Title: RAPID SYNTHESIS AND USE OF $^{18}$F-FLUOROMISONIDAZOLE AND ANALOGS

Abstract

The present invention involves a rapid synthesis of $^{18}$F-FMISO and analogs thereof. New precursors such as 1-(2'-nitro-1'-imidazolyl)-2-O-acetyl-3-O-tosylpropanol, glycerol-1,3-ditosylate-2-O-acetate, 1-(2'-nitro-1'-imidazolyl)-2,3-O-diacyctyl-4-O-tosylbutanol and threitol, 1,4-di-tosylate-2,3-O-diacyctate, are also important aspects of the invention. A further aspect of the invention is the development of a hydrophilic PET ligand to image tumor hypoxia. Erythroxylosyl analogue of 2-nitroimidazole (Ts-ETNIM) was prepared from a mixture of 2-nitroimidazole, disosylthreitol and cesium carbonate at 60 °C for 1 hr. Ts-ETNIM was isolated at 70 % yield. $^{18}$F-fluoroerythroxylosynitroimidazole (FETNIM) was then prepared from Ts-ETNIM and K$^{18}$F/kryptofix®. The yield for $^{18}$F-FETNIM was 26-30 % (60 min, decay corrected). Results of biodistribution and PET studies indicate that $^{18}$F-FETNIM has the potential to detect tumor hypoxia and is indicated to be less neurotoxic.
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BACKGROUND OF THE INVENTION

10 Field of the Invention

Tumor cells are more sensitive to radiation in the presence of oxygen than in its absence Powers et al., 1963). Even a small percentage of hypoxic cells within a tumor could limit response to radiation (Hall, 1988). Hypoxic radioresistance has been demonstrated in many experimental and animal tumors and hypoxia has been directly demonstrated in certain tumor types in man (Moulder et al., 1984; Peters et al., 1982). The occurrence of hypoxia in human tumors has in most cases been inferred from histologic findings and from evidence of hypoxia in animal tumor studies. In vivo demonstration of hypoxia has required tissue measurements with oxygen electrodes and the invasiveness of this technique has limited its application. Most attempts to increase the radiosensitivity of tumors by administration of chemical radiosensitizers have not been successful (Gatenby, et al., 1988; Kayama et al., 1991; Maor et al., 1981). However, there has been no clinically applicable means of demonstrating tumor hypoxia and it has not been possible to identify the patients who could potentially benefit from radiosensitizing therapy. Potential advantages of neutrons over more conventional radiation include a lesser dependence on oxygenation of the tumor and a lower variability of cell neutron sensitivity around the cell cycle.
3-[^18F]Fluoro-1-(2'-nitro-1'-imidazoyl)-2-propanol ([^18F]-fluoromisonidazole; FMISO) has been used with positron emission tomography (PET) to differentiate metabolically active hypoxic tumor from well-oxygenated metabolically active tumor. ^18F-FMISO is metabolized by intracellular nitroreductases and acts as a competing electron acceptor at low oxygen levels. The ^18F-FMISO is reduced and subsequently incorporated into metabolically active hypoxic cells by covalent bonding to various macromolecules. Recent studies have shown that PET, in view of its ability to monitor cell oxygen content through ^18F-FMISO, has a high potential to predict treatment response. (Koh et al., 1992; Valk et al., 1992; Martin et al., 1989; and Rasey et al., 1989.)

Assessment of tumor hypoxia with ^18F FMISO prior to radiation therapy provides a rational means of selecting patients for treatment with chemical radiosensitizing drugs. Such selection of patients permits more accurate evaluation of radiosensitizing drugs, since their use could be limited to patients with hypoxic tumors, who could potentially benefit. In addition, it is possible to differentiate radiotherapy modalities (neutron versus photon radiotherapy) by correlating [^18F]FMISO results with tumor response.

The synthesis of [^18F]FMISO reported by others showed no resemblance to the present inventive methods. Most studies used a two-part, two-step synthetic procedure, whereas the precursor of the present invention has a nitroimidazole moiety. Although there is a great demand in PET for [^18F]FMISO, a simple and efficient synthesis to produce sufficient radioactivity of [^18F]FMISO is difficult. Prior studies used [^18F]epifluorohydrin to react with 2-nitroimidazole (Hwang et al., 1989; Jerabek et al., 1986; Grierson et al., 1989). The reaction takes a longer time (90 min.)
and provides a lower radiochemical yield. One aspect of
the present invention concerns a more rapid synthesis of
[\textsuperscript{18}F]\textsuperscript{FMISO}.

Three classes of agents have found practical use for
therapy against hypoxic tumors (Hall, 1994). These are
(1) radiosensitizers of hypoxic cells, (2)
chemopotentiation agents and (3) bioreductive drugs.
Radiosensitizers are chemical or pharmacologic agents
that increase the lethal effects of radiation when
administered in conjunction with it. Nitroimidazoles
were reported to potentiate the cytotoxic effects when
combined with other chemotherapeutic agents (e.g.
cisplatin, bleomycin, cyclophosphamide and nitrosourea).

