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FLUOROAZOMYCIN ARABINOSIDE (FAZA): Synthesis, ^{2}H and ^{3}H-labelling and preliminary biological evaluation of a novel 2-nitroimidazole marker of tissue hypoxia.

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SUMMARY

1-α-D-(5-Fluoro-5-deoxyarabinofuranosyl)-2-nitroimidazole (fluoroazomycin arabinoside; FAZA) 6, a putative PET imaging agent when labelled with ^{18}F, was synthesized by fluorination of 1-α-D-(2,3-di-O-acetylarabinofuranosyl)-2-nitroimidazole 3 with DAST followed by deprotection. The C-5'-deuterated and tritiated analogues were prepared by $NaCNBD_3$ or $NaCNBT_3$ reduction of the protected C-5'-carbonyl intermediate 5, followed by C-5' fluorination and deprotection, to afford C-5' deuterated and C-5' tritiated FAZA, respectively. Preliminary *in vivo* biodistribution studies in a murine tumour model, and pharmacokinetic studies in rats indicated that ^{3}H-FAZA has biodistribution, tumour uptake and pharmacokinetic properties similar to those of ^{123}I-IAZA, a clinically-proven radiopharmaceutical for SPECT-imaging of hypoxic tissues.

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INTRODUCTION

One promising non-invasive approach to assess hypoxia is to utilize appropriately-labelled radiosensitizers which will bind to oxygen-deficient tissues, but not to normally-oxygenated tissues. Selective binding of reactive reduction intermediates of these radiopharmaceuticals to the macromolecular fraction of hypoxic tissues has been postulated to be the basis for concentrating these compounds in target (hypoxic) tissues (9). Nuclear medicine imaging can then be used to determine the hypoxia-dependent biodistribution of the labelled compound. Azomycin-based compounds undergo single-electron reduction of the nitro group to form an oxygen-sensitive radical anion that, upon further reduction in the absence of oxygen, forms reactive intermediates that bind covalently to tissue macromolecules (10). One of these compounds, [^{123}I]-IAZA, has been extensively studied in patients with solid malignant tumours (11,12,13), peripheral vascular disease (14) and rheumatoid arthritis (15). Other SPECT agents include ^{99m}TcO-PnAO-1-(2-nitroimidazole) (16) and ^{99m}Tc-HL-91 (17). Positron emission tomography (PET) has an important role, and has several advantages over SPECT, in non-invasive investigations of hypoxia in pathological conditions. Positron emitting agents in the literature include [^{18}F]fluoromisonidazole (18,19) and [^{18}F]fluoroerythronitroimidazole (20).

The synthesis of 1-α-D-(5-fluoro-5-deoxy-arabinofuranosyl)-2-nitroimidazole (FAZA) **6** is now reported, with a view to developing a new fluorinated PET imaging agent. FAZA has been labelled with deuterium to model the tritiation reaction, and with tritium to investigate its oxygen dependent binding in EMT-6 tumors in mice and its pharmacokinetics in rats.

EXPERIMENTAL

Materials. All chemicals used were of reagent grade. Anhydrous solvents, when used, were dried over an appropriate drying agent and freshly distilled. The progress of reactions was monitored on Whatman MK6F (250 μm) thin layer chromatographic (tlc) silica gel micro-plates. The products were purified by column chromatography using Merck silica gel 60 (particle size 70-200 and 230-400 mesh ASTM). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer in deuterated chloroform or methanol. Chemical shifts are reported in δ (ppm) downfield with

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respect to tetramethylsilane as an internal standard. ^{19}F NMR chemical shifts are reported in δ(ppm) with respect to trifluoroacetic acid. Melting points were recorded on a Buchi capillary melting point apparatus and are uncorrected. The protons and carbons of the sugars are represented by a single prime (') and a double prime ("), respectively. Elemental analysis and/or high resolution mass spectrometry was performed as a commercial service by the Department of Chemistry, University of Alberta. Sodium cyanoborotritide ($NaCNBT_3$) was purchased from Amersham International, Canada and was used for selective tritiation at the C-5'- position of FAZA. High Performance Liquid Chromatography (HPLC) on a Whatman magnum C-18 (4.5 x 30 mm) column, with uv detection at 323 nm, was used to purify the tritiated products. The radioactive fractions were counted on a Beckman Model 9000 liquid scintillation counter.

