SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL 1,3-OXAZOLIDINE NUCLEOSIDE ANALOGUES

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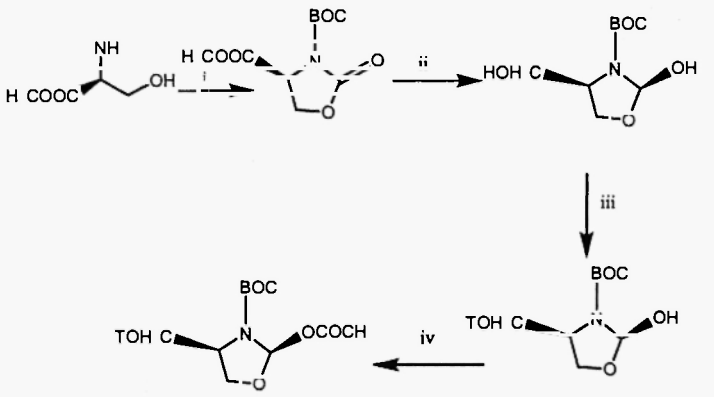
Abstract : The synthesis of novel 1,3-oxazolidine pyrimidine nucleoside analogues are described. These analogues are all derived from the key stereochemically defined intermediate N-tert-butoxy-carbonyl-2-hydroxymethyl-1,3-oxazolidine-4-ol which was accessible in 57% yield starting from L-serine methylester hydrochloride. The heterocyclic bases eg; uracil, thymine etc are efficiently introduced onto the 1,3-oxazolidine by the Vorbruggen procedures. The antimicrobial activity of novel 1,3-oxazolidine nucleoside analogues are highlighted. The compounds **7d** and **7e** showed significant activity against bacteria and fungus.
Key Words: 1,3-oxazolidine nucleosides, Antimicrobial activity.

Introduction

The potent activity displayed by 3′- azido-3′-deoxythymidine (AZT) against human immunodifficiency virus [1] (HIV) provides impetus for the development for the novel nucleoside analogues.[2] Unfortunately, those compounds with the natural stereochemistry possess undesirable pharmacological properties and are susceptible to the development of resistant strains of HIV. In an attempt to overcome some of these detrimental side effects, the carbohydrate moiety of 2′, 3′-dideoxy nucleoside analogues has been replaced by other five membered rings.[3] It has been demonstrated that hetero substitution of these rings has a profound effect on the biological activity of the resultant nucleoside analogues as displayed by (-)-2′-deoxy-3′-thiacytidine (3 TC).[4] Antiviral nucleoside analogs containing more than one heteroatom in the sugar ring have received much attention because of their potent anti-HIV and anti-HBV activities. At present, β-L-(-)-2'-deoxy-3'-thiacytidine (Lamivudine) is clinically used for treatment of AIDS and AIDS-complex. Interestingly, this compound showed much lower cytotoxicity than its antipode the D-enantiomer although both are almost equipotent against the replication of HIV-1 and -2 *in vitro*.

Oxazolidinones are the new class of antimicrobial agents with activity against broad spectrum of gram positive pathogens including staphalococci, streptococci and enterococci.[5-7] The oxazolidinone structure is relatively simple and allows for diverse synthetic modification. Because of this and because of the importance of oxazolidinones as antimicrobial agents, we have efficiently introduced the heterocyclic bases eg; uracil, thymine etc onto the 1,3-oxazolidine by the Vorbruggen procedures[8] and reported the antimicrobial activity of these compounds.

Our synthetic strategy was based on the preparation of N-tert-butoxycarbonyl-1,3-oxazolidine-4-one **(2)** followed by the reduction and acylation to give 4-acyloxy-1,3-oxazolidine. Thus compound **2** has been prepared by condensing di-tert-butyl-di-carbonate with L-serine methyl ester hydrochloride(**1**) in the presence of dimethylaminopyridine (DMAP) and triethylamine.[9] The lactam and the ester functional groups present has been reduced by using $Li(et)_3BH$ at -78^0 C to get the desired N-tert-butoxycarbonyl-2-hydroxymethyl-1,3-oxazolidine-4-ol**(3)**in quantitative yield.[10-12] Protection of primary alcoholic group using trityl chloride has been carried out in the presence of pyridine to give trityl derivative (**4**). This was subjected to acetylation in the presence of acetic anhydride, dimethylaminopyridine and triethylamine to give N-tert-butoxycarbonyl-2-hydroxymethyl- 4-acyloxy-1,3-Oxazolidine (**5**)[13] (Scheme-1)

