enhance the activity of a compound relative to the analogous B-ring unsubstituted compound. For example, the inhibitory activity of **14** is clearly enhanced by the addition of a 4-nitro in the B-ring, **33**. Similar results are observed for **16** (a 5-fold improvement for **32**). Even poorly active compounds such as **19** display improved potency (a 7-fold improvement for **34**). The combination of a suitable 2-substituent in the A-ring (i.e., methoxy or fluoro) with a 4-halo or 4-nitro moiety in the B-ring led to the most active compounds in the series. The most potent compound, **36**, possesses activity ($EC_{50} = 0.13\,\mu M$) which approaches that of FTC ($EC_{50} = 0.03\,\mu M$). EC_{90}s were determined for selected compounds (see Table 1). Values ranged down to approximately 1 μM for **36**. The dose–response curves exhibit varying slopes; the EC_{90}s are thus not proportional to EC_{50}s across the series.

This series has been found to be generally nontoxic in the HepAD38 cell line. Only two compounds were found to be toxic to the point that no useful therapeutic window was observed (**12** and **15**). Most compounds show no toxic effects up to the highest concentrations tested. For **36** $TC_{50}/EC_{50} > 469$. One compound, **6**, has been studied in an additional cell line (HepG2) and found to be similarly nontoxic.[18] This analogue has also shown antiviral activity that is specific for HBV. Anti-HBV activity is similar against both wild type HBV and the YVDD lamivudine resistant variant[19] for all compounds described in Table 1. No effect on viral replication ($EC_{50} > 81\,\mu M$) was observed against woodchuck HBV, duck HBV, HIV-1, HSV-1, Newcastles disease virus or vesicular stomatis virus.[18]

While the precise mode of action of this series has not been proven, preliminary mechanistic studies have implied that these compounds inhibit HBV by interfering with the packaging of the pgRNA into immature core particles.[18]

Conclusion

We have shown that phenylpropenamide derivatives are potent inhibitors of hepatitis B virus replication in the HepAD38 cell line. Inhibitory data coupled with toxicity data offer significant potential for these compounds as a non-nucleosidic, non-immunomodulatory therapy for HBV infection. This class of compounds is also easily synthesized in two steps in good yields.

Acknowledgements

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