Synthesis and Hypoxia Selective Radiosensitization Potential of β-2-FAZA and β-3-FAZL: Fluorinated Azomycin β-Nucleosides

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Abstract: [18]F-Labelled fluoroazomycin arabinoside ([18]F]FAZA) is a 2-nitroimidazole (azomycin) based PET tracer used extensively in cancer clinics to diagnose tumour hypoxia. The hypoxia-specific uptake and rapid blood clearance kinetics of FAZA contribute to good tumor-to-background ratios (T/B ratios) and high image contrast. However, FAZA, an α-configuration nucleoside, is not transported by cellular nucleoside transporters. It enters cells only *via* diffusion, therefore not achieving the high uptake and T/B ratios characteristic of actively transported radiopharmaceuticals. The present work describes the synthesis, physicochemical properties and preliminary assessment of the radiosensitization properties of two novel azomycin nucleosides, 1-β-D-(2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole (β-2-FAZA) and 1-β-D-(3-deoxy-3-fluorolyxofuranosyl)-2-nitroimidazole (β-3-FAZL) (fluorination yields 60% and 55%, respectively). The tosylated precursors required to synthesize the corresponding F-18 labeled radiopharmaceuticals are also reported. The partition coefficients (*P*) for β-2-FAZA (1.0) and β-3-FAZL (0.95) were marginally lower than reported for FAZA (1.1). The radiosensitization properties of both these compounds are similar to that of FAZA, with sensitizer enhancement ratios (SER) of ~1.8 for HCT-116 cells.

Key Words: Tissue hypoxia, cancer, azomycin nucleosides, FAZA, β-analogs, radiosensitization.

INTRODUCTION

Tissue hypoxia, a common feature of many solid malignant tumors, is the result of inadequate oxygen supply to the affected tissues due to poor vascularization and local microcirculation problems including arterial damage and insufficient angiogenesis [1]. An increase in the hypoxic cell fraction of solid tumors decreases the response to therapeutic ionizing radiation [2-4] and may confer resistance to pharmacologic anticancer treatment [2,5,6]. In addition, hypoxia induces genetic instability resulting in augmented tumour aggressiveness [6,7] and diminishes cytotoxic functions of immune cells that infiltrate a tumour [7,8]. These factors together contribute to survival of cancer cells and enhance the chances of metastasis.

Hypoxic cancer cells can bio-reductively activate radiosensitizers *via* reductive enzymes that are upregulated in hypoxic cells [9]. Desirable properties of a hypoxia-sensitive radiosensitizer include minimal *in vivo* metabolism other than reductive bio-activation, short residence time in non-target tissues, rapid renal excretion and low hepatobiliary clearance. Increasing the lipophilicity of a radiosensitizer [10] will increase non-specific accumulation in lipoidal tissues and contribute to selective toxicities (e.g. neuropathies), whereas lowering lipophilicity will increase renal clearance and reduce accumulation in the liver. Effective 2-nitroimidazole based radiosensitizers have reduction potentials (E^1_7) around -390 mV, an electron affinity considered to be optimal for selectivity and sensitivity [11].

When used for imaging focal hypoxia, the radiolabelled radiosensitizer should also clear rapidly from the vascular compartment to reduce the general whole-body radiation doses and to increase hypoxia-specific contrast in the tumour image through reduction in background radioactivity. Hypoxia-selective properties of these molecules, when explored with advanced imaging techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI), can provide *in vivo* visualization of biological processes and metabolic pathways at cellular and molecular levels [1,12], and provide valuable information to monitor therapeutic efficacy and develop new treatment modalities. [18]F]Fluoromisonidazole ([18]F]FMISO) (Fig. **1**), a fluorinated 2-nitroimidazole developed in the early nineteen eighties, was one of the first PET tracers used to diagnose oncological hypoxia. It is currently an important clinical tool in diagnostic oncology [13]. Extensive pharmacokinetic and hypoxia-selective uptake validation studies have been performed with this tracer [14-18]. Other nitroimidazole-based PET tracers that have found limited clinical utility include EFI [19] and FETNIM [20].

[18]F-Labeled fluoroazomycin arabinoside ([18]F]FAZA), a sugar-based azomycin derivative, has recently gained substantial interest among clinicians as a hypoxia marker of choice [21-23]. Currently it is going through clinical trials in several cancer centres [24-27]. FAZA displays a hypoxia-specific uptake with tumour-to-background ratios (T/B ratios) superior to [18]F]FMISO due to its faster clearance from

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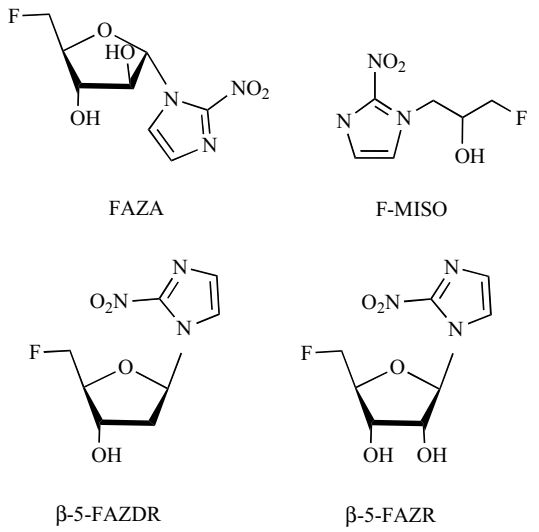


Fig. (1). Structures of FAZA, FMISO, β-FAZDR and β-FAZR

blood [28,29]. However, FAZA, an α-nucleoside, is not actively transported across the cell membranes into cells *via* naturally occurring nucleoside transporters. Instead, like most other azomycin-based radiosensitizers, FAZA permeates cellular membranes *via* diffusion, which leads to a short 'residence time' in hypoxic cells. Furthermore, it is possible that moving the fluorine substituent from C-5' to C-2'/3'fluorine will enhance the chemical stability of the nucleoside bond, and importantly, will also open the sugar-C-5 hydroxyl for phosphorylation by nucleoside kinases. The latter effect could play a role in increasing cell residence times, thereby increasing availability of the drug for bioreductive activation. Several *β*-azomycin nucleosides have been reported recently, including 1-*β*-D-(5-deoxy-5-fluoro-2-deoxyribofuranosyl)-2-nitroimidazole (FAZDR) [30,31], and 1-β-D-(5-deoxy-5-[^{18}F]fluororibofuranosyl)-2-nitroimidazole (β-5-FAZR) [32], but none of these compounds address both the objective of β-nucleoside configuration and fluorination at C-2' or C-3'.

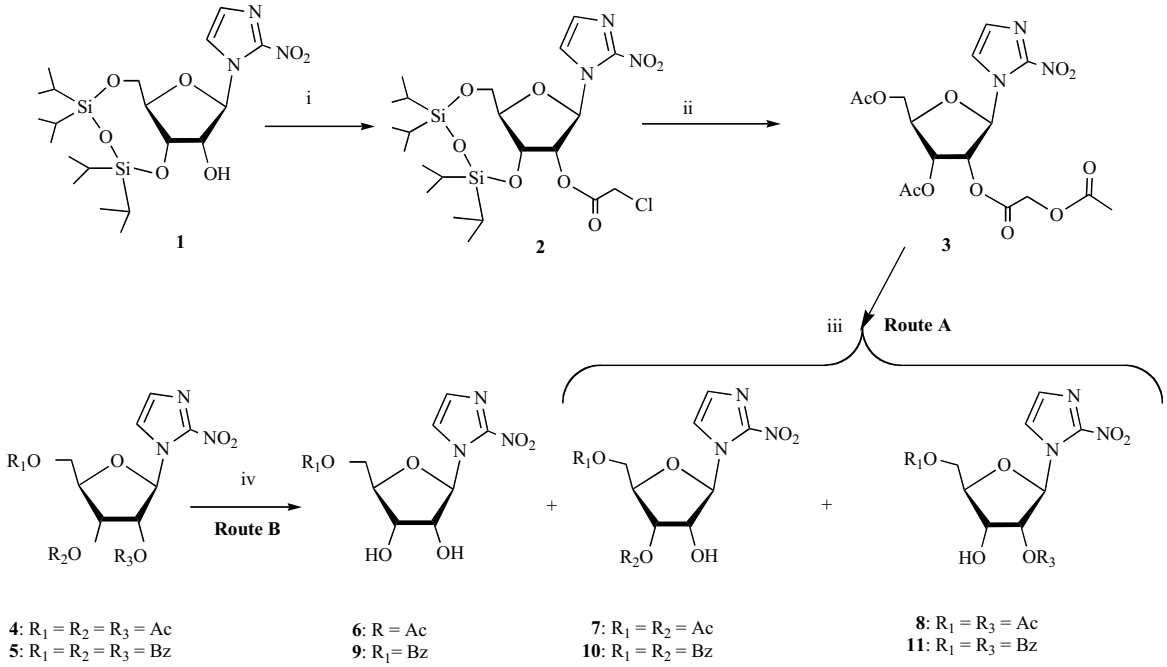
The synthesis, physicochemical parameters and preliminary radiosensitization properties of two novel azomycin nucleosides, 1-β-D-(2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole (β-2-FAZA) and 1-β-D-(3-deoxy-3-fluoroly-xofuranosyl)-2-nitroimidazole **(**β-3-FAZL), are now reported.