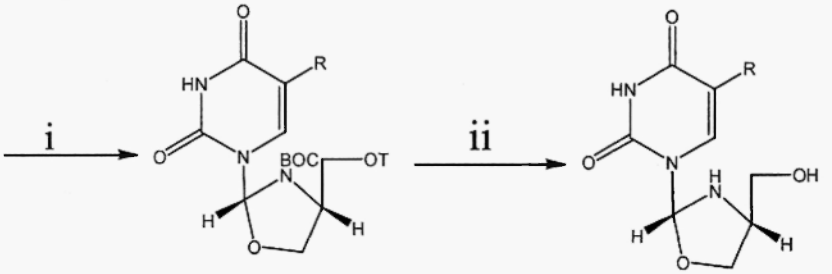


ents and Conditions:

i) (BOC) O, DMAP, (Et) N, RT ii) Li(et) BH, THF, - C iii) Trityl Chloride, Pyridine, Reflux iv) (CH CO) O, DMAP, (Et) N, RT.

Scheme-1

The Compound **5** is suitable for coupling with silylated pyrimidine bases under refluxing conditions in CH_2CN and in the presence Stannic Chloride as lewis acid (Scheme-2). This gave the desired nucleoside analogues as an anomeric mixture in moderate yields.[14-15]. The ^1H NMR spectra of the nucleoside analogues (**7a-f**) showed multiplet due to the aromatic protons in the region 6.55-8.10. The signals due to the NH protons were appeared in the region at 10.0 and the C^1-H proton of the oxazolidine moiety caused a singlet in the region 5.05 indicating the attachment of the oxazolidine moiety and the formation of configuration. While singlet due to the OH protons in these compounds was noticed at 2.0. The N-tert-butoxycarbonyl (BOC) and trityl (T) groups are deprotected by stirring it with trifluoroacetic acid at room temperature. The structures of the synthesized compounds have been characterized by the spectroscopic data and elemental analysis. The results are summarized in Table-1.



i) Silylated pyrimidine base, TMSOTf or SnCl ,CH CN,O C ii) TriflouroAcetic acid, RT.

Base= **a**)R=H Uracil, **b**) R=CH Thymine, **c**) R=SH, -ThioUracil, **d**) R=F -Flourouracil, **e**) R=Br -BromoUracil, **f**) R=Cl -ChloroUracil etc

Scheme-2

Antimicrobial Activity

All the synthesized nucleosides were screened for their antimicrobial activity against bacteria *E.coli* (gram negative), *S.aureus* (gram positive) and fungi viz *A. niger*, *C.albicans* and *A.flavus*, at the conc of 100 µg/ml, using Norfloxacin and Griseofulvin respectively as reference compounds. These investigations have been performed by disc diffusion method by Verma et al.[16] The test compounds were dissolved in dimethyl formamide and different aliquots were placed in each cup. Incubation was carried out at 37^0 C for 24 hr. The results are summarized in table-2.

