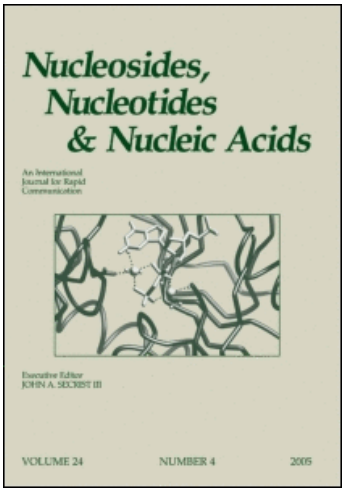

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STRUCTURE-ACTIVITY RELATIONSHIP OF PYRIMIDINE HETEROSUBSTITUTED NUCLEOSIDE ANALOGUES

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ABSTRACT

The structure-activity relationship of sixteen 3-deaza, C-4 substituted pyrimidines and imidazo[1,2-c]pyrimidine bases of 1,3-oxathiolanes and 1,3-dioxolanes revealed good anti-HBV activity in 2.2.15 cells transfected with human hepatitis B virus of the imidazo[1,2-c]pyrimidine nucleosides **21**, **25** and **29**. Two procedures for the preparation of C-4 substituted analogues are reported based on nucleophilic displacement of a sulfonamide or imidazole by a variety of nitrogen nucleophiles.

INTRODUCTION

The incorporation of a heteroatom such as sulfur or oxygen into the carbohydrate moiety of 2',3'-dideoxynucleoside analogues[1] and their 4'-thio derivatives[2] has a profound effect on the biological activity of the resulting heterosubstituted analogue. Recent studies in our laboratories and elsewhere have demonstrated that certain pyrimidine and purine analogues of natural bases are exceptionally potent inhibitors of viral and tumor proliferation.

Three relevant elements of the structure-activity relationship (SAR) have so far been reported. First, greater selectivity against HIV and HBV was obtained with β-L enantiomers of 1,3-oxathiolanes such as (-)-2'-deoxy-3'-thiacytidine (3TC™, Epivir™, lamivudine).[3] Epivir™ in combination with Retrovir™ (AZT) has recently emerged as the most effective first-line treatment of HIV infection in AIDS patients.[4] Lamivudine is

in advanced stages of clinical trials for hepatitis B infections causing a rapid decline in plasma virus load in chronic hepatitis B patients.[5] Moreover, the corresponding dioxolane analogue has potent anticancer[6] and antiviral properties.[2,7] Second, substitution of the H-5 moiety of cytosine by fluorine generally maintains the potency level in dioxolane and oxathiolane analogues.[8] However, reduced selectivity towards HIV replication *in vitro* was noted with the 2'-deoxy-3'-oxa-4'-thio analogues (dOTC) in a number of cell lines.[2] Third, the β-D dioxolane purine analogues of adenine, guanine and 2,6-diaminopurine have selective activity towards HIV.[9] The latter nucleoside, a prodrug of the guanine analogue, has also been shown to be a potent inhibitor of HBV *in vivo*.[10]

The SAR studies, thus far, have demonstrated that the nature of the carbohydrate moiety, the heterocyclic base and the absolute configuration are all important contributors to biological activity. To explore further the rather limited SAR of this important class of chemotherapeutic agents as well as the specificity of cytosine containing heterosubstituted nucleoside analogues, we describe herein the synthesis and biological results of three classes of analogues modified at either N-3 or C-4 moieties of cytosine or at both sites.

RESULTS

So far, two modifications of the cytosine base of BCH-189 (**16**) have been reported and include replacement of the C-2 carbonyl group by SO_2[11] as well as substitution at the C-5 position with halogens and methyl moieties.[10] The latter modifications resulted in analogues with activity against HIV and HBV replications.[2,7,10]

Deaza Analogues. The 3-deaza analogues of BCH-189 were synthesized with the rationale to maintain the base pairing role of the ring nitrogen without altering, significantly, the electrophilicity of the target molecules. The requisite 3-deaza-3-fluorocytosine and 3-deaza-5-fluorocytosine bases were conveniently prepared from pentafluoropyridine[12] and coupled with 2-benzoyloxymethyl-1,3-oxathiolane and -dioxolanes containing an anomeric acetate group in the presence of trimethylsilyltriflate as a Lewis acid promoter in dichloromethane.[13] As expected, the coupling proceeded in good yields (60-70%) to provide a mixture of cis and trans anomers which were readily separated by chromatography on silica gel and deprotected with methanolic ammonia. The 3-deaza analogues **1-3** (FIG. 1) together with their trans isomers (not shown) were