RESULTS AND DISCUSSION

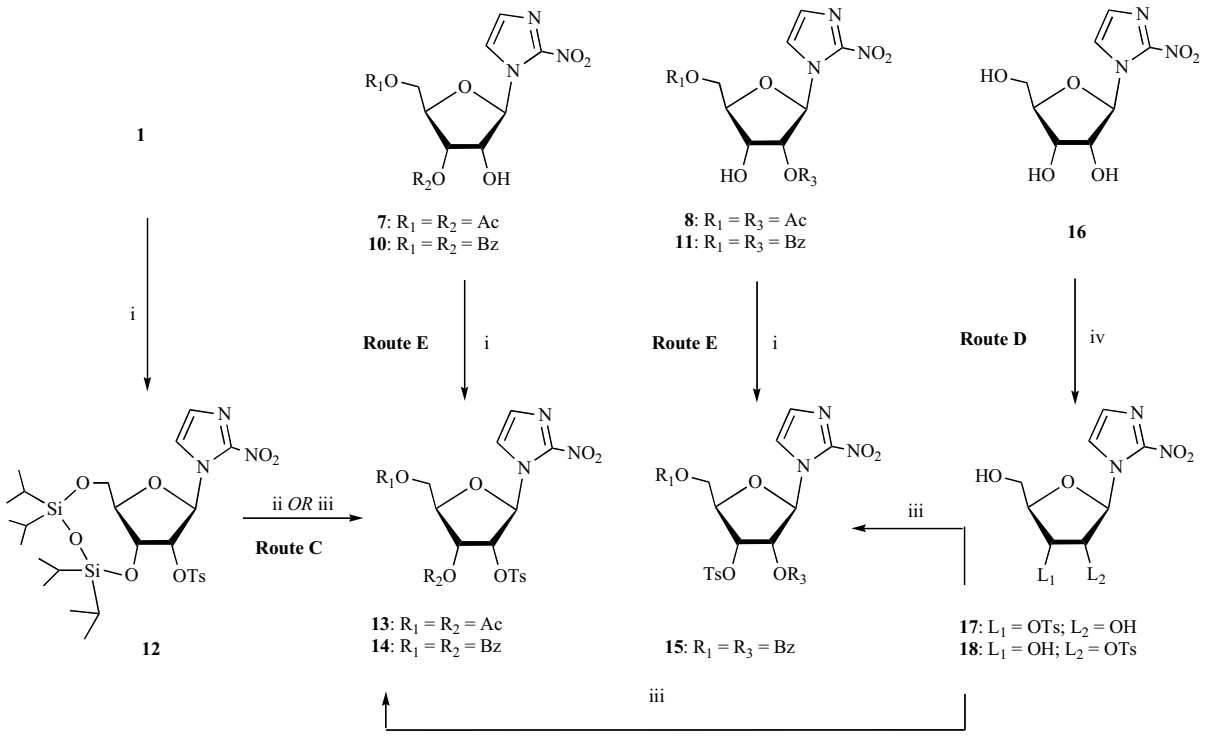
Synthesis of the target fluorinated molecules **20** and **22** was approached with the future development of the corresponding ^{18}F-labeled derivatives in mind. Therefore, the current work includes the syntheses of the tosylated precursors **13**-**15,** which would also be synthons for F-18 labeled **20** and **22** (Schemes **1** and **2**).

Synthons 1-β-D-(3,5-di-O-acetylribofuranosyl)-2-nitroimidazole, **7**, or 1-β-D-(3,5-di-O-benzoylribofuranosyl)-2-nitroimidazole, **10**, were used to synthesize **20**, and 1-β-D-(2,5-di-O-acetylribofuranosyl)-2-nitroimidazole, **8**, or 1-β-D-(2,5-di-O-benzoylribofuranosyl)-2-nitroimidazole, **11**, were developed to prepare the corresponding 3'-fluorinated analog **22** (Scheme **3**).

Syntheses of **20** and **22** can be achieved either by direct fluorination of an –OH group placed in the required configuration, or by nucleophilic substitution of an easily removable leaving group, e.g., tosyl; in both cases the reacting precur-



Scheme 1. Synthesis of selectively-protected azomycin ribosides.

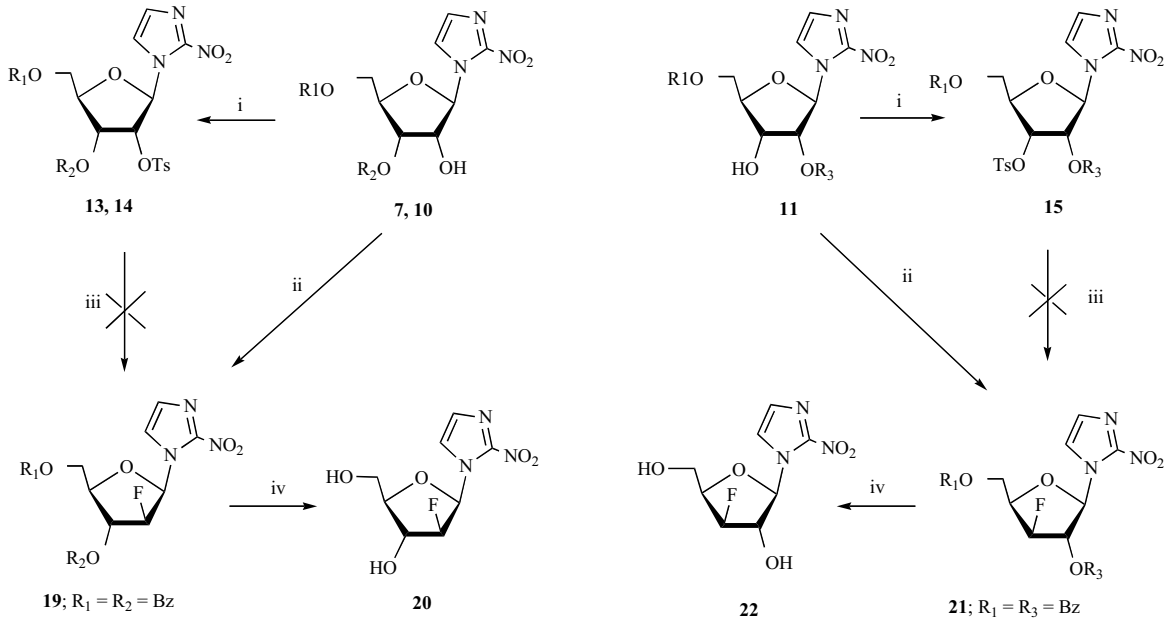


Reagents and conditions: i = TsCl/pyridine; ii = TBAF/Ac_2O/pyridine (compound **13**); iii = TBAF/Benzoyl chloride/pyridine (compound **14**); iv = Dibutyltin oxide/CH_2Cl_2/TsCl/TBAF

Scheme 2. Synthesis of the radiofluorination synthons **13**, **14** and **15**.

sors require selective protection of those hydroxyl groups that are not to undergo fluorination. Therefore, synthesis of **20** warranted selective protection of –OH groups at C-3' and C-5', while –OH groups at C-2' and C-5' were protected to obtain **22**. Compounds **7**, **8**, **10** and **11** were synthesized *via* two routes (Scheme **1**). *Route A* involved silylation of the 3'- and 5'-OH groups of **16** by tetraisopropyldisilanyloxydichloride (TIPDS chloride) to afford **1** in 75 % yield [33]. It was planned that the –OH group at C-2' would be converted to an ester that, after *in situ* acetylation of 3',5'-silyl groups, could be easily removed. Thus, the reaction of **1** with chloroacetic anhydride afforded **2** in 66 % yield. When 'desilylative acetylation' of **2** with acetic anhydride was attempted, it resulted in acetylation not only at 3'- and 5'- positions, it also reacted with chloroacetyl group (at C-2') to afford 1-β-D-(3,5-di-O-acetyl-2-O-acetoxyacetylribofuranosyl)-2-nitroimidazole, **3**, in 61 % yield. Appearance of three new singlets (*s*) at δ 2.17, 2.18 and 2.19 ppm, and shift of methylene protons from δ4.21 to 4.42, confirmed the formation of this molecule. Treatment of **3** with aqueous pyridine, equilibrated at pH 6-7 by adding few drops of 0.1N HCl solution, at 22° C gave a mixture of 1-β-D-(3,5-di-O-acetylribofuranosyl) -2-nitroimidazole, **7**, and 1-β-D-(2,5-di-O-acetylribofuranosyl) -2-nitroimidazole, **8**. Formation of **8** presumably occurs due to intramolecular migration of 3'-acetyl group, which is a very common occurrence in the molecules containing vicinal *cis* OH groups [34]. Compounds **7** and **8** were separated by column chromatography. Alternatively, **7** and **8** were prepared by selective deacetylation at 2'- or 3-' of 1-β-D-(2,3,5-tri-O-acetylribofuranosyl)-2-nitroimidazole, **4**, (*Route B*). Reaction of **4** with hydrazine, pre-dissolved in glacial acetic acid: pyridine (1:4; v/v), also afforded a mixture of 2'- and 3'-deacetylated products **7** and **8** in a combined yield of 90 %. The temperature of this reaction played a major role in the fate of these products since the same reaction at elevated temperatures (80 °C) led to deacetylation at both 2'- and 3'-positions and resulted in the formation of 1-β-D-(5-O-acetylribofuranosyl)-2-nitroimidazole, **6**, exclusively, in merely 2 h, in 70 % yield. Similar effects were seen with the corresponding tri-O-benzoyl analog. Attempted debenzoylation of 1-β-D-(2,3,5-tri-O-benzoylribofuranosyl)-2-nitroimidazole, **5**, afforded a mixture of 1-β-D-(5-O-benzoylribofuranosyl)-2-nitroimidazole, **9** (10 %), 1-β-D-(3,5-di-O-benzoylribofuranosyl)-2-nitroimidazole, **10**, and 1-β-D-(2,5-di-O-benzoylribofuranosyl)-2-nitroimidazole, **11** (52 % combined yield of **10** and **11**). The appearance of two doublets of doublet (*dd*) for H-5' protons (sugar moiety) downfield at δ 4.68 and 4.74 in the ^{1}H NMR spectrum of **9** confirmed its formation during attempted debenzoylation. The appearance of a downfield *dd* signal for H-3' (δ 5.36) in the case of **10**, and for H-2' (δ 5.61) in the case of **11**, respectively, reaffirmed the structural identity of these molecules.