Syntheses:

1-α-D-(5-O-tert-Butyldiphenylsilyl-2,3-di-O-acetylarabinofuranosyl)-2-nitroimidazole; **2.** 1-α-D-Arabinofuranosyl-2-nitroimidazole (α-AZA) **1** (545 mg; 2.05 mmol) was dissolved in anhydrous pyridine (2.5 mL), then *tert*-butyldiphenylchlorosilane (0.51 mL, 2.3 mmol) was added under an argon atmosphere. The reaction mixture was stirred at 22 °C overnight. A tlc check at this time showed complete conversion of AZA to its 5'-silylated derivative. Acetic anhydride (0.83 mL, 8.0 mmol) was added to the reaction mixture and stirring was continued for an additional 4 h, after which the excess acetic anhydride was decomposed by adding ice to the mixture. The contents were evaporated *in vacuo*, dissolved in dichloromethane (25 mL) and washed with water (15 mL x 2). The organic phase was collected, dried over anhydrous sodium sulfate, filtered and evaporated to collect the crude product which was purified on a silica gel column using a linear gradient of ethyl acetate (10% to 90%) in toluene, to yield 675 mg (60%) of **2**: m.p. 53°C; ^1H NMR ($CDCl_3$) δ 1.11 (*s*, 9H, *tert.*-butyl), 2.03 (*s*, 6H, two CH_3), 3.87 (*d*, $J_{4',5'}$=5.0 Hz of *d*, J_{gem}=11.5 Hz, 1H, ***H-5'***), 3.92 (*d*, $J_{4',5''}$=5.0 Hz of *d*, J_{gem}=11.5 Hz, 1H, ***H-5''***), 4.50 (*d*, $J_{3',4'}$=2.0 Hz of *t*, $J_{5',4'}$=$J_{5'',4'}$=5.0 Hz, 1H, ***H-4'***), 5.36 (*d*, $J_{2',3'}$=1.5 Hz of *d*, $J_{4',3'}$=2.0 Hz, 1H, ***H-3'***), 5.40 (*d*, $J_{1',2'}$=2.0 Hz of *d*, $J_{3',2'}$=1.5 Hz, 1H, ***H-2'***), 6.58 (*d*, $J_{2',1'}$=2.0 Hz, 1H, ***H-1'***),

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7.20 (*d*, $J_{4,5}$=1.0 Hz, 1H, ***H-5***) and 7.42 (*d*, $J_{5,4}$=1.0 Hz, 1H, ***H-4***) ppm; anal. for $C_{28}H_{33}SiN_3O_8$ (567.651); calc. (%) C, 59.24; H, 5.86; N, 7.40; found C, 59.29; H, 6.01 and N, 7.05.

1-α-D-(2,3-Di-O-acetylarabinofuranosyl)-2-nitroimidazole; **3.** **2** (565 mg, 1.0 mmol) was dissolved in methanol (25 mL). Ammonium fluoride (50 mg, 1.35 mmol) was added, and this mixture was heated under reflux for 60 min, when tlc indicated that the reaction was near completion. Excess solvent was evaporated *in vacuo* and the viscous residue was purified on a silica gel column using hexane:ethyl acetate (80:20 v/v) as eluent to give 242 mg (74%) of pure diacetyl AZA, **3** as a semisolid mass; ^{1}H NMR ($CDCl_3$) δ 2.04 (*s*, 3H, acetyl C***H***$_3$), 2.20 (*s*, 3H, acetyl C***H***$_3$), 3.88 (*d*, $J_{4',5'}$=4.5 Hz of *d*, J_{gem}=12.0 Hz, 1H, ***H-5'***), 3.95 (*d*, $J_{4',5''}$=5.0 Hz of *d*, J_{gem}=12.0 Hz, 1H, ***H-5''***), 4.53 (*d*, $J_{3',4'}$=3.0 Hz of *d*, $J_{5',4'}$=4.5 Hz of *d*, $J_{5'',4'}$=5.0 Hz, 1H, ***H-4'***), 5.18 (*d*, $J_{4',3'}$=3.0 Hz of *d*, $J_{2',3'}$=1.5 Hz, 1H, ***H-2'***), 5.47 (*d*, $J_{3',2'}$=1.5 Hz of *d*, $J_{1',2'}$=2.0 Hz, 1H, ***H-2'***), 6.68 (*d*, $J_{2',1'}$=2.0 Hz, 1H, ***H-1'***), 7.22 (*d*, $J_{4,5}$=1.0 Hz, 1H, ***H-5***) and 7.38 (*d*, $J_{5,4}$=1.0 Hz, 1H, ***H-4***) ppm; ^{13}C NMR ($CDCl_3$) δ 20.54 (*C*H$_3$), 62.20 (***C-5'***), 76.49 (***C-3'***), 81.52 (***C-2'***), 88.02 (***C-4'***), 92.72 (***C-1'***), 121.87 (***C-5***), 128.57 (***C-4***), 146.20 (***C-2***), 168.87 and 169.61 (two *C*=O) ppm; anal. for $C_{12}H_{15}N_3O_8$ (329.261); calc. (%) C, 43.77; H, 4.59; N, 12.82; found C, 43.59; H, 4.50 and N, 12.65.

