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An Efficient Synthesis Tetrazole and Oxadiazole Analogues of Novel 2'-Deoxy-C-Nucleosides and their Antitumor Activity

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Abstract:
Various tetrazole and oxadiazole C-nucleoside analogues were synthesized starting from pure α- or β-glycosyl-cyanide. The synthesis of glycosyl-cyanide as key precursor was optimized on gram-scale to furnish crystalline starting material for the assembly of C-nucleosides. 1,2,4-Oxadizole C-nucleosides were synthesized via two independent routes. First, both anomers of glycosyl-cyanide were transformed into tetrazole nucleosides followed by acylative rearrangement to furnish 1,2,4-oxadiazoles in high yields. Second, the glycosyl-cyanide was converted into an amidoxime which upon ring closure offered an alternative pathway for the assembly of 1,2,4-oxadizoles in an efficient manner. These protocols offer an easy access to otherwise difficult to synthesize C-nucleosides in good yield and protecting group compatibility. These C-nucleosides were evaluated for their antitumor activity. This work paves a path for facile assembly of library of new chemical entities useful for drug discovery.

Key Words:
C-Nucleoside, Cyano-sugar, Hoffer's Chloro-sugar, 1,2,4-oxadiazole, Anomers, Antitumor

Introduction:
Unlike natural and synthetic N-nucleosides, C-nucleosides [1,2] are stable to enzymatic and acid-catalyzed hydrolysis of the glycosidic bond. Therefore, C-nucleosides offer a distinct advantage over the N-nucleosides for design of biologically active molecules. C-Nucleosides [3,4,5] have also attracted the interest of researchers looking for hydrogen-bond interactions alternative to those produced in the classical Watson–Crick model. Among well-known antitumor C-nucleosides, pyrazofurin,[6] showdomycin [9] and tiazofurin[10] are five-membered heterocyclic structures showing excellent biological activity (Figure- 1). Despite of their remarkable activity profile, lack of specificity and neurotoxicity prohibited the clinical progress of these nucleosides. More recently, remdesivir (GS-5734)[7,8] has shown promise for the treatment of COVID-19. Immucillins is yet another important class of C-nucleosides advancing into clinical trials as inhibitor of purine nucleoside phosphorylase. These observations have motivated us to revisit the study of C-nucleosides, with a particular interest of designing 2'-deoxyribose analogues of various five membered heterocycle and their antiviral and antitumor activity.

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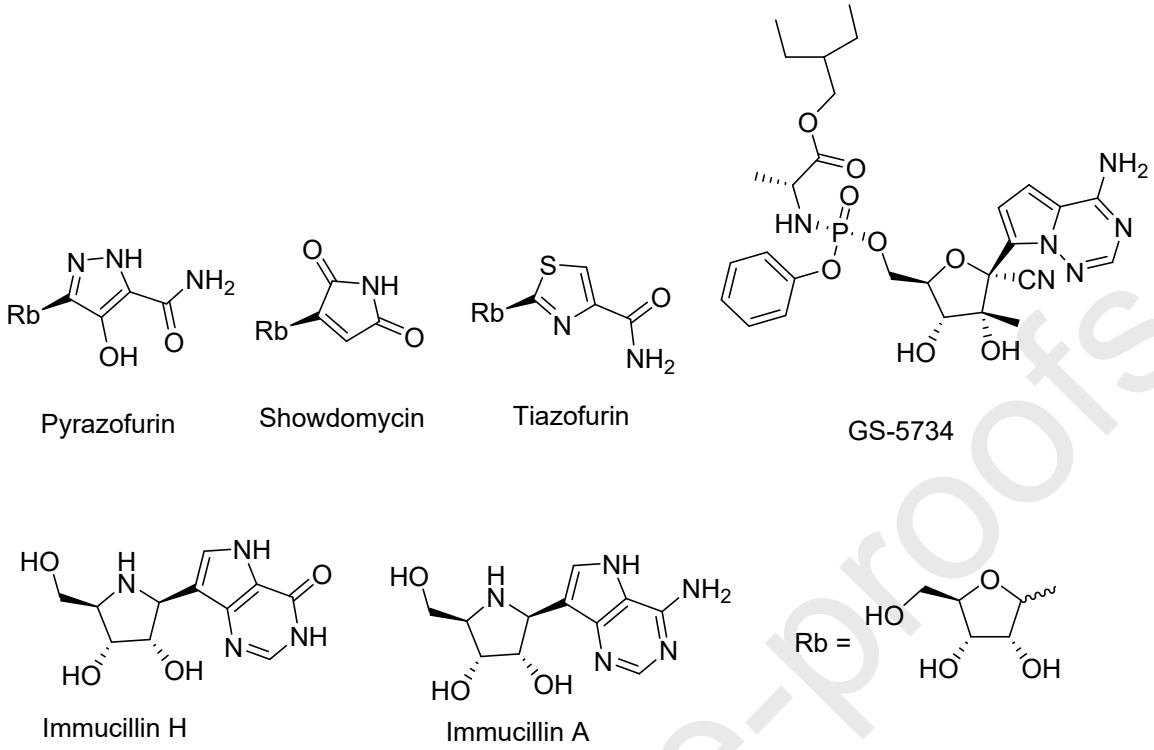