The syntheses of **13**, **14** and **15,** the tosylated derivatives of **7**, **10** and **11**, respectively, were done *via* three routes (Scheme **2**). *Route C* followed the course of tosylation of **1,** the 3',5'-O,O-TIPDS derivative of AZR which, on reaction with toluenesulfonyl chloride at 55 °C, gave 1-β-D-(3,5-O,O-tetraisopropyldisyloxy-2-O-toluenesulfonylribofuranose)-2-nitroimidazole, **12** (75 % yield)**.** *In situ* 'desilylative acetylation' of this compound **12** was performed using TBAF and acetic anhydride, and afforded **13** in high yield (97 %). Disappearance of isopropyl proton resonances and the appearance of two new singlets at δ 2.17 and 2.18 in the ^{1}H NMR



Reagents: i = TsCl/pyridine; ii = DAST/CH_2Cl_2/pyridine; iii = NH_4F/2-pentanone or Tetrabutylammonium fluoride/THF; iv = 2.0 M.NH_3/MeOH

Scheme 3. Synthesis of **20** and **22** from their respective synthons.

spectrum of **13** confirmed its structural identity. An alternate method to prepare these tosylated derivatives involved direct tosylation of **16** *via* stannylation of 2'- and 3'-OH groups (*Route D*). Thus, AZR, **16**, on treatment with dibutylstannyl oxide and toluenesulfonyl chloride at 22 °C, afforded a mixture of 3'-O-tosyl AZR, **17**, and the 2'-O-tosyl analog, **18** (**17**:**18** isomeric ratio is 45:55 as determined from the ^{1}H NMR spectrum). This mixture was purified by column chromatography. Downfield shifts of the *dd* signal for H-3' (δ 4.93) in the ^{1}H NMR spectrum of **17**, and a *dd* signal for H-2' (δ 4.84) in case of **18,** confirmed their respective formation. The presence of additional signals related to the introduction of a tosyl group provided additional strength for assignment of their structures. Compounds **17** and **18** were individually treated with benzoyl chloride in anhydrous pyridine and afforded **15** and **14**, respectively. Tosylation of **7**, **10** and **11** was also performed individually in anhydrous pyridine (*Route E*) and afforded **13**, **14** and **15,** respectively, in 90-95 % yield. These tosylated compounds are the synthons for F-18 labeled **20** and **22**. Routes C and E were the best yielding procedures for the synthesis of the tosylated analogs **13**-**15**.

Compounds **10** and **11** were chosen for the synthesis of the target fluorinated molecules **20** and **22**. DAST treatment of **10** and **11,** separately, gave the corresponding fluorinated compounds 1-β-D-(3,5-di-O-benzoyl-2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole, **19** (60 % yield), and 1-β-D-(2,5-di-O-benzoyl-3-deoxy-3-fluorolyxofuranosyl)-2-nitroimidazole, **21** (55 %), respectively, (Scheme **3**). Substitution of an –OH group with fluorine significantly affects the ^{1}H and ^{13}C chemical shifts not only at the point of substitution, its impact is also strongly evident on other nearby atoms. A comparative analysis of ^{1}H and ^{13}C NMR spectra of molecules **11** (an –OH substituted molecule) with **21** (corresponding fluorinated molecule) demonstrated that the introduction of fluorine at C-3' not only shifted H-3' downfield (0.62 ppm) and C-3' (16.20 ppm), it also deshielded H-2' (0.09 ppm) and H-4' (0.1 ppm). However, H-1' in this molecule was shifted slightly upfield (0.11 ppm). Similar effects were evident in ^{1}H NMR spectrum of **19**, which demonstrated the appearance of a *ddd* for H-2' proton at δ 5.67 ($J_{2',F}$ = 48.5 Hz). Fluorine-induced *–I effects* were also observed on H-3' (δ 5.70; $J_{3',F}$ = 12.8 Hz) and H-1' (δ 6.83; (dd, $J_{1',F}$ = 18.0 Hz).

Additional spectral information (^{19}F and ^{13}C NMR spectra) provided further confirmation of the structure of these molecules. Debenzoylation of **19** and **21** by methanolic ammonia afforded **20** and **22**, respectively, and resulted in strong upfield movement of the proton resonances at those carbons. Thus, H-2' in **20** moved upfield by 0.39 ppm, and in **22** by 0.27 ppm. The deshieding effect of neighboring benzoyl groups on fluorine also disappeared as the ^{19}F chemical shifts in **20** and **22** moved upfield by 3.07 and 2.08 ppm, respectively, in comparison to corresponding benzoylated fluoro compounds **19** and **21**. The effect of fluorination on neighboring hydrogen and carbon atoms is in complete accordance with the literature reports [35]; the subtle changes in ^{1}H, ^{13}C and ^{19}F NMR chemical shifts of **19**-**22** are summarized in Table **1**.

In summary, the synthesis of target compounds **20** and **22** was attempted *via* the DAST reaction on their respective hydroxyl precursors **7**, **10** and **11** (Scheme **3**), synthesized *via* two synthetic pathways (Scheme **1**). The tosylated derivatives **13**-**15**, the precursors to synthesize radiofluorinated **20** and **22**, were synthesized *via* three different synthetic pathways (Scheme **2**).

Radiosensitization studies with **20** and **22** were performed in HCT116 colorectal carcinoma cells (Fig. **2**) at concentrations of 100 μM. The data show that the radiosensitization potential of both β-2-FAZA and β-3-FAZL is comparable to FAZA, since their oxygen enhancement ratios were found to be ~1.8. The radiosensitization properties of

Table 1. ^{1}H, ^{13}C and ^{19}F NMR Chemical Shifts of Fluorinated Compounds 19-21 and the Precursor 11

Compound	Chemical Shift (δ ppm)									Coupling Constants (J) Hz/Cm		
	H-1	H-2	H-3	H-4	C-1	C-2	C-3	C-4	F	$J_{2',F}$	$J_{3',F}$	$J_{1',F}$[a] / $J_{4',F}$[b]
11	6.79	5.61	4.60	4.57								
19	6.83	5.67	5.70	4.64	88.60	89.71	76.44	82.05	-38.19	49	13	
20	6.78	5.28	4.35	3.99	88.48	96.90	73.70	85.38	-35.12	51	11	
21	6.68	5.70	5.22	4.67	91.98	81.35	92.64	80.24	-38.34	12	51	30
22	6.36(s)	4.51	4.95	4.67					-36.26	17	51	33

[a]Compounds **19** and **20**; [b]Compounds **21** and **22**.

20 and **22** fall within the range of a potentially useful hypoxia radiosensitizer and are similar to that for β-5-IAZR in EMT-6 cells [36].

The partition coefficient (*P*) of a compound is a measure of its relative lipophilicity and is an indicator of the potential to diffuse across the cell membranes. Although usually determined by measuring the solubility of a compound in water-saturated 1-octanol and 1-octanol saturated water and expressed as the log10 of the concentration ratio, HPLC retention times on a reversed phase HPLC column offer a more convenient method to determine *P* provided that appropriate reference compounds are available. In general, lipophilic compounds will have longer retention times. Retention times of β-2-FAZA and β-3-FAZL and MISO, FMISO, β-AZA, α-IAZA and α-FAZA, other well known clinical hypoxia markers, were determined on a reversed phase HPLC column (25 x 0.9 cm, 10 ODS3 column). Their partition coefficients [21,37-39] (*P* values) were plotted in relation to their retention times (Fig. **3**). The results are reported in Table **2**. Based on this comparison, β-2-FAZA and β-3-FAZL have estimated LogP values of 1.0 and 0.95, respectively, and are slightly less lipophilic than α-FAZA (*P* = 1.1). This falls within the range (*P* ≅ 0.1-10) of lipophilicities of the compounds that would be compatible with good tissue perfusion by diffusion [10,39].

EXPERIMENTAL

Chemistry

All chemicals used were reagent grade. β-AZR was prepared using a literature procedure [34]. Solvents were dried over appropriate drying agents and freshly distilled before use. The progress of synthetic reactions was monitored by thin layer chromatography (tlc; 90:10, v/v, solvent system B) or hexanes:EtOAc (7:1, v/v, solvent system C; 3:2, v/v, solvent system D and 1:1, v/v, solvent system E) as developing solvents. Column chromatography was performed on Merck silica gel 60 (particle size 70-200 and 230-400 mesh ASTM). Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer in deuterated chloroform ($CDCl_3$) or deuterated methanol (CD_3OD), depending on the solubility of the product. Chemical shifts are reported in ppm downfield with respect to tetramethylsilane as an internal standard. The protons and carbons of the sugar moiety and nitroimidazole are represented by a single prime (') and no prime, respectively. When necessary, high resolution mass spectra (HRMS) were recorded using an AEI-MS-12 mass spectrometer.

1-β-D-(3,5-O,O-tetraisopropyldisyloxy-2-O-chloroacetylribofuranosyl)-2-nitroimidazole (2)

Chloroacetic anhydride (58.5 mg, 0.4 mmol) was added to a cold solution of 1-β-D-(3,5-O,O-tetraisopropyldisyloxyribofuranosyl)-2-nitroimidazole [33], **1**, (48.7 mg. 0.1 mmol) in anhydrous pyridine (1.0 mL) and the mixture was stirred for 16 h at 0 °C. At this time the tlc of the reaction mixture showed complete consumption of **1**. The solvent and the excess reagent were evaporated under reduced pressure on a rotary evaporator, the impure viscous mass was loaded on to a silica gel column and pure product was eluted by hexanes/EtOAc (8.5:1.5, v/v) which afforded pure **2**. Rf 0.20 (solvent system C); Yield 37 mg (66 %); M.P. 132-133 °C; ^{1}H NMR ($CDCl_3$); δ ppm: 0.99-1.2 (m, 16H, four isopropyl groups),), 4.03 (dd, $J_{4',5'}$ = 2.4, Jgem = 14.0 Hz, 1H, H-5'), 4.15 (dd, $J_{3',4'}$ = 9.5 Hz, $J_{5',4'}$ = 2.4 Hz, 1H, H-4'), 4.21 (s, 2H, CH_2Cl), 4.33 (s, Jgem = 14.0 Hz, 1H, H-5'), 4.51 (dd, $J_{4',3'}$ = 9.5 Hz, $J_{2',3'}$ = 4.6 Hz, 1H, H-3'), 5.55 (d, $J_{3',2'}$ = 4.6 Hz, 1H, H-2' 6.41 (s, 1H, H-1'), 7.20 (s, 1H, imidazole H-4), 7.48-7.53 (ddd, J = 7.6 Hz, 7.9 Hz and 0.9 Hz, 4H, H-3 and H-5 of two phenyls), 7.52 (s, 1H, imidazole H-5); HRMS (EI^+): for $C_{22}H_{38}N_3O_8NaSi_2Cl$ Calc. 586.17782, Found 586.17798 (M^+Na, 76.5 %); MS ES$^+$ 586 (M^+Na).