1-α-D-(5-Fluoro-5-deoxy-2,3-di-O-acetylarabinofuranosyl)-2-nitroimidazole; **4.** Diethylamino sulfur trifluoride (DAST; 0.13 mL; 0.96 mmol) in a round bottom flask was cooled over an ice bath, with complete exclusion of moisture. A solution of **3** (0.24 g; 0.87 mmol) in anhydrous dichloromethane (10 mL) was added drop-wise through a dropping funnel, with stirring, to the DAST solution so that the temperature of the reaction mixture remained below 5 °C. Stirring was continued at this temperature for 30 min, the contents were allowed to warm to the room temperature (22 °C), and the mixture was stirred for another 30 min. Tlc at this time showed complete disappearance of the starting material and the presence of a new product. The reaction was quenched by adding methanol (200 μL) to the mixture, the solvent was evaporated on a rotary evaporator, and the viscous residue was dissolved in dichloromethane (25 mL) and washed with cold water (10 mL x 2). The organic phase was dried over anhydrous sodium sulfate, filtered

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and evaporated *in vacuo* to yield impure 4, which was purified on a silica gel column using ethyl acetate:toluene (20:80 v/v) as eluent, to yield 99 mg. (41%) of pure 4 as a semisolid; ^{1}H NMR ($CDCl_3$) δ 2.07 and 2.24 (two *s*, each for 3H of two C*H*$_3$), 4.64 (*d*, $J_{3',4'}$=3.0 Hz of *d*, $J_{5',4'}$=8.0 Hz of *d*, $J_{5'',4'}$=5.0 Hz of *d*, $J_{F,H}$=19.5 Hz, 1H, ***H-4'***), 4.65 (*d*, $J_{H,F}$=47.5 Hz of *m*, 2H, ***H-5'*** and ***H-5''***), 5.15 (*d*, $J_{2',3'}$=1.5 Hz of *d*, $J_{4',3'}$=3.0 Hz, 1H, ***H-3'***), 5.23 (*broad dd*, 1H, ***H-2'***), 6.65 (*d*, $J_{2',1'}$=1.5 Hz, 1H, ***H-1'***), 7.20 (*d*, $J_{4',5'}$=1.0 Hz, 1H, ***H-5***), 7.37 (*d*, $J_{5,4}$=1.0 Hz, 1H, ***H-4***) ppm; ^{13}C NMR ($CDCl_3$) δ 20.39 (*C*H$_3$), 75.97 (*d*, $J_{F,C}$=6.2 Hz, ***C-3'***), 81.64 (*d*, $J_{F,C}$=175.7 Hz, ***C-5'***), 82.20 (***C-2'***), 85.95 (*d*, $J_{F,C}$=19.2 Hz, ***C-4'***), 92.93 (***C-1'***), 121.89 (***C-5***), 128.49 (***C-4***), 144.80 (***C-2***), 168.84 and 169.35 (two *s*, for two *C*=O) ppm; anal. for $C_{12}H_{14}FN_3O_7$ (331.253); calc. (%) C, 43.51; H, 4.23; N, 12.69; found C, 43.18; H, 4.16 and N, 12.50.