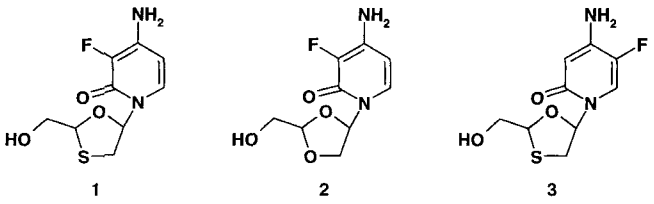
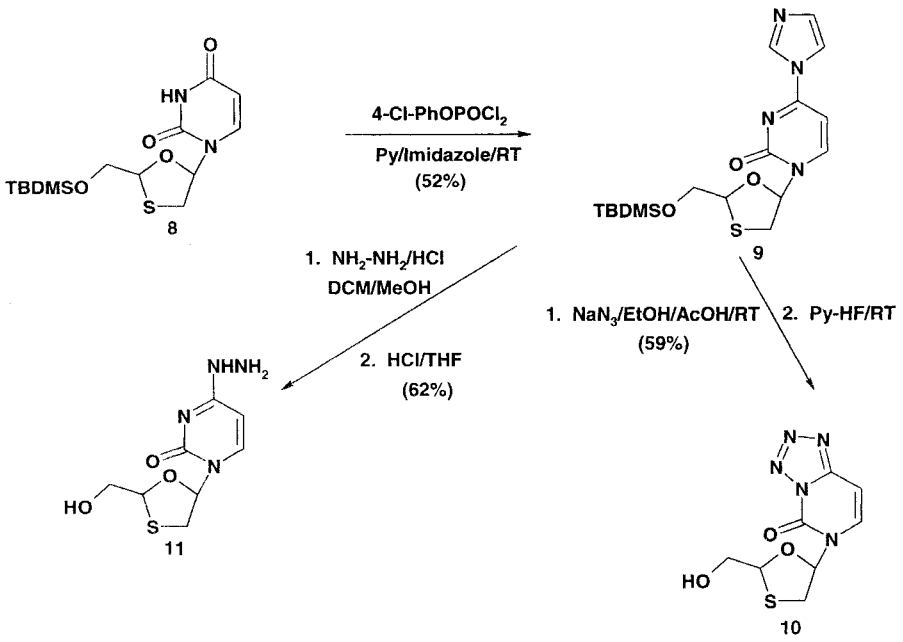


FIG. 1

assayed for activity against HIV-1 in MT-4 cells and HBV in 2.2.15 cells and were found to be devoid of any appreciable activity.

C-4 Analogues. For the synthesis of C-4 substituted analogues two methods were explored. In method A displacement of a toluenesulfonamide group in **4** furnished the N-alkylated derivatives when the reactions were performed in a sealed reaction vessel at 80-100°C for 12 hours. Hydroxylamine, n-butyl and methyl analogues **5-7** (FIG. 2) were prepared in good overall yield (>50%). A milder method based on the generation of a readily displaceable leaving group such as chloro at C-4 was desired to eliminate the need for high temperatures. Thus, reaction of the uracil derivative **8**, with p-chlorophenoxyphosphorylchloride in pyridine containing 5 equivalents of imidazole afforded the imidazolide **9** in 52% isolated yield. Nucleophilic displacements by a variety of nucleophiles such as sodium azide, hydrazine, allyl and propargyl amines afforded the desired C-4 substituted analogues **10-13** in good yields (SCHEME 1). In the case of sodium azide, the corresponding C-4 azido analogue was not isolated as it readily underwent intramolecular cyclization to afford the tetrazolopyrimidine **10**. It is worth mentioning that the presence of small amounts of acetic acid increases the yields of substitution due to the protonation of the imidazole ring, which renders it a better leaving group. Compounds **4**, **5**, **6**, **7** (FIG. 2) and **10**, **12**, **13** and **14** (FIG. 3) did not inhibit the replication of HIV-1 in MT-4 cells at concentrations up to 400 μM. However, in this series, compound **11** had anti-HIV-1 activity in MT-4 cells (EC_{50} 96μM, IC_{50} >400 μM) being about 100 fold less potent than 3TC™.