1-β-D-(3,5-Di-O-acetyl-2-O-acetoxyacetylribofuranosyl)-2-nitroimidazole (3)

Acetic anhydride (59.6 mg, 0.58 mmol) was added to a pre-cooled solution of **2** (41 mg, 0.073 mmol) in anhydrous acetonitrile (1.5 mL) under stirring. A solution of TBAF (0.13 mL; 1M solution in anhydrous THF) was introduced to this reaction mixture under stirring at 22 °C in two portions at an interval of 1 h. A tlc examination of this mixture after 2 h showed no sign of the precursor and the formation of a new product at a lower Rf value. The solvents and the excess acetic anhydride were evaporated under reduced pressure and the impure product was chromatographed on a silica gel column using hexanes/EtOAc as eluent to afford pure **3** as a viscous foam. Rf 0.68 (solvent system B); Yield 18 mg

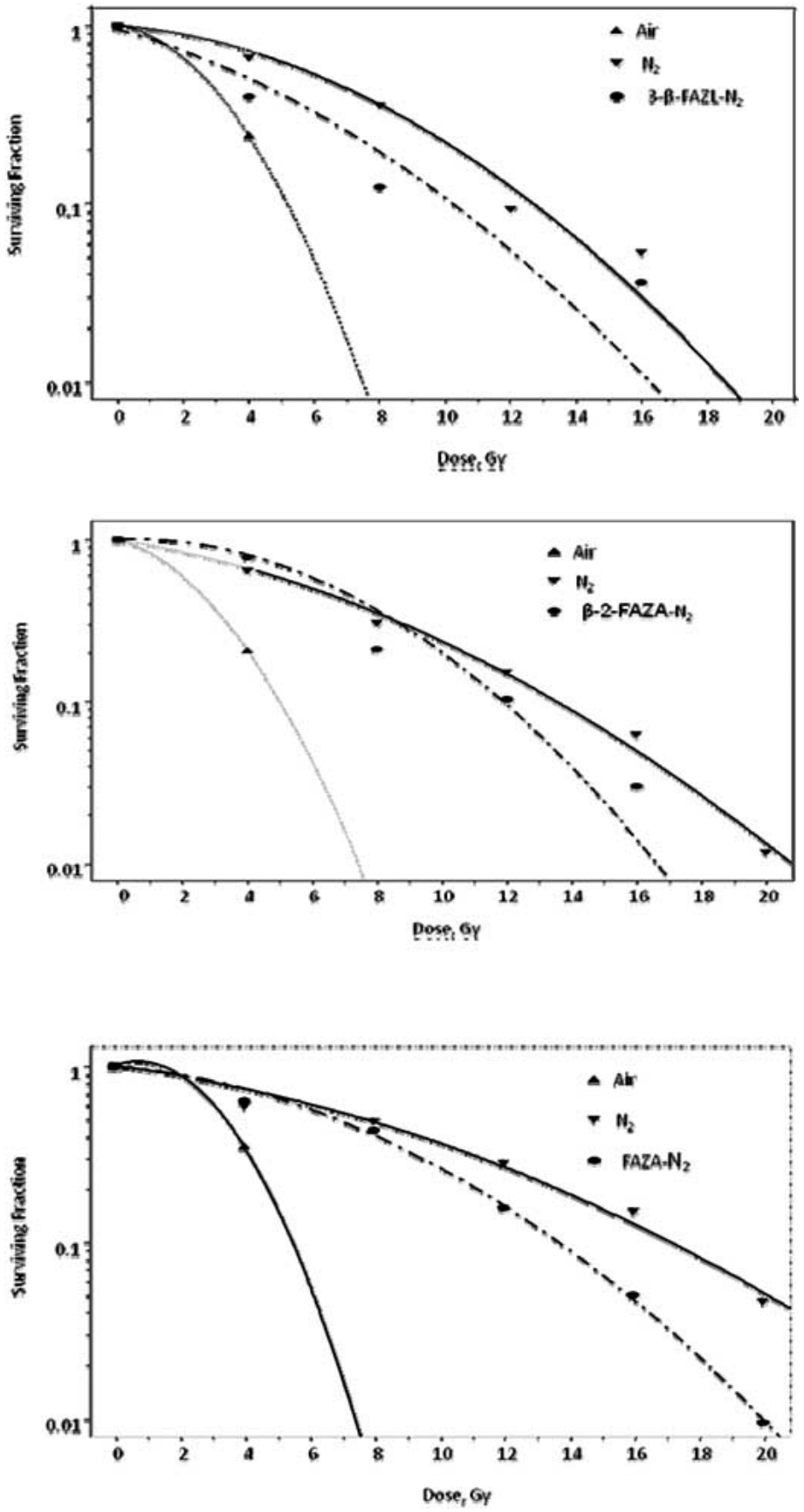


Fig. (2). *In vitro* radiosensitization of HCT116 cells by β-3-FAZL **22** (top), β-2-FAZA **20** (middle), and FAZA (bottom). All data sets are for 100 μM sensitizer concentration.

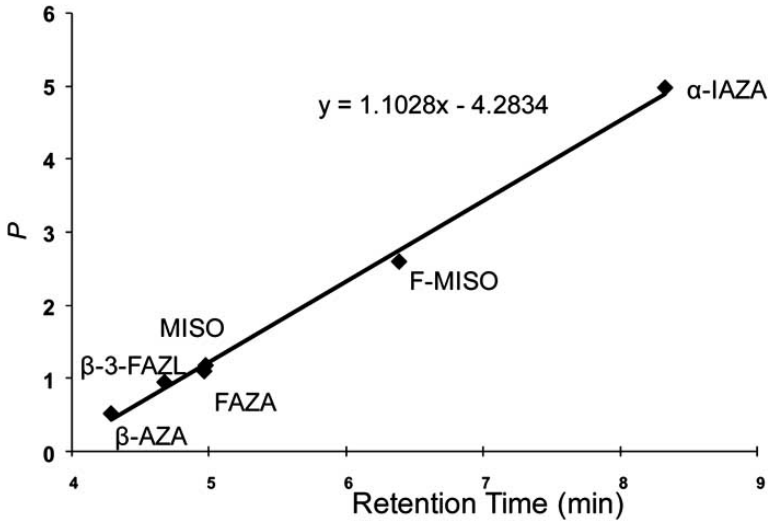


Fig. (3). Estimation of *P* value for β-3-FAZL, **22**, based on a comparison of retention times (R.T.) and *P* values of known radiosensitizers. The solid line is the linear regression line.

(61 %); ^{1}H NMR ($CDCl_3$); δ ppm: 2.17, 2.18 and 2.19 (three s, 9H, 3H for each CH_3), 4.42 (q, ab, 2H, CH_2), 4.50 (dd, $J_{3',4'}$ = 5.5 Hz, $J_{5',4'}$ = 2.4 Hz, 1H, H-4'), 4.63 (dd, Jgem = 16.1 Hz, 1H, H-5"), 4.66 (d, Jgem = 16.1 Hz, $J_{5',4'}$ = 2.4 Hz, 1H, H-5'), 5.41 (dd, $J_{4',3'}$ = 9.5 Hz, $J_{2',3'}$ = 5.5 Hz, 1H, H-3'), 5.48 (dd, $J_{1',2'}$ = 4.0 Hz, $J_{3',2'}$ = 5.5 Hz, 1H, H-2'), 6.65 (d, $J_{2',1'}$ = 4.0 Hz, 1H, H-1'), 7.24 (s, 1H, imidazole H-4), 7.55 (s, 1H, imidazole H-5); MS (ES^+): for $C_{16}H_{19}N_3O_{11}Na$ Calc. 452.0 Found 452.1 (M^+Na).

Table 2. Partition Coefficients (*P*) of β-2-FAZA (20), β-3-FAZL (22) and Several Known Clinically-Used Hypoxia Markers, as Determined by Reverse Phase HPLC

Compound	Retention Time (min)	Partition Coefficient (*P*)
β-AZA	4.28	0.52
β-3-FAZL **22**	4.67	0.95
β-2-FAZA **20**	4.69	1.0
FAZA	4.96	1.1[21]
MISO	4.97	1.18
FMISO	6.38	2.6[12]
α-IAZA	8.32	4.98[36]

1-β-D-(2,3,5-Tri-O-acetylribofuranosyl)-2-nitroimidazole (4)

Compound **16** (61 mg; 0.25 mmol) was dissolved in anhydrous pyridine (1 mL) and acetic anhydride (0.28 mL; 2.7 mmol) was added to it at 22 °C under stirring. The reaction was continued for 16 h and then the solvent was removed by rotary evaporation under reduced pressure. The impure viscous mass was purified by silica gel chromatography on a column using hexanes:EtOAc as eluent and afforded pure **4** as a foam. Rf 0.46 (solvent system E); Yield 98 mg (100 %); ^{1}H NMR ($CDCl_3$) δ ppm: 2.11 and 2.18 (two s, 9H, three CH_3), 4.40 (dd, Jgem = 12.4 Hz, $J_{4',5''}$ = 6.2 Hz, 1H, H-5"), 4.43 (dd, Jgem = 12.4 Hz, $J_{5',4'}$ = 2.9 Hz, 1H, H-5'), 4.50 (ddd, $J_{3',4'}$ = 7.0 Hz, $J_{5',4'}$ = 2.9 Hz, $J_{4',5''}$ = 6.2 Hz, 1H, H-4'), 5.32 (dd, $J_{4',3'}$ = 7.0 Hz, $J_{2',3'}$ = 5.5 Hz, 1H, H-3'), 5.48 (dd, $J_{1',2'}$ = 3.3 Hz, $J_{3',2'}$ = 5.5 Hz, 1H, H-2'), 6.61 (d, $J_{2',1'}$ = 3.3 Hz, 1H, H-1'), 7.24 (s, 1H, imidazole H-4), 7.60 (s, 1H, imidazole H-5); HRMS (EI^+): for $C_{14}H_{17}N_3O_9Na$ Calc. 394.08570, Found 394.08581 (M^+Na, 97 %); MS ES^+ 394 (M^+Na).

1-β-D-(2,3,5-Tri-O-benzoylribofuranosyl)-2-nitroimidazole (5)

This was prepared starting with 1-β-D-ribofuranose-2-nitroimidazole according to a reported procedure[36].

