Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2715–2717

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2,5-Pyridinedicarboxylic Acid Derivatives as Non-Nucleosidic Reverse Transcriptase Inhibitors of Hepatitis B Virus

JinSoo Lee, HyungSoo Shim, YongKyun Park, SangJin Park, JoonSu Shin, WangYong Yang, HakDong Lee, WhuiJung Park, YongHo Chung and SangWook Lee*

Central Research Laboratory, DongWha Pharm. Ind. Co. Ltd., Seoul 430-017, South Korea

Received 22 April 2002; accepted 29 June 2002

Abstract—2,5-Pyridinedicarboxylic acid derivatives were found to be the potent non-nucleoside inhibitors of hepatitis B virus (HBV) with $IC_{50} \leq 0.01\,\mu g/mL$ in a reverse transcriptase inhibitory effect. And they showed the low toxicity compared with the nucleoside analogues.

Introduction

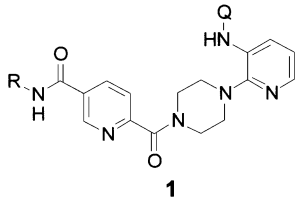
Hepatitis B virus (HBV) infection globally affects 300 million people, which represents about 5% of the earth's population being accompanied by acute or chronic hepatitis. In severe cases, it was reported that HBV is involved in transforming hepatitis into hepatic cirrhosis and hepatocellular carcinoma.[1]

Though α-interferon has been used for the treatment of patients with chronic HBV, its effectiveness rate tends to be low (less than 20%). Recently, as a nucleoside compound, Lamivudine[2,3] (3TC) was approved for the anti-HBV therapy. And, many other nucleosides including Adefovir dipivoxil, Famciclovir, Entecavir and (−)-FCT[4] are currently undergoing clinical trials. But the required prolonged usage of these anti-HBV nucleosides leads almost invariably to resistance problems. They have also other problems such as high treatment cost and adverse effects.

Under these circumstances, there is an urgent need for the development of effective anti-HBV agents as non-nucleosides.

The mechanism of HBV replication differs from that of other DNA viruses in that like retrovirus (e.g. Human immunodeficiency virus type 1), the reverse transcription step is involved. Reverse-transcriptase (RT) is an attractive target enzyme for anti-HBV therapy because it does not exist in human cell indicating that RT inhibitors are highly specific to virus. It is supposed that RT inhibitors inhibit the proliferation of HBV by interfering with the packaging of the reverse transcriptase in active site. HIV-1 RT inhibitors as non-nucleosides have been studied by Upjohn,[5] Merck,[6] etc. but non-nucleosidic HBV RT inhibitors have not been reported until now.

We have devised many classes of HBV RT inhibitors as non-nucleosides on the basis of the structure of non-nucleosidic HIV-1 RT inhibitors and discovered a series of active compounds by screening in the RT polymerase. Our compounds were found to have significant anti-HBV activities that HIV-1 RT inhibitors have not. Among them, 2,5-pyridinedicarboxylic acid derivatives (Fig. 1) showed the potent inhibitory effects. Herein we report our results.

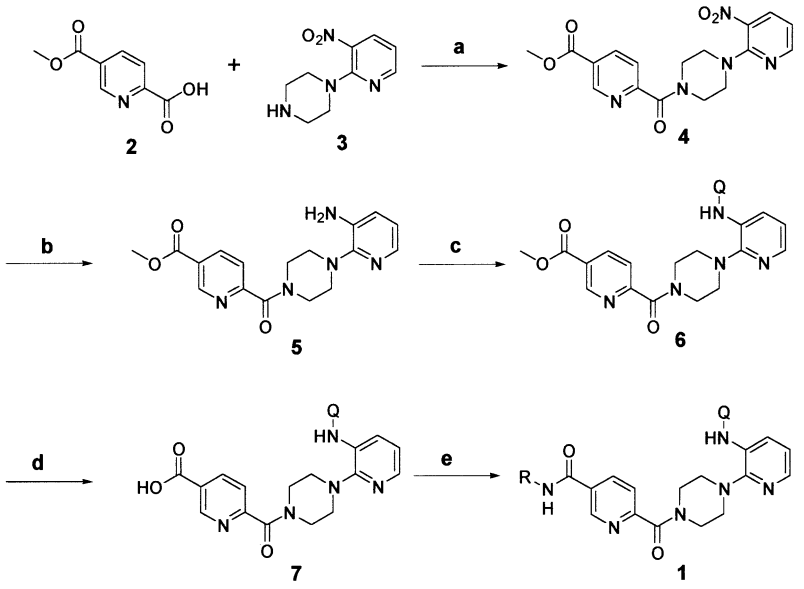


1

Figure 1.

