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DISCOVERY OF IMIDAZO[1,2-*c*]PYRIMIDIN-5(6H)-ONE HETEROSUBSTITUTED NUCLEOSIDE ANALOGUES WITH POTENT ACTIVITY AGAINST HUMAN HEPATITIS B VIRUS IN VITRO

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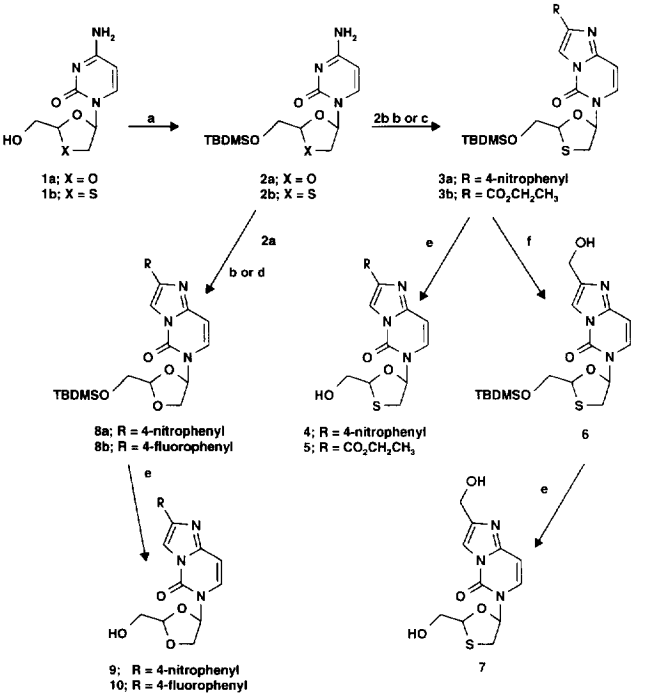
Abstract. The in vitro antihepatitis B virus (HBV) activities of eleven novel imidazo[1,2-*c*]pyrimidin-5(6H)-one dideoxynucleoside analogues in which the sugar ring is 1,3-dioxolane or 1,3-oxathiolane were compared in the chronically HBV-producing human cell line 2.2.15. Seven nucleoside analogues **4**, **9**, **10**, **15**, **16**, **18**, and **24**, of which **16** possesses the *trans* relative stereochemistry, displayed good potency and selectivity towards HBV. The order of decreasing potency at the 90% extracellular DNA inhibition level was **15>16>24≈9>10>18**. None of the tested imidazo[1,2-*c*]pyrimidines inhibited the replication of HIV-1 in MT-4 cells.

Hepatitis B virus (HBV) is a small, partially double-stranded, DNA virus that causes acute and chronic hepatitis in over 300 million people worldwide. The causal relation between chronic HBV infection and hepatocellular carcinoma, the most frequent cancer worldwide, is well established although the exact mechanism by which malignant transformation occurs remains uncertain.[1] HBV is not known to encode virus-specific enzymes for nucleotide synthesis that, consequently, stipulates relying on the host cell for supply of nucleotides for RNA and DNA synthesis. Despite that, some purine and pyrimidine nucleoside analogues are currently amongst the most promising agents against HBV. Several strategies based on nucleoside analogues have evolved for the treatment of HBV infection which include: (1) substitution of a carbon atom in the sugar ring by a heteroatom[2] (2) β-L absolute stereochemistry[2] (3) acyclic purine nucleoside and nucleotide analogues,[3] and (4) liposomal delivery of phospholipid prodrugs.[4] (-)-2'-Deoxy-3'-thiacytidine (3TC™, lamivudine, Epivir), currently in Phase II/III, against chronic HBV infection, was effective in causing a rapid decline in plasma virus load in patients.[5] A number of other nucleoside analogues have recently emerged with potent activity against HBV in tissue cultures and include (-)-2'-deoxy-3'-oxacytidine ((-)-BCH-204),[6,7] (-)-2',3'-dideoxycytidine (β-L-ddC),[8-11] (-)-2',3'-dideoxy-2',3'-didehydrocytidine (β-L-d4C),[12] their 5-fluoro derivatives (-)-FTC,[13] (-)-2'-deoxy-3'-oxa-5-fluorocytidine ((-)-FDOC),[14] β-L-5FddC,[8-11] β-L-Fd4C,[12] 2'-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-FMAU),[15] (+)-2'-deoxy-3'-oxacytidine ((+)-BCH-204),[6,7] ganciclovir,[16] penciclovir,[16] 9-(2-phosphonyl-