1-α-D-(5-Fluoro-5-deoxyarabinofuranosyl)-2-nitroimidazole (FAZA) 6. The acetylated precursor 4 (90.0 mg., 0.27 mmol) was dissolved in a saturated solution of ammonia in methanol and the reaction mixture was stirred overnight at 5 °C. Tlc of the reaction mixture showed complete disappearance of the starting material. The solvent was removed *in vacuo* and the crude mixture was purified on a silica gel column using chloroform:methanol (93:7 v/v) as the elution solvent to give 70 mg (100%) of pure FAZA 6 which was recrystallized from methanol; m.p. 161 °C; ^{1}H NMR (CD_3OD) δ 4.20 (*d*, $J_{H,F}$=57.0 Hz of *m*, 2H, ***H-5'*** and ***H-5''***), 4.50 (*d*, $J_{4',3'}$=2.0 Hz of *d*, $J_{F,H}$=6.0 Hz of *d*, $J_{2',3'}$=1.5 Hz, 1H, ***H-3'***), 4.60 (*m broad*, 1H, ***H-4'***), 4.66 (*m broad*, 1H, ***H-2'***), 6.44 (*d*, $J_{2',1'}$=1.5 Hz, 1H, ***H-1'***), 7.14 (*d*, $J_{4,5}$=1.0 Hz, ***H-5***) and 7.68 (*d*, $J_{5,4}$=1.0 Hz, 1H, ***H-4***) ppm; ^{13}C NMR (CD_3OD) δ 77.21 (*d*, $J_{F,C}$=4.7 Hz, ***C-3'***), 83.64 (***C-2'***), 83.63 (*d*, $J_{F,C}$=169.7 Hz, ***C-5'***), 89.04 (*d*, $J_{F,C}$=20.6 Hz, ***C-4'***), 96.80 (***C-1'***), 125.22 (***C-5***) and 128.25 (***C-4***) ppm; ^{19}F NMR (CD_3OD) δ -147.72 (*d*, $J_{H4',F}$=18.8 Hz of *d*, $J_{H5',F}$=46.0 Hz of *d*, $J_{H5',F}$=46.3 Hz) ppm; anal. for $C_8H_{10}FN_3O_5$ (247.18); calc. (%) C, 38.37; H, 4.08; N, 17.00; found C, 38.11; H, 3.82 and N, 16.61.

1-α-D-(2,3-Di-O-acetyl-4-formylarabinofuranosyl)-2-nitroimidazole; 5. 3 (20 mg, 0.06 mmol) was dissolved in anhydrous dimethyl sulfoxide (2 mL) and dicyclohexyl carbodiimide (DCC, 37.3 mg, 0.18 mmol) was added. The reaction mixture, cooled on an ice bath, was stirred for 10 min,

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then dichloroacetic acid (3.9 mg, 0.03 mmol) was added to this reaction mixture. Stirring was continued for 90 min at room temperature (22 °C), followed by the addition of a methanolic solution of oxalic acid (10.8 mg, 0.121 mmol) and further stirring of the mixture for 30 min. Tlc at this time showed complete conversion of the starting material to a new product. The mixture was filtered through a celite pad and the contents were poured over crushed ice. The product was extracted into dichloromethane (15 mL), dried over anhydrous sodium sulfate, filtered and evaporated to yield 18 mg (91%) of product, which was tentatively characterized as 5 by its proton NMR spectrum. This product was used as directly for either deuteration or tritiation. ^{1}H NMR ($CDCl_3$) δ 2.02 and 2.18 (*two s*, 6H, 3H each for two C*H*$_3$), 4.90 (*d*, $J_{2',1'}$=1.5 Hz of *d*, $J_{3',2'}$=2.5 Hz, 1H, ***H-2'***), 5.42 (*d*, $JH_{4',3'}$=3.0 Hz of *d*, $J_{2',3'}$=2.5 Hz, 1H, ***H-3'***), 6.24 (*d*, $J_{3',4'}$=3.0 Hz of *d*, $J_{5',4'}$=7.5 Hz, 1H, ***H-4'***), 7.15 (*d*, $J_{4,5}$=1.0 Hz, 1H, ***H-5***), 7.35 (*d*, $J_{5,4}$=1.0 Hz, 1H, ***H-5***) and 9.70 (*d*, $J_{4',5'}$=7.5 Hz, 1H, ***H-5'***) ppm.

1-α-D-(2,3-Di-O-acetyl-5-[^{2}H]-arabinofuranosyl)-2-nitroimidazole; **[^{2}H]-3.** 5 (8 mg, 0.024 mmol) was dissolved in ethanol (2 mL) and the pH of this solution was balanced at 4.5 by dropwise addition of acetic acid. Sodium cyanoborodeuteride (7 mg, 0.024 mmol) was added to this solution. After stirring for 3h, tlc showed the formation of a product at the same Rf value as 3. The solvent was evaporated and the mixture purified on a preparative tlc plate to yield 4.5 mg (56%) of 3, ^{1}H NMR ($CDCl_3$) δ 2.07 and 2.24 (two *s*, 6H, 3H each for two C*H*$_3$), 3.98 (*d*, $J_{4',5'}$=6.0 Hz, 1H, **H-5'**), 4.56 (*d*, $J_{3',4'}$=3.0 Hz of *d*, $J_{5',4'}$=6.0 Hz, 1H, ***H-4'***), 5.22 (*d*, $J_{4',3'}$=3.0 Hz of *d*, $J_{2',3'}$=1.5 Hz, 1H, ***H-3'***), 5.51 (*d*, $J_{3',2'}$=1.5 Hz of *d*, $J_{1',2'}$=1.5 Hz, 1H, ***H-2'***), 6.71 (*d*, $J_{2',1'}$=1.5 Hz, 1H, ***H-1'***), 7.27 (*d*, $J_{4,5}$=1.0 Hz, 1H, ***H-5***) and 7.41 (*d*, $J_{5,4}$=1.0 Hz, 1H, ***H-4***) ppm; EI for $C_{12}H_{14}DN_3O_8$, calc. 330.2794; found 330.2739 (M^+ 17.3%).