*Corresponding author. Tel.: +82-31-445-2485; fax: +82-31-446-9556; e-mail: org302@iris.dwcrc.co.kr

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00531-0



Scheme 1. Synthesis of 2,5-pyridinecarboxylic acid derivatives. (a) pivaloyl chloride, triethylamine, methylene chloride; (b) Raney-nickel, H_2, ethyl acetate; (c) acetaldehyde (acetone, isobutylaldehyde) $NaBH_3CN$, methanol; (d) NaOH, methanol, H_2O; (e) pivaloyl chloride, triethylamine, RNH_2, methylene chloride.

Table 1.

Compd[a]	R	Q	Inhibitory activity of HBV–RT(%)			IC_{50} (μg/mL)
			1 μg/mL	0.1 μg/mL	0.01 μg/mL	
8	$HOCH_2CH_2$	H	74	67	54	0.003
9	$HOCH_2C(CH_3)_2$	H	70	62	38	0.038
10	$(CH_3)_2CH$	H	79	62	43	0.023
11	2-Pyridylmethyl	H	72	54	22	0.106
12	$HOCH_2CH_2$	Et	84	69	52	0.007
13	$HOCH_2C(CH_3)_2$	Et	72	56	32	0.068
14	$(CH_3)_2CH$	Et	85	67	54	0.006
15	$CH_3OCH_2CH_2$	Et	75	57	36	0.049
16	2-Morpholinylethyl	Et	66	52	30	0.109
17	2-Pyridylmethyl	Et	70	56	19	0.116
18	$HOCH_2CH_2$	*i*-Pr	54	51	30	0.261
19	$HOCH_2CH_2CH_2$	*i*-Pr	71	60	49	0.012
20	$HOCH_2C(CH_3)_2$	*i*-Pr	80	65	62	0.001
21	$(CH_3)_2CH$	*i*-Pr	65	51	23	0.149
22	$(CH_3)_3C$	*i*-Pr	88	72	61	0.002
23	$(CH_3)_2CHCH_2$	*i*-Pr	83	65	61	0.002
24	Cyclopropyl	*i*-Pr	62	51	5	0.237
25	Cyclopentyl	*i*-Pr	75	65	39	0.029
26	$CH_3OCH_2CH_2$	*i*-Pr	80	68	60	0.001
27	$CH_3OCH_2CH_2CH_2$	*i*-Pr	63	53	28	0.130
28	$(CH_3O)_2CHCH_2$	*i*-Pr	87	65	57	0.005
29	2-Morpholinylethyl	*i*-Pr	70	60	49	0.012
30	2-Pyridylmethyl	*i*-Pr	77	62	53	0.007
31	2-Pyridyl	*i*-Pr	74	55	26	0.085
32	3-Imidazolylpropyl	*i*-Pr	89	72	65	0.001
33	4-Butyrothiolactonyl	*i*-Pr	72	53	23	0.106
34	3-Methylpyrazolyl	*i*-Pr	70	53	20	0.124
35	5-Methylisoxazolyl	*i*-Pr	72	50	25	0.110
36	$HOCH_2CH_2$	*i*-Bu	86	60	54	0.009
37	$HOCH_2C(CH_3)_2$	*i*-Bu	90	71	63	0.001
38	$(CH_3)_2CH$	*i*-Bu	90	66	58	0.005
39	Cyclopropyl	*i*-Bu	87	71	60	0.002
40	$CH_3OCH_2CH_2$	*i*-Bu	85	70	52	0.007
41	$CH_3OCH_2CH_2CH_2$	*i*-Bu	72	51	20	0.123
42	2-(3-Hydroxypyridyl)	*i*-Bu	71	46	13	0.170
43	3-Imidazolylpropyl	*i*-Bu	88	72	60	0.002

[a]Proton NMR and mass spectra were consistent with the assigned structures of all compounds.

Chemistry

All compounds were synthesized through the general procedure shown in Scheme 1. 5-(Methoxycarbonyl)-2-pyridinecarboxylic acid **2** was easily prepared from 2,5-pyridinedicarboxylic acid (=isocinchomeronic acid).[7] Also, 1-(3-nitro-2-pyridyl)piperazine **3** was obtained from 2-chloro-3-nitropyridine with piperazine.

The compound **2** was reacted with pivaloyl chloride to form acid anhydride with good reactivity; then, the intermediate was reacted with the compound **3** to obtain a nicotinic acid ester derivative containing a nitropyridyl group. The compound **4** was reduced to form the derivative containing aminopyridyl group of the compound **5** under high-pressure condition using hydrogen gas in the presence of Raney-nickel.