methoxyethyl)adenine (PMEA) analogues,[17] oxetanocin G,[18] (+)-[2-(hydroxymethyl)-1,3-dioxolane-4-yl]2',6'-diaminopurine ((+)-DAPD),[19] and 1,2-dipalmitoylphosphatidyl dideoxyguanosine (DPP-ddG).[20] Interferon alpha (IFNα) is the only alternative to treatment with antiviral nucleosides of chronic viral hepatitis. However, the response rates to toxic and expensive IFNα are poor and relapses are common.[21] On the basis of the findings that all of the current antiviral nucleosides against HBV contain natural pyrimidine or purine bases which anyway require activation by host enzymes, we explored the antiviral activity of novel base-modified dideoxynucleoside analogues.[22] Herein, we describe the synthesis, biological activities and the structure-activity relationship of imidazo[1,2-*c*]pyrimidin-5(6H)-one dideoxynucleoside analogues, also regarded as 3,7-dideaza-5-azapurines, in which the 3'-methylene group is substituted by sulfur or oxygen and the discovery of analogues with potent and selective activity against HBV in vitro.

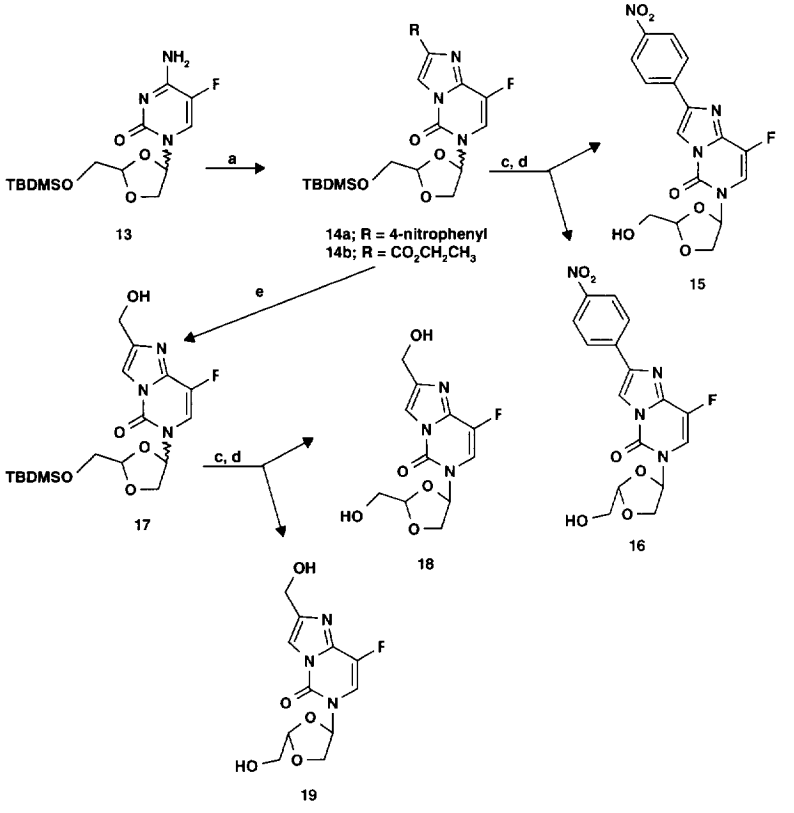
Chemistry. Two synthetic strategies A and B were designed to allow the flexibility in the synthesis of diverse analogues. Method A was retrosynthetically based on dissection of the imidazo[1,2-*c*]pyrimidine nucleoside analogues into the corresponding cytidine nucleoside and electrophilic α-haloketones as depicted in Schemes 1, 2. The overall stereochemistry is derived from that of the cytidine nucleoside. Briefly, protection of the



Reagents: (a) TBDMSCl, CH_2Cl_2, rt, (b) 2-bromo-4'-nitroacetophenone, CH_3OH, reflux, 20 h (c) ethylbromopyruvate, CH_3OH, reflux, 20 h, (d) 2-chloro-4'-fluoroacetophenone, CH_3OH, reflux 48 h (e) TBAF, CH_3CO_2H, THF, rt (f) LAH, THF, 0° C

Scheme 1.