1-α-D-(2,3-Di-O-acetyl-5-[^{3}H]-arabinofuranosyl)-2-nitroimidazole; **[^{3}H]-3.** [^{3}H]-3 was synthesized from 5 (846 μg, 2.59 μmol) in ethanol (100 μL), in a Reactivial ™, at a pre-adjusted pH of 4.5. Ethanol (300 μL) was added to sodium cyanoborotritide (0.86 μmol, 10 mCi) and the contents were transferred to the vial containing the ethanolic solution of 5. The ampoule containing $NaCNBT_3$ was rinsed with additional ethanol (200 μL) and the washings were

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transferred to the reaction mixture. The contents were stirred at room temperature for 3h. This reaction mixture was loaded onto a HPLC C-18 cartridge and eluted using aqueous methanol (50:50 v/v) as eluent at a flow rate of 1.5 mL/min. [^{3}H]-3 appeared at a retention time of 10.6 min. The fractions corresponding to [^{3}H]-3 were collected (2.5 mCi) and diluted with 14.6 mg. of *cold* 3. The solvent (ethanol) was evaporated under a stream of nitrogen and the residue was successively taken up in methanol (500 μL x 3) and evaporated to dryness under a stream of dry N_2 to remove exchangeable tritium. This product was used as such for fluorination.

1-α-D-(5-Fluoro-5-deoxy-2,3-di-O-acetyl-5-[^{3}H]-arabinofuranosyl)-2-nitroimidazole; **[^{3}H]-4.** [^{3}H]-3 was dissolved in anhydrous CH_2Cl_2 (2 mL) on an ice bath. This reaction flask was flushed with argon and diethylaminosulfur trifluoride (13 μL) was added. The solution was stirred at ice temperature for 2 h, then the contents were brought to room temperature. Stirring was continued for a further 30 min. before quenching by the addition of methanol (100 μL). The solvent was evaporated and the impure product was purified on a silica gel column, using chloroform:methanol (100:2.5, v/v), to afford 3.8 mg of pure [^{3}H]-4. This product was deacetylated without further characterization.

1-α-D-(5-Fluoro-5-[^{3}H]-deoxyarabinofuranosyl)-2-nitroimidazole **([^{3}H]-FAZA) [^{3}H]-6.** [^{3}H]-4 was dissolved in a saturated solution of ammonia in methanol (1.5 mL) and the contents were stirred at 5 °C overnight. The solvent was evaporated and the contents purified by HPLC using methanol:water (50:50 v/v) as an eluent at a flow rate of 1.5 mL/min. The elution peak corresponding to [^{3}H]-FAZA was collected and the solvent evaporated to collect chemically pure [^{3}H]-FAZA (1.2 mCi; specific activity 1.42 mCi/mg or 12 GBq/mmol); radiochemical purity as determined by scintillation counting of HPLC eluant fractions was >99 %.

Preliminary Biological Evaluation of [^{3}H]-FAZA; 6:

Biodistribution in tumour-bearing mice. EMT-6 murine mammary tumour cells were implanted subcutaneously into the flanks of recipient Balb/c mice weighing 20-22g (Health Sciences Laboratory Animal Services, U. Alberta). In 14 days, when tumours were 0.5-1 cm in diameter, [^{3}H]-FAZA 6; nominal dose 5 μCi; 0.1 mL in saline) was administered by i.v. injection into the tail