Figure -1

The presence of five-membered heterocyclic system is also a common feature in raltegravir,[11] an antiviral drug for treatment of HIV. Interestingly, the oxadiazole ring system is also present in ataluren[12] and zibotentan[13] used for the treatment of Duchenne muscular dystrophy (DMD) and prostate cancer, respectively (Figure -2). These facts and other reports on the promising biological activity of various regioisomeric oxadiazoles inspired us to synthesise and evaluate the biological activity of novel C-glycosides assembled from 2'-deoxyribose and oxadiazoles.

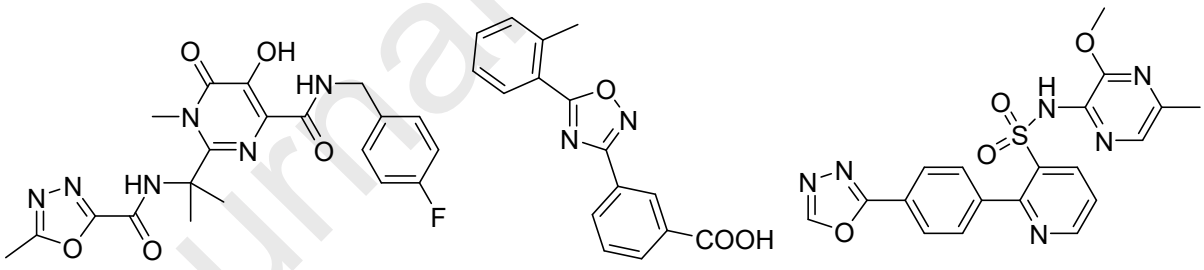


Figure-2

In particular, the oxadiazole ring[14] is an essential part of the pharmacophore favouring ligand binding, act as a flat aromatic linker to place substituents in the proper orientation and finally mimics as bioisoster of esters, amides, carbamates, and hydroxamic esters. The 1,2,3-oxadiazole ring is unstable. 1,2,4-Oxadiazole, 1,2,5-oxadiazole, and 1,3,4-oxadiazole are well known and appear in numerous marketed drugs. We have chosen 1,2,4-oxadiazole and 1,3,4-oxadiazole moiety for C-nucleoside synthesis. We envisioned the synthesis of a common building block by C-C bond formation at C1 and further integration of the heterocycle to assemble these C-nucleosides. We utilized the glycosyl cyanide as the key starting material which is obtained as mixture of α/β-cyanide anomers from 1-chloro carbohydrate by reaction with trimethyl silyl cyanide in the presence of a Lewis acid as catalyst. These two anomers of glycosyl cyanide were transformed into novel C-nucleosides. The main advantage of this strategy resides in availability of stereochemically pure glycosyl cyanide that is transformed into C-nucleosides without anomerization at the C1 position. The C-nucleosides of both anomers of 2'-deoxyriboside of tetrazoles and oxadiazoles synthesised are