Imidazo[1,2-c]pyrimidine Analogues. Further to our studies on base modifications at N-3 and C-4 positions, the structure activity relationship of imidazo[1,2-



SCHEME 1

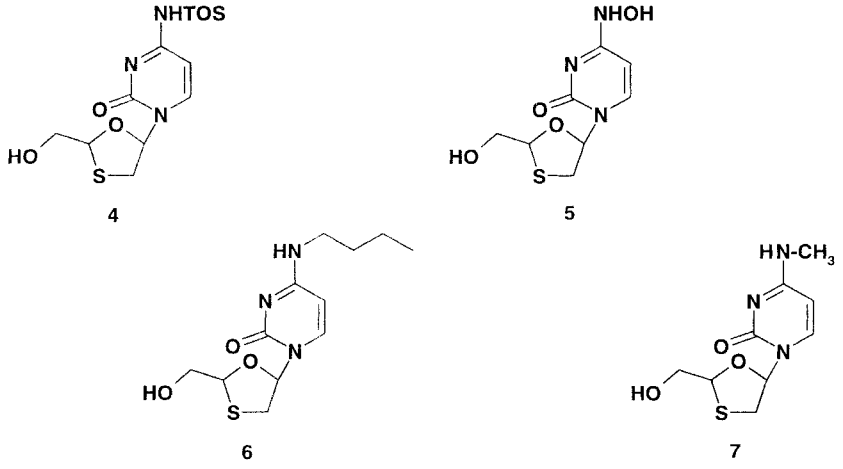


FIG. 2

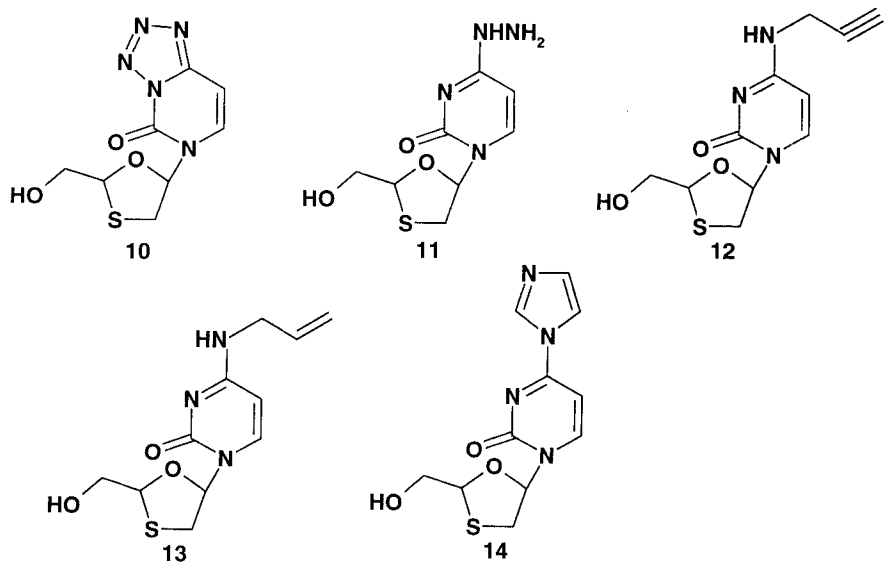
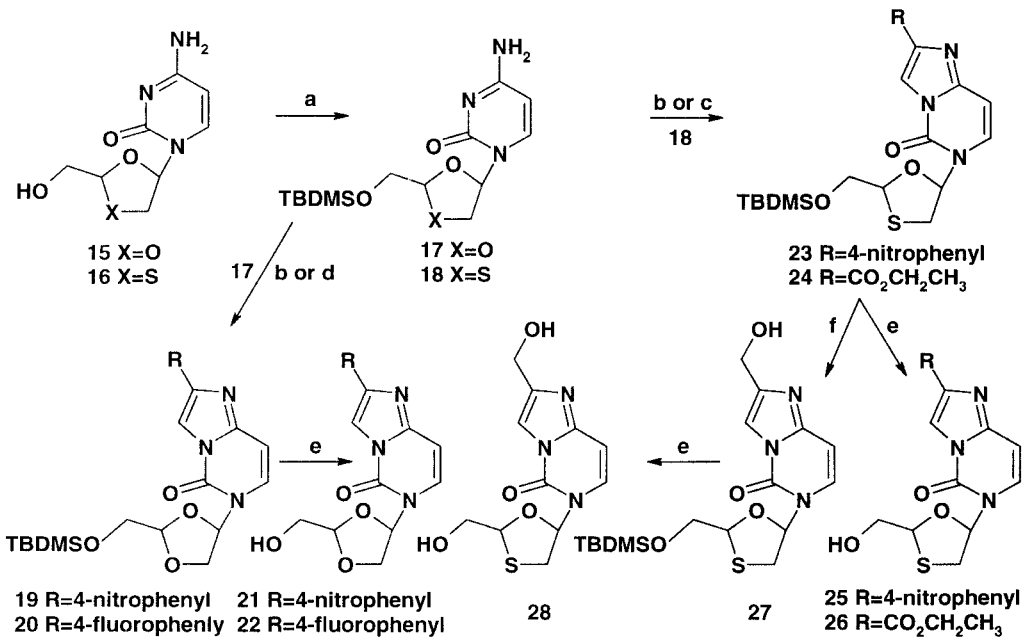