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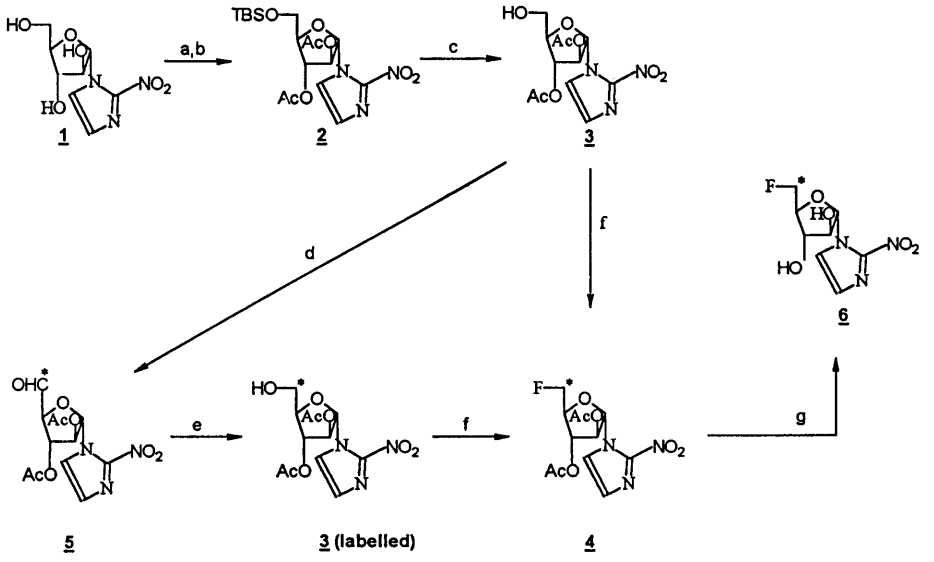

Reagents: a) t-Butyldiphenylsilyl chloride/Pyridine, b) Ac_2O, c) NH_4F, d) Oxidation, e) Reduction ($NaCNBT_3$) f) DAST, g) NH_3/MeOH and *=H/D/T

Scheme: Synthesis of FAZA

vein. Animals were sacrificed at pre-determined intervals by asphyxiation in a carbon dioxide chamber followed by cardiac puncture exsanguination. Tissues were collected, blotted to remove superficial blood, and specimens weighing not more than 250 mg were air-dried for 1 week prior to combustion using a Harvey Ox-300 biological oxidizer. Tritium-containing combustion products from the oxidizer were collected in liquid scintillation fluor (Harvey Instruments) and analyzed by liquid scintillation counting using a Beckman Model 9000 counter. Internal ^3H standards were used to calibrate combustion and counting efficiencies. Biodistribution data are presented as percent of injected dose per g. of tissue (Table 1).

Tracer kinetics in vivo. Tracer kinetics were determined in two male Wistar rats (290-300 g; Health Sciences Laboratory Animal Services, U Alberta). The animals were allowed to recover for 24 h from the jugular catheter implantation surgery prior to the experiment. [^3H]-FAZA (30 μCi in 0.6 ml) was injected as a bolus via the jugular catheter, and blood samples were collected from 1 min to 24 h via the same catheter. Control experiments indicated that cross-contamination between the dose and first blood sample was minimal under these conditions in which the same

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catheter was used for both dosing and sampling. Blood samples were analyzed for tritium content using quantitative combustion and liquid scintillation counting, as described under biodistribution studies (above). Blood radioactivity data are presented in Figure 1. Kinetic data (Table 2) were derived using Winnonlin v 1.1 (Scientific Consulting, Inc.).

Partition Coefficient (P). The *P* value for FAZA was estimated by comparing its retention time on a reverse phase HPLC column to the retention times of AZA, FMISO and IAZA, and interpolating from a plot (Fig. 2) of retention time plotted as a function of 1-octanol : water partition coefficients taken from the literature (21,22).

RESULTS AND DISCUSSION

Biodistribution and tracer kinetics. In the murine EMT-6 tumour model, the radioactive dose was rapidly cleared from the blood, with rapid equilibration of radioactivity among all tissues (Table 1). Although the clearance of radioactive compound from these tissues follows the blood profile with a short lag time, there is a cross-over of levels between blood and tumour within 1h that is indicative of hypoxia dependent binding in this tumor model. Similar patterns of responses have been reported for [^{18}F]-FMISO (28). Accumulation in the liver and kidneys was observed, and was interpreted as indicative of active clearance through these organs as is the case for [^{123}I]-IAZA (28).

Table 1: Percent of Injected Dose per g (%ID/g) in Tissue Following i. v. Injection of ^{3}H-FAZA into Balb/c Mice Bearing Implanted EMT-6 Tumors (n =3, ± S.D.)