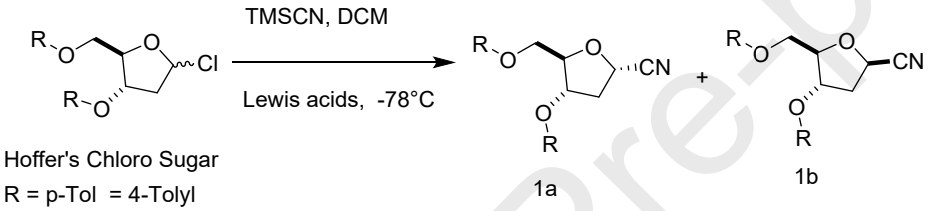
reported for the first time and the methodologies developed are general, which can be applied to construct other structurally diverse anomerically pure C-nucleosides.

Result and Discussion:

Herein, we describe a modular synthesis approach allowing rapid assembly of C-nucleoside library in an efficient manner. Synthesis of a common building block by C-C bond formation at C1 and further integration of the heterocycle was the key feature of our approach. We have divided our study in three parts: (i) synthesis and separation of glycosyl cyanine anomers on multigram scale, (ii) synthesis of 1,2,4-oxadiazole C-nucleoside via amidoxime intermediate and (iii) synthesis of 1,3,4-oxadozle via acylative rearrangement of tetrazole.

(i) Synthesis and separation of glycosyl cyanide anomers on multigram scale:

The glycosyl cyanide is one of the most important type of C-glycosyl intermediate, which is usually obtained as mixture of cyanide anomers from commercially available Hoffer's chloro sugar[15] by reaction with trimethyl silyl cyanide in the presence of a Lewis acid as catalyst (Scheme 1). Synthesis of 2'-deoxy glycosyl cyanine anomers (**1a** and **1b**) have been reported[16] only on small-scale. Since 2'-deoxy glycosyl cyanine anomers (**1a** and **1b**) are the key starting materials for our study, it was essential to optimize the yield and anomeric ratio with an ultimate objective of making it in hundred-gram quantity. Because chloro-sugar is devoid of neighbouring group participation, selective stereochemical outcome is challenging. Hence development of process which is robust and greener was undertaken. We screened various Lewis acids and solvents to improve yield and obtain better ratio of anomers in favour of β-selectivity. β-Anomer **1b** is desirable to produce C-nucleoside having resemblance to the naturally abundant 2'-deoxy-nucleosides.



Scheme-1: Synthesis of glycosyl cyanide

Entry	Lewis Acid	anomeric ratio (β/α)	Isolated Yield (%)
1	$SnCl_4$	3:1	**93**
2	$BF_3.OEt_2$	2:1	**70**
3	TMSOTf	0.59:1	**60**
4	$CeCl_3$	No Reaction (Starting material remain intact)	**-**
5	$ZnCl_2$	2:1	**55**
6	Mg $(ClO_4)_2$	No Reaction (Insoluble in DCM)	**-**
7	$FeCl_3$	5.7:1	**70**

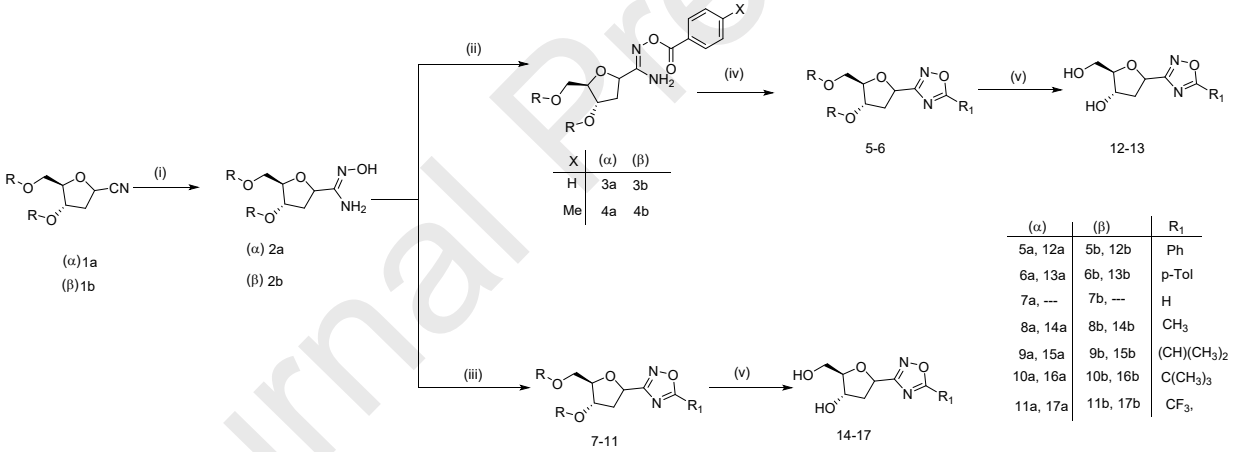
Table-1: Screening of Lewis acid for cyanation of Hoffer's chloro sugar in DCM at -78°C