1-β-D-(5'-O-acetylribofuranosyl) -2-nitroimidazole (6)

A solution of **4** (37 mg; 0.1 mmol) and hydrazine (12.8 mg; 0.4 mmol) in glacial AcOH:pyridine (1:4; v/v; 1 mL) was heated at 80 °C under stirring. A tlc check at this time showed no presence of residual precursor. The reaction was quenched by adding acetone (0.5 mL) and the solvents were removed under reduced pressure. A silica gel purification of the impure product on a silica gel column using EtOAc: hexanes (8:2; v/v) afforded pure **6** as a viscous mass. Rf 0.37 (solvent system B); Yield, 20 mg (70 %); ^{1}H NMR ($CDCl_3$) δ ppm: 2.18 (s, 3H, CH_3), 4.16 (ddd, $J_{3',4'}$ = 5.5 Hz, $J_{5',4'}$ = 2.2 Hz, $J_{4',5''}$ = 4.0 Hz, 1H, H-4'), 4.30 (d, $J_{3',2'}$ = 10.2 Hz, 1H, H-2'), 4.35 (dd, $J_{4',3'}$ = 5.5 Hz, $J_{2',3'}$ = 10.2 Hz, 1H, H-3'), 4.47 (dd, Jgem = 12.8 Hz, $J_{4',5''}$ = 2.2 Hz, 1H, H-5"), 4.56 (dd, Jgem = 12.8 Hz, $J_{5',4'}$ = 4.0 Hz, 1H, H-5'), 6.39 (s, 1H, H-1'), 7.22 (s, 1H, imidazole H-4), 7.65 (s, 1H, imidazole H-5); HRMS (EI^+): for $C_{10}H_{13}N_3O_7Na$ Calc. 310.06457, Found 310.06451 (M^+Na, 98.4%); MS ES^+ 310 (M^+Na).

1-β-D-(3,5-Di-O-acetylribofuranosyl)-2-nitroimidazole (7), and 1-β-D-(2,5-di-O-acetylribofuranosyl)-2-nitroimidazole (8)

Compounds **7** and **8** were synthesized from both compounds **3** (route A) and **4** (route B).

Route A

Compound **3** (100 mg, 0.23 mmol) was treated with 50 % aqueous pyridine (1.0 mL) at pH 6-7 (adjusted by adding drops of 0.1N HCl solution) and then the reaction mixture was stored at 22 °C for 48 h. Afterwards, the solvents were evaporated and the crude mass was purified by column chromatography using EtOAc:hexanes (8:2; v/v) to give a mixture of pure **7** and **8** (40:60 as evident by ^{1}H NMR) in a combined yield of 52 mg (68 %). This mixture was re-purified by preparative thin layer chromatography using EtOAc:hexanes (6:4; v/v) to afford **7** (20 mg) and **8** (19 mg) individually.

Route B

Alternatively, **7** and **8** were obtained from precursor **4** (1 equivalent) during the synthesis of **6**. This synthetic pathway differed from route A with respect to the reaction temperature and the precursor. Thus, **4** (150 mg, 0.4 mmol) was treated with hydrazine (51 mg, 1.6 mmol) in a solution of glacial acetic acid:pyridine (1:4; v/v, 4 mL) at 22 °C for 9 h and afforded a mixture of **7** and **8** (1:1 as seen by ^{1}H NMR) after purification of impure product using EtOAc:hexanes (8:2; v/v) as the eluent. Combined yield of **7** and **8** *via* this route was 93 mg (90 %). Compound **7**: viscous foam; Rf 0.60 (solvent system B); ^{1}H NMR ($CDCl_3$) δ ppm: 2.17 and 2.19 (two s, each for 3H, 2 x CH_3), 4.33 (ddd, $J_{3',4'}$ = 5.3 Hz, $J_{5',4'}$ = 2.2 Hz, $J_{4',5''}$ = 3.8 Hz, 1H, H-4'), 4.46 (dd, Jgem = 12.8 Hz, $J_{4',5''}$ = 2.2 Hz, 1H, H-5"), 4.49 (dd, Jgem = 12.8 Hz, $J_{5',4'}$ = 3.8 Hz, 1H, H-5'), 4.58 (dd, $J_{1',2'}$ = 2.2 Hz, $J_{3',2'}$ = 7.3 Hz, 1H, H-2'), 4.95 (dd, $J_{4',3'}$ = 5.3 Hz, $J_{2',3'}$ = 7.3 Hz, 1H, H-3'), 6.45 (d, $J_{2',1'}$ = 2.2 Hz, 1H, H-1'), 7.23 (s, 1H, imidazole H-4), 7.63 (s, 1H, imidazole H-5) ppm; HRMS (EI^+): for $C_{12}H_{15}N_3O_8Na$ Calc. 352.07553, Found 352.07514 (M^+Na, 97.8 %). Compound **8**: foam; Rf 0.55 (solvent system B); ^{1}H NMR ($CDCl_3$)- δ ppm: 2.25 and 2.18 (two s, each for 3H, 2 x CH_3), 4.34 (ddd, $J_{3',4'}$ = 5.5 Hz, $J_{5',4'}$ = 2.2 Hz, $J_{4',5''}$ = 4.0 Hz, 1H, H-4'), 4.51 (dd, $J_{4',3'}$ = 5.5 Hz, $J_{2',3'}$ = 2.2 Hz, 1H, H-3'), 4.47 (dd, Jgem = 12.1 Hz, $J_{4',5''}$ = 2.2 Hz, 1H, H-5"), 4.56 (dd, Jgem = 12.1 Hz, $J_{5',4'}$ = 4.0 Hz, 1H, H-5'), 5.32 (d, $J_{1',2'}$ = 1.4 Hz, $J_{3',2'}$ = 2.2 Hz, 1H, H-2'), 6.54 (d, $J_{2',1'}$ = 1.4 Hz, 1H, H-1'), 7.22 (s, 1H, imidazole H-4), 7.61 (s, 1H, imidazole H-5) ppm; Analysis for $C_{12}H_{15}N_3O_8$ (329.26); Calc. C 43.77 %, H 4.59 %, N 12.76 %; Found C 43.46 %, H, 4.55 %, N 12.33; MS (ES^+): for $C_{12}H_{15}N_3O_8Na$ Calc. 352.1, Found 352.1 (M^+Na, 100 %).

1-β-D-(5-O-Benzoylribofuranosyl)-2-nitroimidazole (9), 1-β-D-(3,5-di-O-benzoylribofuranosyl)-2-nitroimidazole (10), and 1-β-D-(2,5-di-O-benzoylribofuranosyl)-2-nitroimidazole (11)

Route B was adapted to synthesize **9**-**11** by selective debenzoylation of **5**. Thus, hydrazine hydrate (46 mg; 1.44 mmol) was added to a solution of **5** in anhydrous pyridine (6 mL) and the mixture was stirred at 22 °C for 40 h. Excess of unreacted hydrazine was quenched with acetone (0.2 mL) and the solvents were evaporated under reduced pressure. The impure viscous mass was purified on a silica gel column using hexanes:EtOAc (62.5:37.5; v/v) that afforded pure **9** (81 mg, 19 %) and a mixture of **10** and **11** (285 mg, 52 %) in a ratio of 3:2. Compound **9:** Rf 0.53 (solvent system D); M.P. 55-57 °C; ^{1}H NMR ($CDCl_3$); δ ppm: 4.22 (dd, $J_{4',3'}$ = 7.6 Hz, $J_{2',3'}$ = 4.9 Hz, 1H, H-3'), 4.28 (dd, $J_{3',2'}$ = 4.9 Hz, $J_{1',2'}$ = 0.9 Hz, 1H, H-2'), 4.43 (ddd, $J_{3',4'}$ = 7.6 Hz, $J_{5',4'}$ = 3.1 Hz, $J_{5'',4'}$ = 4.3 Hz, 1H, H-4'), 4.68 (ddd, $J_{4',5''}$ = 4.3, Jgem = 12.5 Hz, 1H, H-5"), 4.74 (dd, $J_{4',5'}$ = 3.1, Jgem = 12.5 Hz, 1H, H-5'), 6.40 (d, $J_{2',1'}$ = 0.9 Hz, 1H, H-1'), 7.06 (s, 1H, imidazole H-4), 7.48-7.53 (ddd, J = 7.6 Hz, 7.9 Hz and 0.9 Hz, 4H, H-3 and H-5 of two phenyls), 7.61-7.66 (ddd, J = 7.6 Hz, 7.9 Hz and 1.3 Hz, 2H, H-4 of two phenyls), 7.76 (s, 1H, imidazole H-5), 8.02-8.05 (dd, J = 7.9 and 0.9 Hz, 4H, H-2 and H-6 of two phenyls) ; HRMS: for $C_{15}H_{15}N_3O_7Na$ Calc. 352.07514, Found 352.07521 (M^+Na, 100 %). Compound **10**: Foam; Rf 0.70 (solvent system D); ^{1}H NMR ($CDCl_3$); δ ppm: 4.71 (ddd, $J_{4',3'}$ = 2.9 Hz, $J_{5'',4'}$ = 4.4 Hz, $J_{4',5'}$ = 3.6 Hz, 1H, H-4'), 4.73 (dd, $J_{5'',4'}$ = 4.4 Hz, J_{gem} = 12.0 Hz, 1H, H-5"), 4.88 (dd, $J_{4',5'}$ = 3.6 Hz, J_{gem} = 12.0 Hz, 1H, H-5'), 4.90 (dd, $J_{3',2'}$ = 8.9 Hz, $J_{1',2'}$ = 2.2 Hz, 1H, H-2'), 5.36 (dd, $J_{2',3'}$ = 8.9 Hz, $J_{4',3'}$ = 2.9 Hz, 1H, H-3'), 6.56 (d, $J_{2',1'}$ = 2.2 Hz, 1H, H-1'), 7.13 (s, 1H, imidazole H-4), 7.6 (s, 1H, imidazole H-5), 7.42-8.08 (m, 10H, two phenyls). Compound **11**: Foam; Rf 0.79 (solvent system D); ^{1}H NMR ($CDCl_3$); δ ppm: 4.57 (ddd, $J_{3',4'}$ = 2.9 Hz, $J_{5',4'}$ = 2.2 Hz, $J_{5'',4'}$ = 5.5 Hz, 1H, H-4'), 4.60 (dd, $J_{4',3'}$ = 2.9 Hz, $J_{2',3'}$ = 4.4 Hz, 1H, H-3'), 4.77 (dd, $J_{4',5''}$ = 5.5, Jgem = 12.8 Hz, 1H, H-5"), 4.83 (dd, $J_{4',5'}$ = 2.2, Jgem = 12.8 Hz, 1H, H-5'), 5.61 (dd, $J_{3',2'}$ = 4.4 Hz, $J_{1',2'}$ = 2.2 Hz, 1H, H-2'), 6.79 (d, $J_{2',1'}$ = 2.2 Hz, 1H, H-1'), 7.11 (s, 1H, imidazole H-4), 7.66 (s, 1H, imidazole H-5), 7.40-8.20 (m, 10H, 2 x phenyls); HRMS: for $C_{22}H_{19}N_3O_8Na$ Calc. 453.07514, Found 453.07519 (M^+Na, 100 %).