Tissue	Time after injection					
	15min	30min	1h	2h	4h	8h
Blood	4.9±2.8	3.5±0.3	0.8±0.02	0.7±0.0	0.4±0.0	0.3±0.1
Liver	10.2±3.6	9.3±0.4	4.0±1.1	2.5±0.2	1.5±0.1	1.3±0.2
Kidney	9.0±3.7	9.1±0.7	3.2±0.9	1.9±0.2	1.04±0.1	0.8±0.2
Tumour	2.4±1.2	3.4±0.4	1.1±0.4	1.0±0.1	1.0±0.7	0.4±0.1
Tumour:Blood Ratio	0.49	0.97	1.38	1.43	2.50	1.33

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The tracer kinetic data of [^{3}H]-FAZA in two rats were best fitted using a two compartment open model. The rapid distribution phase ($t_{1/2\alpha}$ = 4.23 min) was followed by a much slower elimination phase ($t_{1/2\beta}$ = 3.13 h). [^{3}H]-FAZA had a relatively large steady state volume of distribution (V_{ss} = 473.3 mL/h/kg) and a low systemic clearance (CL_{TB} = 1.72 L/kg). These data are comparable to the total radioactivity pharmacokinetics of ^{125}I-IAZA in Sprague Dawley rats (26), the radioiodinated analogue of [^{3}H]-FAZA. The tracer kinetic parameters for both [^{3}H]-FAZA and ^{125}I-IAZA are summarized in Figure 1 and Table 2.

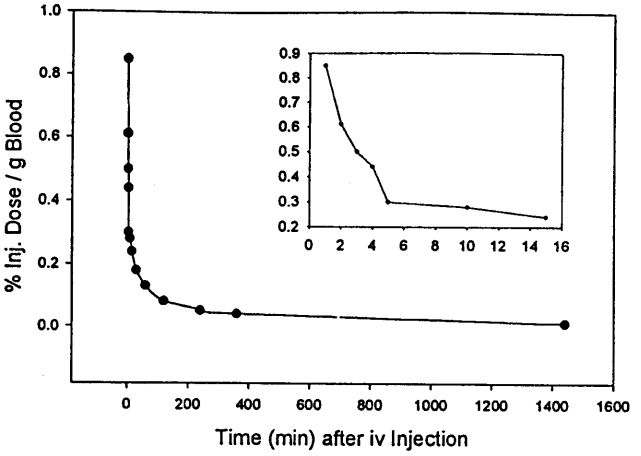

Fig. 1. Tracer pharmacokinetics of [^{3}H]FAZA in rats. Data are the mean from two animals. Data were plotted using SigmaPlot v.3 (Jandel).

Table 2: Total Radioactivity Pharmacokinetic Parameters After i.v. Injection of ^{3}H-FAZA in Sprague Dawley Rats (n=2). Comparative Data for Total Radioactivity after ^{123}I--IAZA i.v. administration are taken from Stypinski, 1997

Pharmacokinetic Parameter*	Total Radioactivity (^{123}I-IAZA, n=3)	Mean Total Radioactivity (^{3}H-FAZA, n=2)
α-phase $t_{1/2}$ (min)	9.2 ± 0.8	4.2
β-phase $t_{1/2}$ (h)	2.5 ± 0.5	3.1
MRT (h)	3.2 ± 0.7	4.1
CL_{TB} (mL/h/kg)	595 ± 179	473
V_{SS} (L/kg)	1.9 ± 0.3	1.7

*MRT is the mean retention time, CL_{TB} is total blood clearance and V_{ss} is the steady state volume of distribution.

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Partition Coefficient. Based on a comparison of the reverse phase HPLC retention times of AZA, IAZA, FAZA and (F-MISO), FAZA is only mildly lipophilic, with an estimated partition coefficient (*P*) of 1.1. This falls well within the range (*P*= 0.1-10) of lipophilicities thought to be compatible with good tissue penetration by diffusion (24, 25). Clinically effective nitroimidazole radiosensitizers with substantially lower partition coefficients are preferred because of their greatly reduced neurotoxicity as compared to compounds like MISO (26, 27).

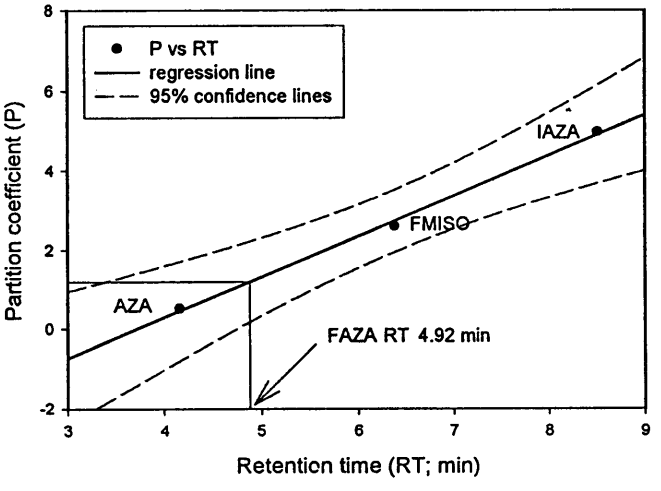

Fig. 2: Correlation between P values and reverse phase HPLC retention times for FAZA and selected 2-nitroimidazoles. The linear regression line was calculated by least squares fit and drawn using SigmaPlot v. 3 (Jandel); $r^{2=}$ 0.99.