Upon screening of various Lewis acids, $FeCl_3$ afforded best β:α = 5.7:1 ratio in 70% yield (entry 7 Table 1). Next solvent screening using nitromethane, toluene, 1,2-dichloroethane, tetrahydrofuran, acetonitrile, 1,2-dimethoxyethane, acetone and dimethyl formamide failed to improve the β:α ratio and yield compared to the reaction performed in dichloromethane. Considering desired β:α ratio, yield (93%) and scalability, $SnCl_4$ was chosen as preferred Lewis acid for the present study. It is important to note that low temperature is essential for β-selectivity and high yield. Further optimization effort is underway in our laboratory to find a robust process with non-toxic Lewis acid.

The two anomers of glycosyl cyanides were easily separated by silica gel column chromatography and anomeric configuration was established by ^{1}H NMR experiments. The $SnCl_4$ protocol (entry 1 Table 1) was scaled-up to furnish 366g of the pure β-anomer required for the transformation into various C-nucleosides containing five membered heterocycles. This route is the largest scale synthesis of glycosyl cyanide **1b** reported to date in high yield.

(ii) Synthesis of 1,2,4-oxadiazole C-nucleoside via amidoxime intermediate:

Nitrile functional group has served as an excellent handle to install several heterocyclic rings. Separately, both anomers of glycosyl cyanide (**1a** and **1b**) were converted into amidoxime (**2a** and **2b**) following Tiemann protocol[17] using hydroxylamine hydrochloride under basic condition. Reaction was performed with $NH_2OH.HCl$ in presence of Hünigs base, instead of using $NH_2OH.HCl$ and Na_2CO_3 was reported by Adelfinskaya et al.[18] Excellent yields were obtained for both anomers. These amidoxime derivatives were then converted into 1,2,4-oxadiazoles derivatives (**5-6** and **7-11**) following two distinct protocols (Scheme 2). First, sequential synthesis of *O*-acylated amidoximes using acetyl chloride followed by cyclization to 1,2,4-oxadiazole ring (**5-6**) using alkaline DMSO solution. Whereas the second protocol involve direct cyclization of amidoximes[19] to 1,2,4-oxadiazoles[20-27] using orthoformate or acid anhydride in presence of $BF_3.Et_2O$ as Lewis acid. The later protocol is shorter and offered higher yields compared to the first route. Deprotection of *p*-tolyl group was accomplished using NaOMe in methanol at room temperature in excellent yield except for **7a** and **7b**. Multiple product formation was observed on TLC for **7a** and **7b**. This phenomenon of multiple product formation can be attributed to the deprotonation[28,29] of acidic C5-H of 1,2,4-oxadiazole by NaOMe followed by ring opening and rearrangement.