primary hydroxyl group as its silyl ether is readily achieved with *t*-butyldimethylsilyl chloride. Condensation of the silyl ether with the corresponding α-haloketones in methanol afforded the imidazo[1,2-*c*]pyrimidines that were readily desilylated with tetrabutylammonium fluoride and acetic acid in tetrahydrofuran. In the case of ethylbromopyruvate, reduction of **3b** with lithium aluminium hydride in THF produced the corresponding hydroxymethyl derivative **6**. Method A was useful for the preparation of **4**, **5**, **7**, **9**, and **10**. Compound **12** (structure not shown), which is the *trans* analogue of **9**, was synthesized in 71% yield by direct condensation of the *trans* dioxolane cytosine nucleoside **11** with 2-bromo-4'-nitroacetophenone in methanol. Scheme 2 illustrates the synthesis of *cis* (**15** and **18**) and *trans* (**16** and **19**) imidazo[1,2-*c*]pyrimidines by starting with a mixture of cytidine dioxolanes **13**, followed by condensation with ethylbromopyruvate or 2-bromo-4'-nitroacetophenone, desilylation and separation.

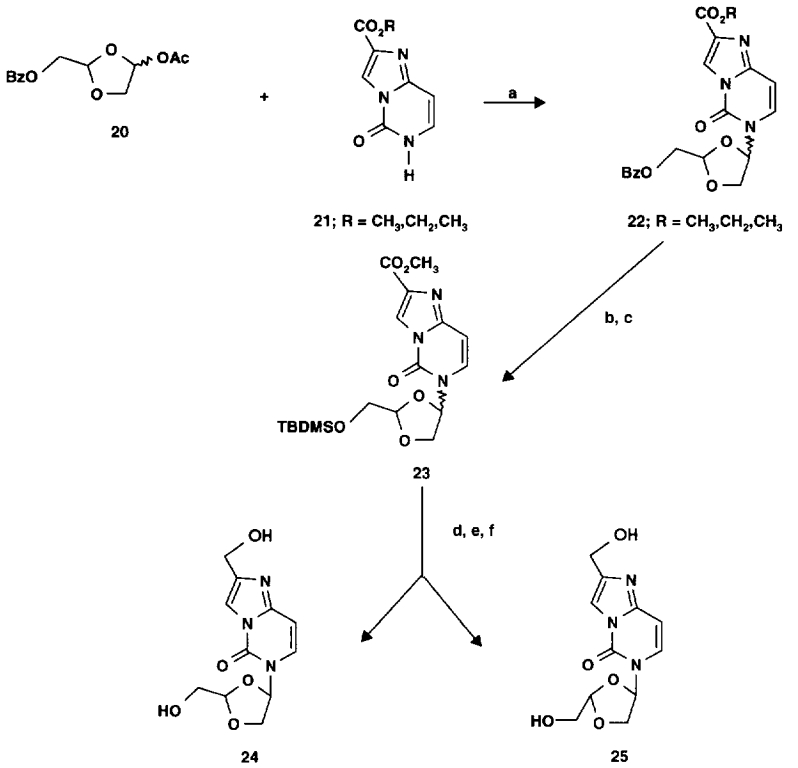


[a] Reagents: (a) 2-bromo-4'-nitroacetophenone, 2,6-lutidine, CH_3OH, reflux, (b) ethylbromopyruvate, 2,6-lutidine, CH_3OH, reflux, (c) TEAF, CH_3CO_2H, THF, (d) flash chromatography, (e) LAH, THF 0° C

Scheme 2

In method B, a performed imidazo[1,2-*c*]pyrimidine base **21** is coupled with the dioxolane sugar **20** in the presence of trimethylsilyltriflate as a Lewis acid promoter to afford a mixture of *cis* and *trans* anomers **22**. Separation of these anomers was best achieved by converting them to their *t*-butyldimethylsilyl ethers **23**

followed by chromatography on silica gel, reduction and deprotection with tetraethylammonium fluoride in acetonitrile. Scheme 3 illustrates the preparation of analogues **24** and **25** utilizing method B.



[a] Reagents: (a) 2 equiv. TMSOTf, CH_2Cl_2, 2,6-lutidine, rt, (b) NaOMe, CH_3OH, 1 h, (c) TBDMSCl, Im., DMF, rt, 1 h, (d) flash chromatography, (e) LAH, THF, O° C, (f) TEAF, CH_3CN