1-β-D-(3,5-O,O-Tetraisopropyldisyloxy-2-O-toluenesulfonyl-ribofuranosyl)-2-nitroimidazole (12)

Tosyl chloride (39 mg; 0.2 mmol) and dimethylaminopyridine (2.5 mg; 0.02 mmol) were added to a solution of **1** (49 mg, 0.10 mmol) in anhydrous pyridine (1.0 mL) and the mixture was stirred in an ice bath. After 30 min, the temperature of the reaction was raised to 55 °C and the stirring was continued at this temperature for an additional 27 h. The reaction showed completion at this time. Evaporation of the solvent followed by silica gel column chromatography of the impure material gave pure **12**. Rf 0.43 (solvent system C); Yield 49 mg (75 %); M.P. 146-147 °C; ^{1}H NMR ($CDCl_3$); δ ppm: 0.86-1.2 (m, 16H, four isopropyl groups), 2.47 (s, 3H, CH_3), 4.02 (dd, $J_{4',5''}$ = 2.9, Jgem = 14.3 Hz, 1H, H-5"), 4.20 (dd, $J_{3',4'}$ = 7.3 Hz, $J_{5'',4'}$ = 2.9 Hz, 1H, H-4'), 4.31 (s, Jgem = 14.0 Hz, 1H, H-5'), 4.49 (dd, $J_{4',3'}$ = 7.3 Hz, $J_{2',3'}$ = 4.0 Hz, 1H, H-3'), 5.21 (d, $J_{3',2'}$ = 4.0 Hz, 1H, H-2'), 6.37 (s, 1H, H-1'), 7.25 (s, 1H, imidazole H-4), 7.36 (d, J = 7.9 Hz, H-3 and H-5 of phenyl), 7.89 (s, 1H, imidazole H-5), 7.92 (d, J = 7.9 Hz, H-2 and H-6 of phenyl); HRMS (EI^+): for $C_{27}H_{43}N_3O_9NaSi_2S$ Calc. 664.21508, Found 664.21529 (M^+Na, 80.7 %); MS ES^+ 664 (M^+Na).

The synthesis of **13-15** proceeded *via* three different routes C, D and E. Route C involved silylation at 3'- and 5'-positions of **1** that afforded **4** which underwent *in situ* desilylative acetylation to give **13**. Route E involved stannylation of 2'- and 3'-OH groups in **16** followed by its tosylation to provide **17** and **18** which, after benzoylation, afforded **15** and **14**, respectively. Alternatively, **13, 14** and **15** were obtained also by tosylation of 2'/3' –OH groups in **7**, **10** and **11**, re-

spectively (route D). The syntheses of these compounds (Scheme **2**) are described below.

Route C: 1-β-D-(3,5-Di-O-acetyl-2-O-toluenesulfonylribofuranosyl)-2-nitroimidazole (13)

TBAF (0.13 mL; 1M solution in THF) was added in two portions (at 1 h apart) to a stirred solution of **4** (42 mg; 0.065 mmol) and acetic anhydride (53 mg; 0.52 mmol) in anhydrous acetonitrile (1.5 mL) and stirred at 22 °C for a total of 8 h. A chromatographic check of the progress of this reaction indicated complete disappearance of the precursor and formation of a new spot at a lower Rf value. The solvents were evaporated and the impure product was chromatographed on a silica gel column using hexanes:EtOAc (1:1; v/v) as eluent that afforded pure **13** as a viscous foam. Rf 0.29 (solvent system E); Yield 30 mg (97 %); ^{1}H NMR ($CDCl_3$); δ ppm: 2.17 and 2.18 (two s, 6H, each for 3H of CH_3) 2.47 (s, 3H, tolyl CH_3), 4.39 (dd, $J_{4',5''}$ = 2.8, Jgem = 13.1 Hz, 1H, H-5"), 4.46 (dd, $J_{4',5''}$ = 3.1, Jgem = 13.1 Hz, 1H, H-5'), 4.53 (ddd, $J_{3',4'}$ = 5.8 Hz, $J_{5'',4'}$ = 2.8 Hz, $J_{5',4'}$ = 3.1 Hz, 1H, H-4'), 5.20 (dd, $J_{2',1'}$ = 2.4 Hz, $J_{2',3'}$ = 7.0 Hz, 1H, H-2'), 5.25 (dd, $J_{3',2'}$ = 7.0 Hz, $J_{3',4'}$ = 5.8 Hz, 1H, H-3'), 6.53 (d, $J_{2,1}$ = 2.4 Hz,1H, H-1'), 7.19 (s, 1H, imidazole H-4), 7.35 (d, J = 8.4 Hz, H-3 and H-5 of phenyl), 7.54 (s, 1H, imidazole H-5), 7.84 (d, J = 8.4 Hz, H-2 and H-6 of phenyl); HRMS (EI^+): for $C_{19}H_{21}N_3O_{10}NaS$ Calc. 506.08399, Found 506.08409 (M^+Na, 95.3 %); MS (ES^+) 506 (M^+Na).

Route D: 1-β-D-(3-O-toluenesulfonylribofuranosyl) -2-nitroimidazole (17) and 1-β-D-(2-O-toluenesulfonylribofuranosyl) -2-nitroimidazole (18)

Dibutyltin oxide (112 mg, 0.45 mmol) and tosyl chloride (128 mg, 0.67 mmol) were added to a stirred suspension of 1-β-D-(ribofuranosyl) -2-nitroimidazole, **16**, (98 mg, 0.40 mmol) in anhydrous CH_3CN (1.5 mL). This was followed by the addition of TBAF (0.04 mL, 1M solution in THF) to this reaction mixture, and the contents were allowed to stir for 16 h at 22 °C. The progress of the reaction was checked at this time which showed complete disappearance of **16** and the formation of two new products at higher Rf values. The solvents were removed on a rotary evaporator and the impure product was purified on a silica gel column using CH_2Cl_2:EtOAc (70/30; v/v) as an eluent to collect pure **17** (55 mg; 32 %) and **18** (45 mg; 27 %) as solids. Compound **17**: Rf 0.46 (solvent system B); M.P., 172-173 °C; ^{1}H NMR ($CDCl_3$); δ ppm: 2.47 (s, 3H, CH_3), 3.56 (dd, $J_{4',5''}$ = 2.6, Jgem = 12.5 Hz, 1H, H-5"), 3.83 (dd, $J_{4',5''}$ = 2.2, Jgem = 12.5 Hz, 1H, H-5'), 4.29 (ddd, $J_{3',4'}$ = 5.1 Hz, $J_{5'',4'}$ = 2.6 Hz, $J_{5',4'}$ = 2.2 Hz, 1H, H-4'), 4.33 (dd, $J_{2',1'}$ = 4.0 Hz, $J_{2',3'}$ = 5.5 Hz, 1H, H-2'), 4.93 (dd, $J_{3',2'}$ = 5.5 Hz, $J_{3',4'}$ = 5.1 Hz, 1H, H-3'), 6.43 (d, $J_{2,1}$ = 4.0 Hz,1H, H-1'), 7.15 (d, $J_{4,5}$ = 1.1 Hz, 1H, imidazole H-4), 7.43 (d, J = 8.4 Hz, H-3 and H-5 of phenyl), 7.84 (d, J = 8.4 Hz, H-2 and H-6 of phenyl), 8.07 (d, $J_{4,5}$ = 1.1 Hz,1H, imidazole H-5); ^{13}C NMR ($CDCl_3$), δ ppm: 20.36 and 20.51 (two CH_3), 60.61 (C-5'), 68.93 (C-4'), 75.68 (C-3'), 82.78 (C-2'), 89.71 (C-1'), 122.3 (imidazole C-4), 127.97 (imidazole C-5), 168.96 and 170.04 (two C=O) ppm; HRMS (EI^+): for $C_{15}H_{17}N_3O_8NaS$ Calc. 422.06286, Found 422.06281 (M^+Na, 95.9 %); MS ES^+ 422 (M^+Na). Compound **18**: Rf 0.34 (solvent system B); M.P., 165-166 °C; ^{1}H NMR ($CDCl_3$); δ ppm: 2.57 (s, 3H, CH_3), 3.67 (dd, $J_{4',5''}$ = 2.1, Jgem = 10.1 Hz, 1H, H-5"), 3.75 (dd, $J_{4',5}$ = 1.8, Jgem = 10.1 Hz, 1H, H-5'), 3.97 (ddd, $J_{3',4'}$ = 5.3 Hz, $J_{5'',4'}$ = 2.1 Hz, $J_{5',4'}$ = 1.8 Hz, 1H, H-4'), 4.20 (dd, $J_{3',2'}$ = 2.4 Hz, $J_{3',4'}$ = 5.3 Hz, 1H, H-3'), 4.84 (dd, $J_{2',1'}$ = 4.3 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-2'), 6.18 (d, $J_{2,1}$ = 4.2 Hz,1H, H-1'), 6.42 (d, $J_{4,5}$ = 0.9 Hz, 1H, imidazole H-4), 6.63 (d, J = 7.0 Hz, H-3 and H-5 of phenyl), 6.92 (d, J = 7.0 Hz, H-2 and H-6 of phenyl), 7.16 (d, $J_{4,5}$ = 1.1 Hz,1H, imidazole H-5); ^{13}C NMR ($CDCl_3$), δ ppm: 20.36 and 20.51 (two CH_3), 60.61 (C-5'), 68.93 (C-4'), 75.68 (C-3'), 82.78 (C-2'), 89.71 (C-1'), 122.3 (imidazole C-4), 127.97 (imidazole C-5), 168.96 and 170.04 (two C=O) ppm; HRMS (EI^+): for $C_{15}H_{17}N_3O_8NaS$ Calc. 422.06286, Found 422.06261 (M^+Na, 95.8 %); MS ES^+ 422 (M^+Na).