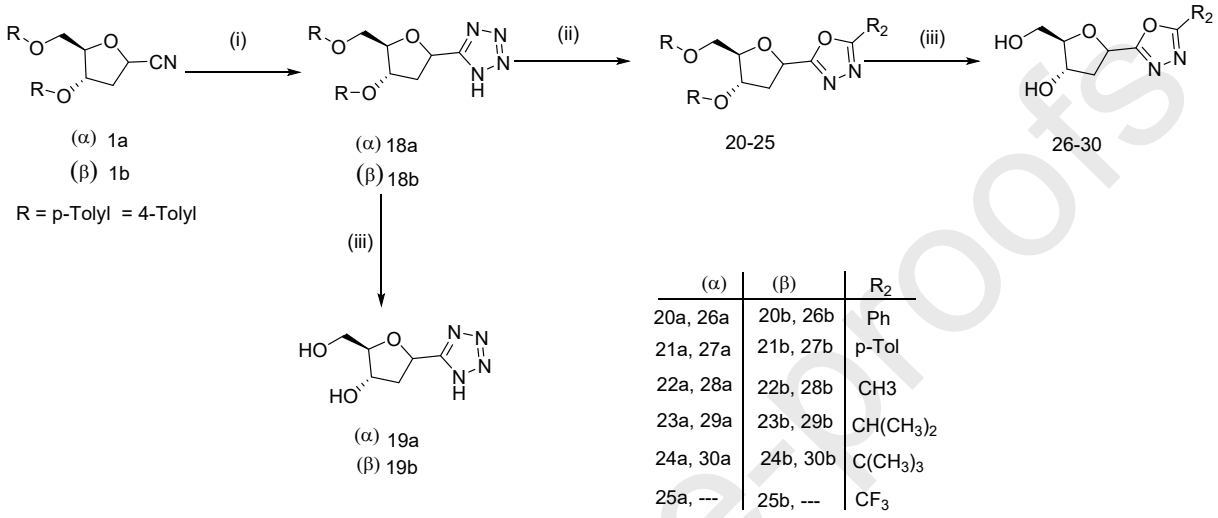


Scheme-2 (Reagents and Conditions): (i) $NH_2OH.HCl$, DIEA, EtOH, reflux, 1h (92-96%); (ii) Acyl chloride ,1,4-dioxane, RT, 16 h (73-97%); (iii) Trimethyl orthoformate, $BF_3.Et_2O$ 110°C, 3 h (or) Acid anhydride, , $BF_3.Et_2O$, 110°C (or) Trifluoroacetic anhydride, DCM, RT, 5 h (80-98%); (iv) KOH, DMSO, RT, 6 h (72-98%); (v) NaOMe, DCM: MeOH (3:2), RT, 16h (48-97%).

(iii) Synthesis of 1,3,4-oxadozle via acylative rearrangement of tetrazole:

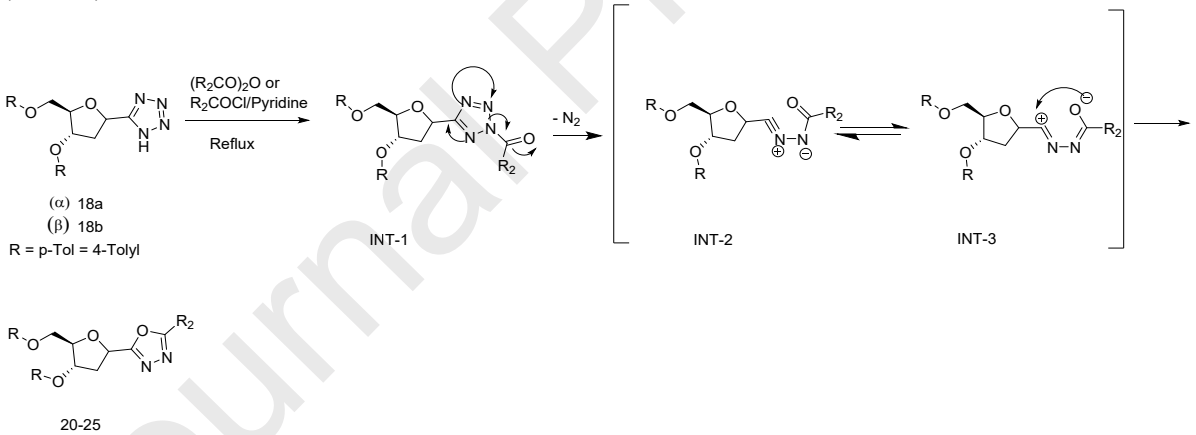
In 1994, Kobe et al.[30] synthesised 5-β-D-ribofuranosyl-1*H*-tetrazole from allononitrile using NaN_3 and $AlCl_3$ in excellent yield. Our attempt to utilize same protocol starting with glycosyl cyanide **1a** or **1b** resulted in low yield of tetrazole derivative along with other unidentified products. Therefore, tetrazole derivatives[31-33] (**18a** and **18b**) were successfully synthesized in good yield from both anomers of glycosyl cyanide (**1a** and **1b**) using azide click reaction with copper and cupric sulphate in DMF at 120°C. Unprotected tetrazole nucleosides (**19a** and **19b**) were obtained by cleaving tolyl protecting group using sodium methoxide in methanol at room temperature (Scheme 3). The conversion of tetrazole to 1,3,4-oxadiazole derivatives[34-38] was achieved either by reacting tetrazole derivatives with carboxylic acid anhydride in presence of hydroquinone under reflux or by reacting with carboxylic acid chloride in pyridine.[39-42] The Deprotection of *p*-tolyl group was executed using NaOMe in methanol at room temperature in excellent yield. However, the deprotection protocol suffers from a drawback for C5-unsubstituted and C5-substitution with electron