Several clinical trials are in progress combining a
radiosensitizer with alkylating agents. It would
increase the efficiency of chemotherapy if the hypoxic
component could be identified by a labeled tracer prior
to the therapy. Bioreductive drugs are not
radiosensitizers, yet, these drugs are reduced
intracellularly in hypoxic cells to form active cytotoxic
agents. Three drugs are primarily used in clinical
trials. These drugs are mitomycin C, triapazamine
(SR4233) and dual-function nitroheterocyclic compounds
(RB6145).

Two nitroimidazole analogues (misonidazole and
etanidazole) are potent radiosensitizers. In clinical
trials, patients with high hemoglobin levels and cancer
of the pharynx showed a great benefit from the addition
of these analogues (Hall, 1994). However, the dose-
limiting toxicity was found to be peripheral neuropathy
that progressed to central nervous system toxicity if
drug administration was not stopped. This neurotoxicity
prevented the use of the drug at adequate dose levels
throughout multifraction treatments.
Therefore, it is necessary to develop a more hydrophilic agent than misonidazole. This would allow the agent to have shorter half-life in vivo and, be expected to show less neurotoxicity. Another aspect of the present invention is the synthesis and evaluation of a new 2-nitroimidazole analogue which is more hydrophilic than FMISO and misonidazole. The synthetic scheme is shown in FIG. 6.

SUMMARY OF THE INVENTION

The present invention includes a first description of new compounds including: 1-(2′-nitro-1′-imidazolyl)-2-0-acetyl-3-0-tosylpropanol; glycerol-1,3-ditosylate-2-0-acetylate; 1-(2′-nitro-1′-imidazolyl)-2,3-acetyl-4-0-tosylbutanol; and threitol 1,4-di-tosylate-2,3-0-di-acetylate.

The present invention also involves a method for preparing 1-(2′-Nitro-1′-imidazolyl)-2-0-acetyl-3-0-tosylpropanol comprising reacting glycerol-1,3-ditosylate-2-0-acetylate with 2-nitroimidazole. This reaction is preferably in dimethylformamide in combination with cesium carbonate. Preferable conditions for this reaction are at a temperature of 50°C and for a time of about 1 hour. The present invention also involves a method for preparing 18F-fluoromisonidazole comprising reacting 1-(2′-nitro-1′-imidazolyl)-2-propanol with 18F complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane or the like. This reaction is preferably in an acetonitrile solvent. Reaction conditions here are preferably from about 90°C to about 100°C.

Methods of the present invention include: A method for preparing 1-(2′-nitro-1′-imidazolyl)-2,3-acetyl-4-0-
tosylbutanol comprising reacting threitol-1,4-di-
tosylate-2,3-0-acetylate with 2-nitroimidazole. Also part of the present invention is a method for preparing a $^{18}\text{F}$-fluoromisonidazole butanol analogue useful for PET imaging and localization of hypoxic tissue. This method comprises reacting 1-(2'-nitro-1'-imidazoly1)-2,3-acetyl-
4-0-tosylbutanol with $^{18}\text{F}$ complexed with 4,7,13,16,21,24-
hexaaxo-1,10-diazabicyclo[8,8,8]hexacosane or the like. The present invention also comprises a method for preparing $^{131}\text{I}$-iodomisonidazole comprising reacting 1-
(2'-nitro-1'-imidazoly1)-2-acetyl-3-0-tosylpropanol with a salt of $^{131}\text{I}$ such as Na$^{131}\text{I}$.

Another aspect of the present invention is the preparation of a more hydrophilic analogue of misonidazole, $[^{18}\text{F}]$fluoroerythro-2-nitroimidazole. This compound is prepared from a novel precursor, (2'-nitro-
1'-imidazoly1)-2,3-isopropylidene-4-tosylbutanol.

The method for preparing (2'-nitro-1'-imidazoly1)-
2,3-isopropylidene-4-tosylbutanol comprises reacting 1,4-
ditosyl-2,3-isopropylidene-D-threitol with 2-
nitroimidazole, and preferably the reacting is in dimethylformamide, and preferably the reacting is in the presence of cesium carbonate. The reacting is also preferably at about 60°C for about 1 hour.

A further aspect of the invention is a method for preparing $[^{18}\text{F}]$fluoroerythro-2-nitroimidazole comprising reacting (2'-nitro-1'-imidazoly1)-2,3-isopropylidene-4-
tosylbutanol with $^{18}\text{F}$ complexed with 4,7,13,16,21,24-
hexaaxo-1,10-diazabicyclo[8,8,8]hexacosane. In this method, the reacting is preferably in acetonitrile.