Table-1: Analytial and spectral data

Compd. No	Yield (%)	m.p in ^{0}C	C,H,N Analysis Anal Calcd for	IR (nujol) cm^{-1}	^{1}H NMR ($CDCl_3$)
2	60	68	$C_{10}H_{15}NO_6$, C,48.98; H,6.17;N,5.71, Found; C48.95;H,6.15;N,5.70	1710-1720 cm^{-1} (C=O of BOC-ester group), 1820 cm^{-1} (C=O group), 1690 cm^{-1} (C=O group).	4.99 (1H,dd), 3.84 (3H, s), 3.64 (1H,dd), 3.32 (1H,dd), 1.51,(9H s, Boc ester).
3	57	79	$C_9H_{17}NO_5$, C,49.31; H,7.76;N, 6.39, Found; C, 49.29; H, 7.74; N; 6.36.	1710-1720 cm^{-1} (C=O of BOC-ester group), 3200-3220 cm^{-1} (OH)	4.99 (1H,dd), 3.64 (2H, dd), 1.51,(9H s,C=O) 2.78 (d,2H)6.68 (1H, s, CH), 2 (2H, s,OH),
4	65	112	$C_{28}H_{31}NO_5$, C,72.86;H,6.77; N,3.03, Found;72.85;H,6.75; N,3.04.	1710-1720 cm^{-1} (C=O of BOC-ester group), 3200-3220 cm^{-1} (OH) 750 cm^{-1} (ArH).	4.99 (1H,dd), 3.64(1H,dd), 1.51,(9H s, Boc ester),2.78 (d,2H) 6.68 (1H, s, CH), 2 (2H, s,OH), 7.19(15H, s, ArH).
5	53	130	$C_{30}H_{33}NO_6$ C,72.55;H,6.61;N,2.78, Found; 72.56; H,6.62; N,2.79.	1710-1720 cm^{-1} (C=O of BOC-ester group), 1690 (C=O of acetoxy gp) 750 cm^{-1} (Ar H)	4.99 (1H,dd), 3.64 (1H,dd), 2.01 (3H,s)2.78 (d,2H) 1.51,(9H,s,Boc ester 6.68 (1H, s, CH), 2 (2H, s,OH), 7.1(15H, s, ArH).
7a	50	----	$C_8H_{11}N_3O_4$. C,45.07;H,5.16;N,19.71, Found;C,45.03;H,5.12; N,19.70.	1890 cm^{-1} (C=O), 1690 cm^{-1}(C=O), 2950cm^{-1} (NH), 3557 cm^{-1} (OH)	10.0(1H,s,NH), 5.05(1H,t CH), 4.05 (2H,t,H_2), 3.84 (2H,t, H_2), 2.0 (1H, s, OH), 2.3 (1H, s, NH), 5.76(1H,d,CH) , 7.79 (1H, d, CH);
7b	45	----	$C_8H_{11}N_3O_4$, C,42.02; H,4.28; N,16.34,S,12.45, Found;C,42.05;H,4.26;N,16.30, S, 12.40.	1890 cm^{-1} (C=O), 1690 cm^{-1}(C=O), 2950 cm^{-1}(NH), 3557 cm^{-1} (OH)	10.0(1H,s,NH), 5.05(1H,t, CH), 4.05 (2H,t,H_2), 3.84 (1H,t, H_2), 2.0 (1H, s, OH), 2.3 (1H,s,NH), 1.93(3H,d,CH_3) 7.57 (1H, s, CH)
7c	52	----	$C_8H_{11}N_3O_4S$, C,42.02;H,4.28;,16.34,S,12.45, Found;C,42.05;H,4.26;N.16.30, S, 12.40	1890 cm^{-1} (C=O), 1690cm^{-1}(C=O), 2950cm^{-1} (NH), 3557 cm^{-1} (OH), 2600 cm^{-1} (SH)	10.0 (1H,s,NH), 1.5 (1H,d,SH) 5.05(1H,t,CH), 4.05(2H,t,H_2), 3.90 (1H,t,H_2), 3.84 (2H,d,H_2), 2.0 (1H, s, OH), 2.3 (1H,s,NH), 4.5 (1H, d, 1H) 7.3 (1H, d, CH)
7d	48	----	$C_8H_{10}FN_3O_4$, C,40.16;H,4.18; N,17.57,F,11.29 Found;C,40.17; H,4.17; N,17.50,F,11.27	1890 cm^{-1} (C=O), 1690 cm^{-1}(C=O), 2950 cm^{-1} (NH), 3557 cm^{-1} (OH)	10.0(1H,s,NH), 5.05(1H,t, CH), 4.06 (2H,t,H_2), 3.90 (1H,t, H_2), 3.84 (2H,d, H_2), 2.0 (1H, s, OH), 2.3 (1H,s,NH), 7.39 (1H, s, CH)
7e	51	----	$C_8H_{10}BrN_3O_4$, C,32.87; H,3.42; N,14.38;Br;27.39 Found;C,32.85;H,3.32; N,14.30;Br;27.38.	1890 cm^{-1} (C=O), 1690 cm^{-1}(C=O), 2950 cm^{-1}(NH), 3557 cm^{-1} (OH)	10.0 (1H,s,NH), 5.05(1H, t,CH), 4.06 (2H,t,H_2), 3.90 (1H,t, H_2), 2.0 (1H, s, OH), 2.3 (1H,s,NH), 8.24 (1H,s,CH)
7f	62	----	$C_8H_{10}ClN_3O_4$, C,38.70;H,4.03;N,16.93;Cl,14.51 Found;C,38.73;H,4.05;N,6.90;Cl,14.5	1890 cm^{-1} (C=O), 1690 cm^{-1}(C=O), 2950 cm^{-1} (NH), 3557 cm^{-1} (OH)	10.0(1H,s,NH), 5.05(1H,t, CH), 4.06 (2H,t,H_2), 3.84 (1H, t, H_2), 2.0 (1H, s, OH), 2.3 (1H,s,NH), 8.24 (1H, s, CH)

Table-2: Effect of compounds 7a-f on bacterial and fungal activity (Dilution: 100μg/ml.)

Compounds	Antibacterial activity		Antifungal activity		
	Zone of Inhibition(mm)		Zone of Inhibition (mm)		
	E.Coli	S.Aureus	A.niger	C.albicans	A.flavus
7a	23	24	24	22	23
7b	25	24	25	24	26
7c	25	26	26	27	27
7d	34	30	31	30	31
7e	32	30	30	30	31
7f	30	29	28	30	29
Norfloxacin	35	32	-------	------	-----
Griseofulvin	-----	------	32	32	32

Experimental

The melting points were determined in open capillary and are uncorrected. The IR spectra were recorded on Shimatzu FT-IR spectrophotometer in nujol.^{1}H NMR spectra was recorded on Hitatchi R-600 spectrometer with TMS as internal standard.