1-β-D-(3,5-Di-O-benzoyl-2-O-toluenesulfonylribofuranosyl)-2-nitroimidazole (14)

Benzoyl chloride (130 mg, 0.90 mmol) was added to a cold stirred (ice bath) solution of **18** (45 mg, 0.11 mmol) in anhydrous pyridine (0.5 mL) and the mixture was stirred and allowed to warm up to 22 °C. At this time the stirring was continued for an additional 5 h and then quenched by adding a piece of ice. The solvent was removed and the impure product was loaded on a silica gel column that was eluted with hexanes:EtOAc (65:35; v/v) to afford pure **14**. Rf, 0.40 (solvent system D); Yield 64 mg (95 %); M.P., 176-177 °C; ^{1}H NMR ($CDCl_3$); δ ppm: 2.65 (s, 3H, CH_3), 4.65 (dd, $J_{4',5''}$ = 3.9, Jgem = 12.2 Hz, 1H, H-5"), 4.78 (ddd, $J_{3',4'}$ = 4.3 Hz, $J_{5'',4'}$ = 3.9 Hz, $J_{5',4'}$ = 3.8 Hz, 1H, H-4'), 4.84 (dd, $J_{4',5}$ = 3.8, Jgem = 12.2 Hz, 1H, H-5'), 5.37 (dd, $J_{3',2'}$ = 4.9 Hz, $J_{3',4'}$ = 4.3 Hz, 1H, H-3'), 5.65 (dd, $J_{2',1'}$ = 3.8 Hz, $J_{2',3'}$ = 4.9 Hz, 1H, H-2'), 6.78 (d, $J_{2',1'}$ = 3.8 Hz,1H, H-1'), 7.10 (s, 1H, imidazole H-4), 7.18 (d, J = 8.0 Hz, H-3 and H-5 of phenyl), 7.45 (s, 1H, imidazole H-5), 7.47-7.70 (m, 8H, 6H of two phenyls and 2H of sulfonylphenyl); HRMS (EI^+): for $C_{29}H_{25}N_3O_{10}$ SNa (630.11529), found 630.11553.

1-β-D-(2,5-Di-O-benzoyl-3-O-toluenesulfonylribofuranosyl)-2-nitroimidazole (15)

A similar procedure, as described for **14**, was followed to benzoylate **17** (56 mg; 0.14 mmol) that afforded **15**. Yield (65 mg, 77 %); Rf, 0.35 (solvent system D); ^{1}H NMR ($CDCl_3$); δ ppm: 2.63 (s, 3H, CH_3), 4.66 (dd, $J_{4',5''}$ = 3.9, Jgem = 12.4 Hz, 1H, H-5"), 4.72 (ddd, $J_{3',4'}$ = 4.6 Hz, $J_{5'',4'}$ = 3.9 Hz, $J_{5',4'}$ = 3.8 Hz, 1H, H-4'), 4.81 (dd, $J_{4',5}$ = 3.9, Jgem = 12.4 Hz, 1H, H-5'), 5.29 (dd, $J_{3',2'}$ = 4.2 Hz, $J_{1',2'}$ = 4.0 Hz, 1H, H-2'), 5.70 (dd, $J_{2',3'}$ = 4.2 Hz, $J_{4',3'}$ = 4.6 Hz, 1H, H-3'), 6.98 (d, $J_{2',1'}$ = 4.0 Hz,1H, H-1'), 7.05 (s, 1H, imidazole H-4), 7.15 (d, J = 8.0 Hz, H-3 and H-5 of phenyl), 7.37 (s, 1H, imidazole H-5), 7.43-7.70 (m, 8H, 6H of two phenyls and 2H of sulfonylphenyl); MS (ES^+): for $C_{29}H_{25}N_3O_{10}SNa$ (M^+Na) 630.

Route E

This alternate route involved tosylation of diacetylated **7** and dibenzoylated **10** and **11**. Thus, TsCl (0.60 mmol) was added to a pre-cooled (0-5°C) solution of **7**/**10**/**11** (0.40 mmol) in anhydrous pyridine (1 mL) and the mixture was stirred at 22 °C for 16 h. Excess tosyl chloride in the reaction mixture was decomposed by adding a few ice pieces to the solution and then the solvents were evaporated under reduced pressure. These compounds were purified chroma-

tographically on a silica gel column using CH_2Cl_2:EtOAc (70/30; v/v). Tosylation of **7** afforded pure **13** (90 %), while **10** and **11**, on tosylation, yielded **14** and **15**, respectively in 95 % yield.

1-β-D-(3,5-Di-O-benzoyl-2-deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole (19)

A catalytic amount of anhydrous pyridine (160 μL) was added to a solution of **9** (110 mg, 0.24 mmol) in anhydrous CH_2Cl_2 (10 mL) and the contents were cooled down to 0-5 °C. DAST (180 mg; 1.2 mmol) was slowly added to this cold reaction mixture under stirring at this temperature and then for 1 h at 22 °C. A second portion of DAST (110 mg; 0.67 mmol) was added (after cooling the reaction mixture again) and then the stirring was continued at 22 °C for an additional 14 h. The progress of the reaction was checked on a tlc plate at this time which showed complete disappearance of the precursor. Excess DAST was decomposed by adding a few drops of MeOH and the solvents were removed in vacuo. The impure viscous mass was purified on a silica gel column using chloroform as a solvent to elute pure **19**. Rf, 0.71 (solvent system A; two runs); Yield 67 mg (60 %); M.P. 162-163 °C; ^{1}H NMR ($CDCl_3$)- δ 4.64 (ddd, $J_{3',4'}$ = 4.3 Hz, $J_{5',4'}$ = 3.1 Hz, $J_{5'',4'}$ = 7.3 Hz, 1H, H-4'), 4.83 (dd, $J_{4',5''}$ = 7.3, Jgem = 12.5 Hz, 1H, H-5"), 4.87 (dd, $J_{4',5'}$ = 3.8, Jgem = 12.5 Hz, 1H, H-5'), 5.67 (ddd, $J_{2',F}$ = 48.5 Hz, $J_{3',2'}$ = 3.1 Hz, $J_{1',2'}$ = 2.7 Hz, 1H, H-2'), 5.70 (ddd, $J_{4',3'}$ = 4.5 Hz, $J_{2',3'}$ = 3.1 Hz, $J_{3',F}$ = 12.8 Hz 1H, H-3'), 6.83 (dd, $J_{2',1'}$ = 2.7 Hz, $J_{1',F}$ = 18.0 Hz, 1H, H-1'), 7.18 (s, 1H, imidazole H-4), 7.43-7.53 (ddd, J = 7.6, 7.9 and 0.9 Hz, 4H, H-3 and H-5 of two phenyls), 7.67-7.68 (ddd, J = 7.6 Hz, 7.9 Hz and 1.3 Hz, 2H, H-4 of two phenyls), 7.60 (s, 1H, imidazole H-5), 8.02-8.05 (dd, J = 7.9 and 0.9 Hz, 4H, H-2 and H-6 of two phenyls) ; ^{19}F NMR ($CDCl_3$), δ ppm: -38.19 ppm (ddd, $J_{2',F}$ = 48.7 Hz, $J_{1',F}$ = 18.0 Hz, $J_{3',F}$ = 12.8 Hz); ^{13}C NMR ($CDCl_3$), δ ppm: 63.04 (C-5'), 76.44 (d, $J_{F,C3'}$ = 20.8 Hz, C-3'), 82.05 (C-4'), 88.60 (d, $J_{F,C1'}$ = 17.6 Hz, C-1'), 89.71 (d, $J_{F,C2'}$ = 194.5 Hz C-2'), 128.47-129.87 (phenyl carbons), 133.40 (imidazole C-4), 134.13 (imidazole C-5), 143.81 (imidazole C-2) 164.99 and 166.02 (two C=O) ppm; HRMS EI$^+$: for $C_{22}H_{18}N_3O_7FNa$ Calc. 478.10210, Found 478.10231 (M^+Na, 93.5 %); MS ES$^+$ 478 (M^+Na).

1-β-D-(2-Deoxy-2-fluoroarabinofuranosyl)-2-nitroimidazole (20)

A solution of NH_3 in MeOH (0.6 mmol of 2.0 M solution) was added to **19** (55 mg, 0.12 mmol) and stirred at 22 °C for 4 h. This was followed by the addition of another aliquot (0.2 mmol) of NH_3/MeOH to this reaction mixture and the stirring was continued for another 5 h. The solvent was evaporated on a rotary evaporator after this time and the impure product was purified on a silica gel column using $CHCl_3$:MeOH (92.5:7.5; v/v) as eluent that afforded pure **20**. Rf 0.33 (solvent system B; two runs); Yield, 27 mg (92 %); m.p., 183-185 °C; ^{1}H NMR (CD_3OD) δ 3.78 (dd, $J_{4',5''}$ = 4.8, Jgem = 12.4 Hz, 1H, H-5"), 3.88 (dd, $J_{4',5'}$ = 4.8, Jgem = 12.4 Hz, 1H, H-5'), 3.99 (ddd, $J_{3',4'}$ = 4.4 Hz, $J_{5',4'}$ = 4.8 Hz, $J_{5'',4'}$ = 4.6 Hz, 1H, H-4'), 4.35 (ddd, $J_{4',3'}$ = $J_{2',3'}$ = 4.4 Hz, $J_{3',F}$ = 19.0 Hz 1H, H-3'), 5.28 (ddd, $J_{2',F}$ = 52.4 Hz, $J_{3',2'}$ = $J_{1',2'}$ = 4.4 Hz, 1H, H-2'), 6.78 (dd, $J_{2',1'}$ = 4.4 Hz, $J_{1',F}$ = 10.8 Hz, 1H, H-1'), 7.18 (s, 1H, imidazole H-4), 7.99 (s, 1H, imidazole H-5) ppm; ^{19}F NMR (CD_3OD) δ ppm: -35.12 ppm (ddd, $J_{2',F}$ = 51.3 Hz, $J_{1',F}$ = 18.3 Hz, $J_{3',F}$ = 11.0 Hz); ^{13}C NMR (CD_3OD) δ ppm: 61.35 (C-5'), 73.70 (d, $J_{F,C3'}$ = 24.2 Hz, C-3'), 85.38 (d, $J_{F,C4'}$ = 5.5 Hz, C-4'), 88.48 (d, $J_{F,C1'}$ = 16.5 Hz, C-1'), 96.9 (d, $J_{F,C2'}$ = 194.4 Hz C-2'), 125.06 (imidazole C-4), 128.5 (imidazole C-5), 132.5 (imidazole C-2)) ppm; Analysis for $C_8H_{10}N_3O_5F$ (247.18), Calc. C 38.87 %, H 4.08 %, N 17.00 %; Found. C 38 %, H 4.02 %, N 16.88 %.