FIG. 3

c]pyrimidines was explored.[14] These analogues are ethenocytosine derivatives but can also be regarded as 3,7-dideaza-5-azapurines glycosylated at N-1. One synthetic strategy to this class of nucleosides 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 SCHEME 2. Protection of the 5'-hydroxyl group of **15** and **16** as its silyl ether group is readily achieved to afford **17** and **18** respectively. Condensation of **17** with the corresponding α-haloketones in methanol gave the imidazo[1,2-c]pyrimidines **19** and **20** which were desilylated with TBAF in THF to afford nucleosides **21** and **22**. Similar transformation of **18** produced **23** and **24** which were readily converted to the deprotected nucleosides **25**, **26** and **28**. The dioxolane analogue **29** was prepared from **17** using the same procedure depicted in SCHEME 2 for compound **28**.

The nucleosides **21**, **25**, **26**, **28** and **29** (FIG. 4) were inactive in the anti-HIV-1 assays in MT-4 cells. However, their anti-HBV activity was assessed in hepatoma cell line 2.2.15 transfected with human HBV.[15] Compounds **21**, **25** and **29** emerged as good inhibitors of extracellular HBV with **21** and **29** being more selective than the control ddC, whereas **28** had moderate activity and **26** was inactive (TABLE 1).



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

SCHEME 2

TABLE 1. Anti-HBV activities of imidazo[1,2,c]pyrimidines

Compound	Extracellular HBV Virion EC_{90} (μM)	Citytoxicity IC_{50} (μM)	SI
21	11	486	45
25	4.8[1]	>27	>5.6
26	>100	>100	—
28	275	456	1.7
29	11	618	59
ddC	7	233	34

[1] EC_{50}

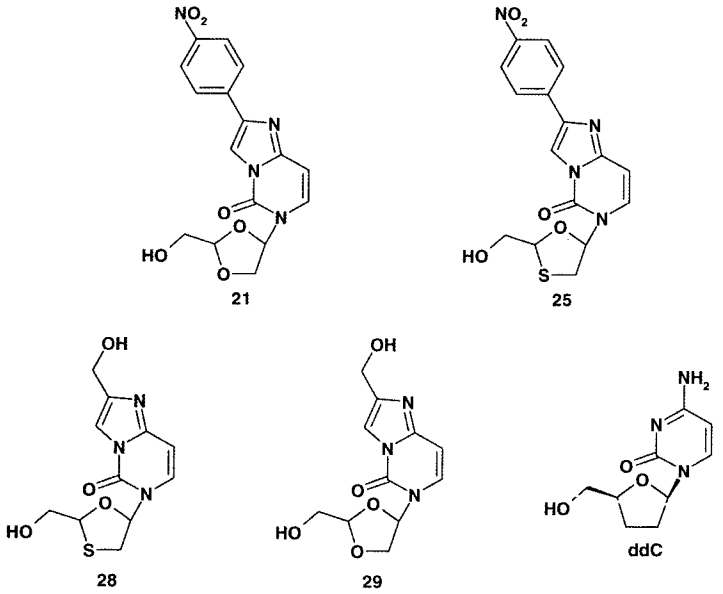


FIG. 4

DISCUSSION

The above results demonstrate that minor modifications at N-3 and C-4 positions eliminate the anti-HIV-1 activity of BCH-189. The specific reasons for this have not been investigated but are likely related to the lack of recognition by kinases or due to their biochemical metabolism. The encouraging inhibitory effects of the imidazo[1,2-c]pyrimidines **21** and **29** against HBV suggest the need for further SAR in this new series of base modified heterosubstituted nucleoside analogues.

In summary, the structure-activity relationship of pyrimidine modified 1,3-oxathiolanes and -dioxolanes led to the discovery of a new class of anti-HBV agents which warrant further studies including stereochemical considerations. Substitution at the N-3 or N-4 resulted in appreciable reduction of anti-HIV-1 activity.

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