Scheme 3

Biological Evaluation. The anti-HBV activity of the imidazo[1,2-*c*]pyrimidine nucleosides was assessed in confluent cultures of the human hepatoma cell line 2.2.15 that chronically produces infectious HBV, according to the procedure of Korba and Milman.[23] The concentrations required to inhibit 50% and 90% of extracellular and intracellular replicating HBV DNA (EC_{50}, EC_{90}), 50% cytotoxic concentration (CC_{50}) by neutral red uptake[23] and selectivity indices (CC_{50}/EC_{90}) relative to 2',3'-dideoxycytidine (ddC) are shown in Table 1. Five of the six imidazo[1,2-*c*]pyrimidine bases when coupled to 1,3-dioxolane or 1,3-oxathiolane rings produced active nucleoside analogues of which **9**, **10**, **15**, **16**, **18**, and **24** were more selective than the control. The order of decreasing potency at the 90% extracellular DNA inhibition level was **15** >**16** >**24** ≈**9** >**10** >**18**. Nucleoside **15**, with *cis* relative stereochemistry, emerged as the most active analogue inhibiting effectively both extracellular and intracellular levels of HBV replications. In these assays, compound **15** has selectivity indices comparable to that of the potent anti-HBV agent β-L-5FddC measured in the same assay.[14] Interestingly,

compound **15** is about twofold more active than its *trans* isomer **16**. Fluoro substitution at C-8 enhanced the potency of both compounds **9** and **12** containing the powerful electron withdrawing 4-nitrophenyl moiety (see compounds **15** and **16** respectively), but reduced the activity of the hydroxymethyl containing analogue **24** in comparison to **18**. All analogues were substantially less toxic in cultures of 2.2.15 than the control as determined by the uptake of neutral red dye.

Table 1. Inhibitory Effect of Imidazo[1,2-*c*]pyrimidine nucleosides against HBV in 2.2.15 Cells in (μM)

	Extracellular HBV virion[a]		Intracellular HBV RI[a]		Cytotoxicity	Selectivity Index[b]	
Compound	EC_{50}	EC_{90}	EC_{50}	EC_{90}	CC_{50}	Virion	RI
4	4.8 ± 0.5	ND[c]	ND[c]	ND[c]	> 26.7	>5.6	
5	> 90	> 90	ND[c]	ND[c]	> 90	ND	
7	77.7 ± 9.2	275.6 ± 29.3	ND[c]	ND[c]	456 ± 29	1.7	
9	2.3 ± 0.3	10.9 ± 1.1	ND[c]	ND[c]	486 ± 53	45	
24	2.0 ± 0.3	10.5 ± 1.1	ND[c]	ND[c]	618 ± 52	59	
18	15.1 ± 1.8	33.3 ± 3.2	29.1 ± 3.5	66.6 ± 6.0	1059± 105	36	16
10	4.2 ± 0.6	25.2 ± 2.6	ND[c]	ND[c]	850 ± 70	34	
15	1.9 ± 0.3	5.9 ± 0.8	7.7 ± 0.8	13.6 ± 2.7	1390 ± 117	180	103
12	12.5 ± 1.5	107.6 ± 11.9	ND[c]	ND[c]	517 ± 47	5.7	
16	3.7 ± 0.5	8.8 ± 1.1	12.8 ± 1.1	25.0 ± 2.9	587 ± 51	46	24
19	> 105	> 105	ND[c]	ND[c]	1389 ± 109		
ddC	1.5 ± 0.2	6.9 ± 0.6	3.2 ± 0.4	10 ± 0.9	233 ± 20	34	23

[a] Values are means ± standard deviations. EC_{50} and EC_{90} were determined on day 9. The mean extracellular HBV DNA in untreated cells on day 9 was 94 pg/mL. The mean replicative intermediates (intracellular HBV DNA) in untreated controls on day 9 was 78 pg/μg cell DNA. [b] Selectivity index = CC_{50}/EC_{90}. [c] ND not determined.

Since the replicative cycle of HBV involves a cytoplasmic reverse transcription step of a pregenomic RNA intermediate similar to that of the human immunodeficiency virus (HIV), many dideoxynucleoside analogues such as 3TC™ (Epivir),[2] (-)-FTC,[13] (+)-BCH-204,[6, 7] (-)-BCH-204,[6,7] β-L-ddC,[8-11] ddC,[8-11] β-L-5FddC,[8-11] 5FddC,[11] β-L-Fd4C,[12] and β-L-d4C[12] exhibit potent inhibitory activities against both HIV and HBV. Therefore, compounds **4**, **7**, **12**, and **16** were tested against HIV-1 in MT-4 (human T helper) cells. None of the above nucleosides displayed any appreciable anti-HIV-1 activity at concentrations up to 300 μM. In conclusion, this work demonstrates the unprecedented antiviral properties of a new class of dioxolane and oxathiolane nucleoside analogues containing imidazo[1,2-*c*]pyrimidines, thus outlining a new strategy for discovery of antiviral agents based on modifications in the base moiety of nucleoside analogues.

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