Table 3: A Comparison of Retention Times of AZA, IAZA, FMISO and FAZA Using (1:1, v/v; 1 mL/min) on a Waters Radial Pak LC Cartridge (8.0 mm) and Their Partition C

Compound	Retention Time (min.)	P value
AZA	4.16	0.52
IAZA	8.51	4.98
FMISO	6.38	2.60
FAZA	4.92	1.10*

* P value derived from fig. 2

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Chemistry. FAZA 6 was synthesized with the objective of developing a PET radiotracer that would be similar to, but less lipophilic than IAZA and fluoromisonidazole (FMISO). The synthesis of FAZA started from α-AZA, which was selectively protected at the C5 hydroxyl of the arabinose moiety by silation with *tert.*-butyldiphenylsilane. This was followed by *in situ* acylation of the arabinosyl hydroxyls at C-2 and C-3 to give 5-*tert.* butyldiphenylsilyl-2,3-di-O-acetyl α-AZA 2. Desilylation of 2 using ammonium fluoride generated 3 in reasonable yield (74%). This intermediate 3 is the key synthon leading to both unlabelled and isotopically labelled FAZA. The synthesis of *cold* FAZA from 3 was straight forward; 3 was treated with DAST in an inert atmosphere to replace the free OH group at C-5 of the sugar with fluorine, to form the di-acetylated intermediate 4, which was deacetylated with ammonia in methanol to afford FAZA 6 in excellent yield. The introduction of fluorine leads to characteristic fluorine-carbon (24) and fluorine-proton coupling at C-5' ($J_{F,H}$= 47.5 Hz and $J_{F,C}$=175.7 Hz), C-4' ($J_{F,H}$=19.5 Hz and $J_{F,C}$=19.2 Hz) and C-3' ($J_{F,C}$=6.2 Hz). The ^{19}F NMR spectrum of 6 showed a *ddd* resonance at δ -147.72 arising from fluorine coupling with the protons at C-5' and C-4'.

The preparation of isotopically labelled FAZA followed a slightly different route since the objective was selective deuteration/tritiation at C-5'. Initially C-5' oxidation was attempted with Jones reagent, which involves highly acidic reaction conditions. This resulted in cleavage of the nucleosidic bond of 3, and produced a complex mixture of decomposition products. Therefore, 3 was oxidized using Swern's oxidation procedure, which is milder and specifically oxidizes primary hydroxyls to aldehydes, in this case forming 5 in satisfactory yield. This intermediate was characterized by its ^1H NMR spectrum, which showed the presence of an aldehydic proton at δ 8.75 and loss of the C-5' protons (H-5' and H-5"). This intermediate 5 was not purified, but was reduced directly. Sodium cyanoborohydride (deuterated or tritiated depending on the product desired) was found to be the reducing agent of the choice (23), since sodium borohydride also reduced the protective acetyl groups, making the reaction procedure complicated. The reduction was done in ethanol (pH 4.5) at ambient temperature and was complete in about 4 h. The reduced

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product appeared at the same retention time (10.7 min) as 6, on a C-18 column under similar elution conditions. 3 was treated with DAST to yield the corresponding [^{3}H]-4, which on deprotection with NH_3/MeOH, gave [^{3}H]-FAZA. The overall radiochemical yield for tritiation (tritiation, fluorination and deblocking) was 12%.

SUMMARY

The synthesis and labelling of FAZA, a new, moderately hydrophobic nitroimidazole nucleoside are reported. Preliminary biodistribution data are indicative of the potential utility of FAZA for studies of hypoxia in oncology and other pathologies. When labelled with ^{18}F, FAZA may have some advantages over ^{18}FMISO because of its lower lipophilicity; this may produce higher tissue perfusion and more rapid clearance from blood, thereby providing higher hypoxia (tissue):blood differentials at short time intervals after injection, that are more compatible with the relatively short half-life of ^{18}F (109 min).

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