withdrawing groups. In both cases, multiple product formation was observed due to the ring opening of the oxadiazole ring. This decomposition can be explained by the nucleophilic addition of NaOMe to C5-carbon and ring opening.[43] The postulated mechanism[44] of this conversion is illustrated in Scheme-4. 5-Substituted tetrazole undergoes N2-acylation upon treatment with acylation reagent due to steric bulk of 5-substitution. This unstable intermediate (INT-1) then ring opens via nitrogen extrusion and formation of *N*-acyl nitrilimine as putative intermediates (INT-2 and INT-3). These intermediates are then cyclized to form 1, 3, 4-oxadiazoles (**20**-**25**) in good yield. Structural elucidation of the new compounds described in this study was based on NMR and mass spectral data.



(α)	(β)	R_2
20a, 26a	20b, 26b	Ph
21a, 27a	21b, 27b	p-Tol
22a, 28a	22b, 28b	CH3
23a, 29a	23b, 29b	$CH(CH_3)_2$
24a, 30a	24b, 30b	$C(CH_3)_3$
25a, ---	25b, ---	CF_3

Scheme-3 (Reagents and Conditions): (i) NaN_3, Cu, $CuSO_4$, DMF, 120°C, 16 h (87-90%); (ii) $(R_2CO)_2O$, hydroquinone, reflux, 1 h (or) R_2COCl , Pyridine, 90°C, 2 h (78-97%); (iii) NaOMe, DCM : MeOH (3:2), RT, 16 h (46-97%).



Scheme-4: Plausible reaction mechanism for 2-substituted 1,3,4-oxadiazole ring formation from **18a** or **18b**

Biological Activity:

We tested a set of 12 C-nucleosides both α– and β-anomers for their in-vitro cytotoxicity activity in five tumor cell lines,[45]namely HeLa, MDA-MB-231 (breast cancer), PANC-1 (pancreatic cancer), PC3 (prostate cancer) and SK-OV-3 (ovarian cancer) using the MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Doxorubicin was used as a positive control to validate the MTT assay [46]. Majority of the compounds failed to exhibit significant cytotoxicity against five cell lines tested at 10 μmol concentration. The results are summarized in the graph shown below. Compounds **15b** and **28a** exhibited modest (9-11%) inhibition of breast cancer cell line MDA-MB-231. Whereas in case of SK-OV-3, we observed 10-14% inhibition exerted by five C-nucleosides (**19b**, **17b**, **12b**, **14b** & **14a**) (Figure 3).