1-β-D-(2,5-Di-O-benzoyl-3-deoxy-3-fluoro-lyxofuranosyl)-2-nitroimidazole (21)

DAST (90 mg; 0.6 mmol) was added to a solution of **11** (60 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (5 mL) and anhydrous pyridine (80 μL) under stirring at 0-5 °C. After 1h, the temperature of the reaction was raised to 22 °C. A tlc examination of the reaction mixture at this time showed that the fluorination of **11** was not complete. A second portion of DAST (70 mg; 0.45 mmol) was added (after cooling down the reaction mixture again) and then the stirring was continued at 22 °C for an additional 14 h. At the end of the reaction, excess DAST was quenched by adding a few drops of MeOH. The solvents were removed in vacuo and the impure product was purified by silica gel chromatography using chloroform as a solvent that eluted pure **21**. Rf, 0.67 (solvent system A; two runs); Yield 35 mg (55 %); ^{1}H NMR ($CDCl_3$)- δ 4.67-4.86 (m 3H,1H of H-4' and 2H of H-5), 5.22 (dd, $J_{3',F}$ = 50.9 Hz, $J_{4',3'}$ = 1.9 Hz, $J_{2',3'}$ = 1.1 Hz, 1H, H-3'), 5.70 (dd, $J_{4',3'}$ = 4.5 Hz, $J_{3',2'}$ = 1.1 Hz, $J_{2',F}$ = 12.5 Hz 1H, H-2'), 6.68 (s, 1H, H-1'), 7.15 (s, 1H, imidazole H-4), 7.30-7.60 (m, 6H of two phenyls and 1H, imidazole H-5), 7.67-7.68 (ddd, J = 7.6 Hz, 7.9 Hz and 1.3 Hz, 2H, H-4 of two phenyls), 7.60 (s,), 8.00 (d, J = 7.3 4H, H-2 and H-6 of two phenyls) ; ^{19}F NMR ($CDCl_3$), δ ppm: -38.34 ppm (ddd, $J_{3',F}$ = 51.3 Hz, $J_{4',F}$ = 30.1 Hz, $J_{2',F}$ = 12.8 Hz); ^{13}C NMR ($CDCl_3$), δ ppm: 60.76 (d, $J_{F,C5'}$ = 9.9 Hz C-5'), 80.24 (d, $J_{F,C4'}$ = 30.8 Hz, C-4'), 81.35 (d, $J_{F,C2'}$ = 19.8 Hz,C-2'), 91.98 (C-1'), 92.64 (d, $J_{F,C3'}$ = 187.9 Hz C-3'), 127.99 (imidazole C-4), 128.30-134.25 (phenyl carbons), 129.00 (imidazole C-5), 145.81 (imidazole C-2) 164.36 and 165.99 (two C=O) ppm; HRMS EI$^+$: for $C_{22}H_{18}N_3O_7FNa$ Calc. 478.10210, Found 478.10225 (M^+Na, 93.4 %); MS ES$^+$ 478.1 (M^+Na).

1-β-D-(3-Deoxy-3-fluorolyxofuranosyl)-2-nitroimidazole (22)

A solution of NH_3 in MeOH (0.6 mmol of 2.0 M solution) was added to **21** (55 mg, 0.12 mmol) and stirred at 22 °C for 4 h. This was followed by the addition of another aliquot (0.2 mmol) of NH_3/MeOH to this reaction mixture and the stirring was continued for another 5 h. The solvent was evaporated on a rotary evaporator after this time and the impure product was purified on a silica gel column using $CHCl_3$:MeOH (92.5:7.5; v/v) as an eluent that afforded pure **22**. Rf 0.29 (solvent system B; two runs); Yield, 27 mg (92 %); m.p., 183-185 °C; ^{1}H NMR (CD_3OD) δ 3.97 (dd, $J_{4',5''}$ = 2.2, Jgem = 11.7 Hz, 1H, H-5"), 4.03 (dd, $J_{4',5'}$ = 2.6 Hz, Jgem = 11.7 Hz, 1H, H-5'), 4.51 (dd, $J_{3',2'}$ = 2.6 Hz, $J_{F,2'}$ = 18.0 Hz, 1H, H-2'), 4.67 (dddd, $J_{5',4'}$ = 2.6 Hz, $J_{4',5''}$ = 2.6 Hz, $J_{4',3'}$ = 6.2 Hz, $J_{4',F}$ = 27.0 Hz 1H, H-4'), 4.95 (ddd, $J_{3',F}$ = 51.0 Hz, $J_{3',2'}$ = 1.5 Hz, $J_{1',2'}$ = 4.4 Hz, 1H, H-3'), 6.36 (s, 1H, H-1'), 7.13 (s, 1H, imidazole H-4), 7.63 (s, 1H, imidazole H-5) ppm; ^{19}F NMR (CD_3OD) δ ppm: -36.26 ppm (ddd, $J_{3',F}$ = 51.3 Hz, $J_{4',F}$ = 33.0 Hz, $J_{2',F}$ = 17.3 Hz); ^{13}C NMR (CD_3OD)

δ ppm: 63.04 (C-5'), 76.44 (d, $J_{F,C3'}$ = 20.8 Hz, C-3'), 85.38 (d, $J_{F,C4'}$ = 5.5 Hz, C-4'), 88.48 (d, $J_{F,C1'}$ = 16.5 Hz, C-1'), 96.9 (d, $J_{F,C2'}$ = 194.4 Hz C-2'), 125.06 (imidazole C-4), 128.5 (imidazole C-5), 132.5 (imidazole C-2)) ppm; MS (ES^+) for $C_8H_{10}N_3O_5FNa$ Calc. 247.0, found 270.0 (M^+ Na).

Radiosensitization Studies

Cell radiosensitization was determined using a ^{60}Co x-ray source together with a clonogenic survival assay[22]. Briefly, HCT116/100 colorectal carcinoma cells (300,000 cells in 3 mL DMEM/F12 medium per T60 Petri dish) were incubated under 5% CO_2 in air at 37 °C for 20 h, then test substance (stock solution 10 mM in 95 % ethanol) was added to achieve a concentration of 100 μM, and incubation continued for 24 h. The dishes were assigned to either the control (normoxic) or hypoxic groups. Those in the hypoxic group were de-gassed to hypoxia by 6 consecutive vacuum and nitrogen fill cycles in a vacuum chamber. The Petri dishes (hypoxic and normoxic controls) were incubated for 30 min on an oscillating shaker at R/T X 60 cycles per min and then irradiated in a ^{60}Co γ-irradiator at 0 (control), 4, 8, 12, 16 and 20 Gy in N_2 (hypoxic sub-group) and air chambers (normoxic sub-group). The cells were then recovered from each dish by two consecutive washes with PBS followed by the addition of trypsin (500 μL) and quenching with fresh medium (4.5 mL). Cells were then plated at densities from 100 to 15000 cells/5 mL medium for normoxic conditions and 100 and 5000 cells/5 mL medium for hypoxic conditions. The cells were incubated for 10 to 14 days at 37 °C under 5% CO_2, then stained with methylene blue or crystal violet in ethanol, clones counted and surviving fractions were calculated. Data for β-2-FAZA, β-3- FAZL and FAZA, all at 100 μM in HCT116 cells are presented in Fig. (**2**). Tests were done in triplicate.

Partition Coefficients

The partition coefficients (*P*) for β-2-FAZA and β-3-FAZL were calculated by determining their retention times and comparing with the retention times and partition coefficients (*P*) of MISO[37], FMISO[21], β-AZA[36], α-IAZA[36] and α-FAZA[21] which were determined on a reversed phase radial pak column chromatography using an isocratic composition of the solvents (MeOH/ H_2O; ¼=v/v) at a flow rate of 1.5 mL/min. The results are presented in Table **1** and Fig. (**3**). Based on this comparison, β-2-FAZA and β-3-*lyxo*-FAZL have estimated log partition coefficients of 1.0 and 0.95, respectively, and are slightly less lipophilic than α-FAZA (*P* = 1.1)[22].

CONCLUSION

β-2-FAZA, **20**, and β-3-FAZL, **22**, analogues of FAZA with β-nucleoside configuration, were synthesized by DAST fluorination of **10** and **11**, respectively. The concept behind this development was to exploit the β-nucleoside configuration to promote their interaction with nucleoside transporters and, as a result, in 'active' permeation across hypoxic cell membranes. Furthermore, incorporation of fluorine at secondary carbon atoms (C-2' and C-3', respectively) is anticipated to provide improved chemical stability to the nucleosidic bond in these molecules which is prone to the cleavage in the cellular environment[41]. Preliminary *in vitro* radiosensitization studies with these compounds against HCT116/100 colorectal carcinoma cells indicate that both β-2-FAZA and β-3-FAZL are equipotent to FAZA, the current clinical marker of hypoxia, as radiosensitizers, and their lipophilic properties are suitable for good tissue perfusion. Evaluation of their facilitated transport across cell membranes and *in vivo* radiosensitization potency in a hypoxic model are warranted.

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