And, aminopyridyl group of the compound **5** was reductively alkylated with acetaldehyde, acetone, or isobutyraldehyde in the presence of sodium cyanoborohydride under acidic condition to form nicotinic acid ester derivatives containing alkylaminopyridyl group of the compound **6**. The nicotinic acid derivatives of **7** were obtained by hydrolysis of nicotinic acid ester derivatives **6**. The target compounds **1** were prepared using pivaloyl chloride and several amines with acid compounds **7**.

Results and Discussion

The inhibitory activities of each test compound were shown in the following Table 1.[8] Although general structure–activity relationship of this series of compounds to HBV RT inhibition was not elucidated from these data, the compounds **36**–**43**, in which 'Q' is substituted by the isobutyl group, have the excellent activities ($IC_{50} \leq 0.01\,\mu g/mL$) with two exceptions (**41** and **42**). And in each class, the result was the most excellent when 'R' was isopropyl or *tert*-butyl, 3-imidazolylpropyl. And also in general introduction of heterocyclic groups into 'R' position was less effective except that of 3-imidazolylpropyl.

The cytotoxicity test was carried out using HepG2 cell in vitro. As a result, 2,5-pyridinedicarboxylic acid derivatives have low cytotoxicity with $CC_{50} \geq 100\,\mu M$, thus being quite safe compared with $CC_{50} = 50\,\mu M$ of Lamivudine.

In conclusion, we revealed that several synthetic compounds have a HBV RT inhibitory effect as non-nucleosides for the first time. And also they showed low cytotoxicity. It presents great potentiality of a new, effective and safe drug without any resistant problems and adverse effects of present nucleoside agents.

Acknowledgements

The authors are grateful to the MOST of Korea for financial support. We also thank Professor K. H. Chung at Seoul National University for helpful discussions.

References and Notes

1. Alter, M. J.; Shapiro, C. N. In *Therapies for Viral Hepatitis*; Schinazi, R. F., Sommadossi, J.-P., Eds.; International Medical: London, 1998; pp 3–8.
2. Deinstag, J. I. *New Engl. J. Med.* **1999**, *341*, 1657.
3. Lai, C.-L.; Chien, R.-N.; Leung, N. W. Y.; Chang, T.-T.; Guan, R.; Tai, D.-I.; Ng, K.-Y.; Wu, P.-C.; Dent, J. C.; Varber, J.; Stephenson, S. L.; Gray, D. F. *New Engl. J. Med.* **1988**, *339*, 61.
4. Furman, P. A.; Schinazi, R. F. In *Therapies for Viral Hepatitis*; Schinazi, R. F., Ed.; International Medical: London, 1988; p 273.
5. Dueweke, T. J.; Poppe, S. M.; Romero, D. L.; Swaney, S. M.; So, A. G.; Downey, K. M.; Althaus, I. W.; Reusser, F.; Busso, M.; Resnick, L.; Mayers, D. L.; Lane, J.; Aristoff, P. A.; Thomas, R. C.; Tarpley, W. G. *Antimicrob. Agents Chemother*. **1993**, *37*, 1127.
6. Thomas, J. T.; Terry, A. L.; Catherine, M. W.; Susan, F. B.; Steven, D. Y.; William, M. S.; William, C. L.; Mark, E. G.; Julie, A. O.; Richard, G. B.; Carl, F. H.; William, A. S.; Emilio, A. E.; Joel, R. H.; Paul, S. A. *J. Med. Chem*. **1994**, *37*, 2444.
7. *Nippon Kagaku Kaishi* **1967**, *88*, 553.
8. Yoon, S. J. U.S. Patent 5,968,781, 1999; Incubate recombinant HBV polymerase expressed in *E. coli* system, template/primer hybrid poly (A) oligo (dT)15, dTTp, DIG-UTP, biotin-UTP and inhibitor in streptavidin coated well plate. Digoxigenin- and biotin-labled nucleotides in optimized ratio are incorportated into one and the same DNA molecule, which is freshly synthesised by reverse transcription activity of the HBV polymerase. The detection and quantification of synthesized DNA molecule as a parameter for HBV polymerase activity or inhibitory activity follows ELISA protocol: First, biotin- labeled DNA binds to the streptavidin of well plate. In next, antibody to digoxigenin, conjugated to peroxidase, binds to the DIG-labeled DNA. In the final step, the peroxidase substrate ABTS is added. The absorbance of the sample can be detected using ELISA reader at 405 nm and the activity of enzyme or inhibitor is determined by relative absorbance.