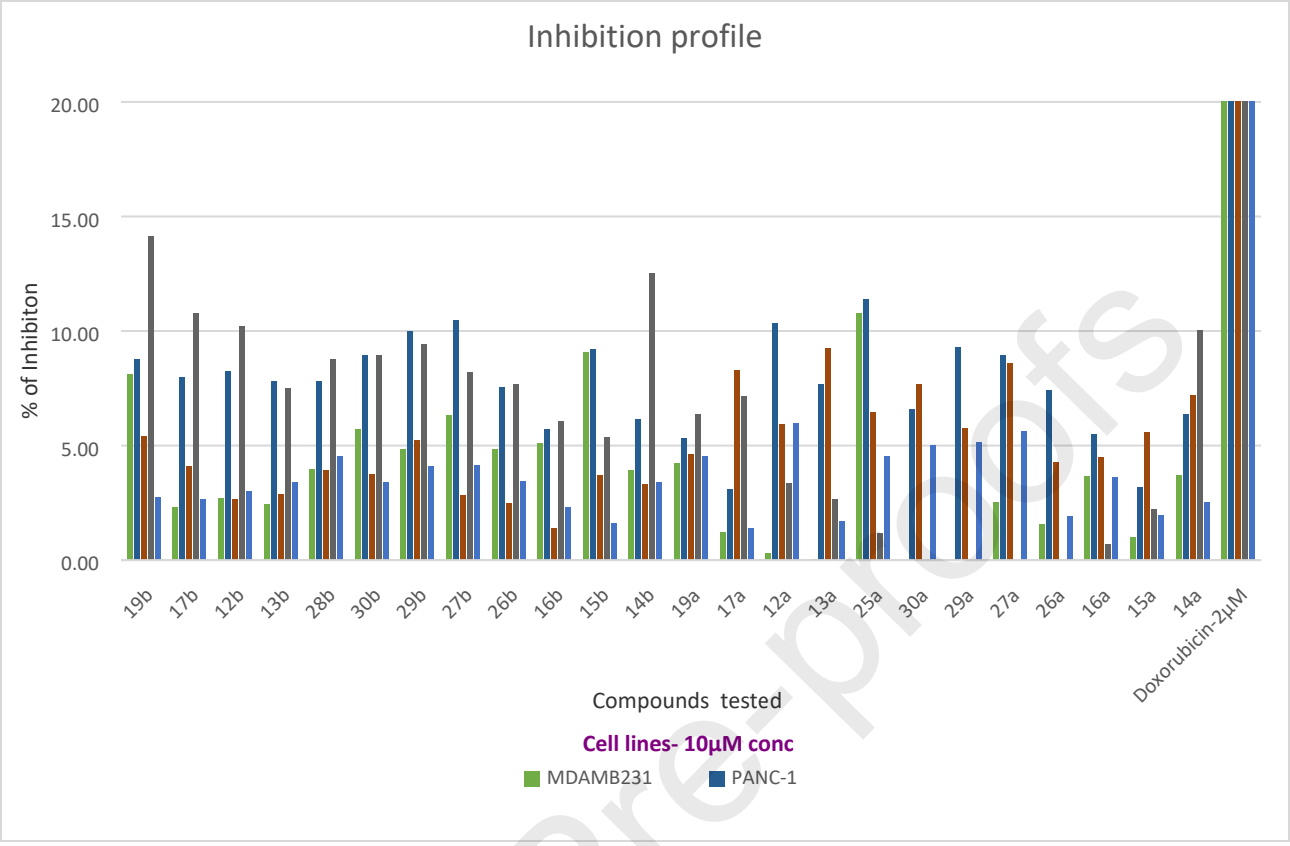


Figure 3: Inhibition profile for 12 C-nucleosides against 5 cell lines. Doxorubicin was used as a control which exhibited >90% inhibition at 2μM.

Summary:
Various regio-isomeric five-membered oxadiazoles based 2'-deoxy-C-nucleosides were synthesized for the first time in good yield and high purity. All C-nucleosides were assembled from pure α- or β-anomer of glycosyl cyanide. The synthesis of glycosyl cyanide as key starting material was established on large-scale and in excellent yield. The easy accessibility of glycosyl cyanide further allows its utility in design of therapeutic oligonucleotides. [47]The synthetic methodologies developed in this study are general and offer future scope to generate other nucleoside analogues for SAR study. Biological evaluation was carried out for synthesised compounds and shows reasonable cytotoxicity in five different tumor cell lines. Studies on antiviral activity of these compounds is in progress and it will be published elsewhere.

Declaration of Competing Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Data:
Experimental data and product characterization is available as SI material.

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