1 N-tert-butoxycarbonyl-4-(methoxy carbonyl)-1,3-oxazolidin-2-one (2)
Di-tert-butyl-di-carbonate (3 equiv) was dissolved in 4ml of MeCN (or toluene) and placed in an ice bath and 0.5 equiv of DMAP and 3 equiv of triethylamine were added. After 5 minutes Serine methyl ester hydrochloride (0.083g,0.5 mmol) was added in portions for during 2 mins and the reaction was allowed to run for 1 hour more. At the end of the reaction chloroform was added and the solution was washed with 5% HCl (2x20 ml), dried with $MgSO_4$ and evaporated to give the compound **2** as a white solid in good yield.

2 N-tert-butoxycarbonyl-4-(hydroxyl methyl)-1,3-oxazolidin-2-ol (3)
Compound **2** was dissolved in THF (20ml), cooled to -78^0 C was added 3 eq of 1M solution of $Li(et)_3BH$. The reaction requires 10 to 60 mins for completion. Excess of reagent was destroyed by the addition of saturated solution of NH_4Cl at -78^0 C and the reaction mixture was extracted with dichloromethane (3x15ml) and dried over anhydrous $MgSO_4$ and evaporated to dryness followed by the purification by flash chromatography (hexane-ethyl acetate 7:2) afford the compound **3** as light yellow oil.

3 N-tert-butoxycarbonyl-4-(trityloxy methyl)-1,3-oxazolidine-2-ol (4)
Compound **3** (0.8979g, 4mmol) and trityl chloride (triphenylmethylchloride) (1.4g,5mmol) were dissolved in 20 ml of pyridine and the mixture was heated at 100^0 C (steam bath) with swirling for 30 mins. The reaction mixture was cooled at room temperature and then poured in to 100 ml of ice water. The slurry was stirred vigorously during quenching. The solid was filtered and washed thoroughly with water until it is free from pyridine. Dry the solid separately. Finally recrystallization from acetone-toluene to give a white solid of O-trityl derivative.

4 N-tert-butoxycarbonyl-2-hydroxymethyl- 4-acyloxy-1,3-oxazolidine (5).
A solution of trityl derivative **4** in CH_2Cl_2 was treated with acetic anhydride (2.6gm,26.3 mmol), Triethylamine (2.6gm,26.3 mmol) and a catalytic amount of 4-DMAP at room temperature for 3 hrs. The

resultant mixture was washed with 5% HCl (3x15ml) and extracted with CH_2Cl_2 (3x20ml) and evaporated to dryness and purified by silica gel column chromatography with 5% chloroform: Ethyl acetate 7:2 to get white solid.

5 General procedure for the synthesis of 1,3-oxazolidine nucleoside analogues (7a-f)

A mixture of pyrimidine base (2.35 mmol) in Hexamethyldisilazane (10 ml) and CH_3CN was heated under reflux for 5 hrs. After removal of solvent by vacuum pump, a solution of compound **5** (0.786 mmol) in 15 ml of CH_3CN was added to the reaction flask containing the silylated pyrimidine bases and then Stannic Chloride (1.4 mmol) was added dropwise at room temperature. After 16 hrs the reaction was quenched with 1 ml of saturated $NaHCO_3$ and the resultant mixture was concentrated. The crude mixture was exctracted with CH_2Cl_2 (3x20ml) , washed with aq. $NaHCO_3$, dried over $MgSO_4$, filterd and concentrated. The crude product was purified by silica gel column chromatography with ethyl acetate:hexane (7:2) to give nucleoside analogues (**6a-f**) as yellow oil. Finally BOC and trityl groups were deprotected by stirring it with triflouroacetic acid at room temperature afford **7a-f** in moderate yield.

Conclusions

In conclusion, we have presented short and efficient preparations of the 1,3-oxazolidine nucleoside analogues which as a consequence of their low toxicity should prove to be important antimicrobial agents. The novel 1,3-oxazolidine nucleoside analogues have been synthesized using Vorbruggen procedure by condensing silylated pyrimidine bases with the acylated 1,3-oxazolidine moiety in the presence of lewis acid to get the desired products in the moderate yield. The antimicrobial activity of these compounds have been studied and in that compounds **7d** and **7e** with fluoro and bromo substituents showed significant activity against *E.coli, S.aureus, A. niger, C.albicans* and *A.flavus* among the six compounds screened.

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