An embodiment of the present invention is a method of use of $[^{18}\text{F}]$fluoroerythro-2-nitroimidazole for assessing tumor hypoxia. The method of use preferably
comprises administering to a patient an effective amount of $[^{18}\text{F}]$fluoroerythro-2-nitroimidazole and subjecting the patient to positron emission tomography or single photon emission computed tomography.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 schematically shows the synthesis of $[^{18}\text{F}]$fluoromisonidazole and its analogue.

FIG. 2 schematically shows the synthesis of -(2'-nitro-1'-imidazolyl)-2,3-O-acetyl-4-O-tosyl butanol.

FIG. 3 shows a PET image of a patient with nasopharyngeal cancer (NPC) after receiving 10 mCi each of ($^{18}\text{F}$)FMISO prepared according to the present invention.

FIG. 4 shows a PET image of a second patient with nasopharyngeal cancer (NPC) after receiving 10 mCi each of ($^{18}\text{F}$)FMISO prepared according to the present invention.

FIG. 5 schematically shows the radiosynthesis of ($^{131}\text{I}$)iodomisonidazole.

FIG. 6 schematically shows the synthesis of $[^{18}\text{F}]$FETNIM.

FIG. 7 shows the PET images of tumor bearing rabbits 1 hour post-injection with $[^{18}\text{F}]$FETNIM.

FIG. 8 shows the autoradiogram and photographic studies of mammary tumor bearing rats treated with $[^{18}\text{F}]$FETNIM, $[^{18}\text{F}]$FDG and $[^{18}\text{F}]$FMISO.
FIG. 9 is a histogram showing tumor-to-tissue ratios of [18F]FETNIM in tumor bearing rats.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present precursors for the preparation of [18F]FMISO or its butanol analog are of value clinically and scientifically. The precursor for [18F]FMISO is 1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol. Such a precursor can be radiolabeled in a short time with 18F (t½=109.7 min.), 123I (t½=13.3 hours) or 131I. The first two radioisotopic ligands are useful to detect hypoxic tumor volume by positron emission tomography (PET) and single photon emission computed tomography (SPECT).

[18F]FMISO was prepared as schematically shown in FIG. 1. Glycerol-1,3-ditosylate (compound 2) was obtained by treating glycerol with tosyl chloride according to a previously reported method (Benbouzid et al., 1988). Compound 3 (2-acetyl glycerol-1,3-ditosylate) was prepared by reacting glycerol-1,3-ditosylate with anhydrous acetic anhydride and BF3·etherate (yield 100%). In a mixture of compound 3, 2-nitroimidazole and Cs2CO3 in dry DMF at 50°C, compound 4 [1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol] was prepared. (When this reaction was carried out at 100°C for 16 hr, the undesired compound 5 resulted.) HPLC showed the retention time of compound 4 to be 9.83 minutes (UV 310 nm, C-18 reverse phase column eluted with H2O:MeOH; 0-80% at a flow rate of 1.5 ml/min.

The butanol FMISO analog was analogously prepared.
Compound 1 ([\(^{18}\text{F}\)]fluoromisonidazole) is prepared just prior to use by reacting compound 4 with [\(^{18}\text{F}\)]KRYPTOFIX® 222.

Reagents and conditions for the steps in FIG. 1 and FIG. 2: i) TsCl (2eq), Pyridine, 0°C, 48h (90%); ii) Ac_{2}O (1.5 eq) BF\(_3\)·Et\(_2\)O (in anhydrous ether, 0°C, 30 min (100%), iii) 2-nitroimidazole, (0.9 eq), Cs\(_2\)CO\(_3\) (0.9 eq), DMF, 50°C, 1h (48%); iv) [\(^{18}\text{F}\)]KRYPTOFIX\(^*\), CH\(_3\)CN, 95°C, 10 min., H\(^+\), 90°C, 5 min. (12-18%).

Accomplished syntheses of misonidazole and misonidazole analogue precursors, [\(^{18}\text{F}\)]fluoromisonidazole (FMISO), [\(^{13}\text{I}\)]iodomisonidazole ([\(^{13}\text{I}\)IMISO) and [\(^{18}\text{F}\)]fluoromisonidazole analogues are described in the Examples below.

The synthesis scheme for [\(^{18}\text{F}\)]FETNIM is depicted in FIG. 6. Biodistribution studies of [\(^{18}\text{F}\)]FETNIM were performed at 1, 2 and 4 hours in mammary tumor-bearing rats (10 μCi, iv, N=3/time interval). Autoradiograms were performed at 1 hour postinjection of [\(^{18}\text{F}\)]FETNIM, and a comparison has been made with [\(^{18}\text{F}\)]fluoromisonidazole (FMISO). Intratumoral oxygen tension measurements were performed using the Eppendorf computerized histographic system. Twenty to twenty-five pO\(_2\) measurements along each of 2 to 3 linear tracks were performed at 0.4 mm intervals on each tumor. PET imaging studies of [\(^{18}\text{F}\)]FETNIM were performed in rabbits bearing V2 tumors (N=3, 4 mCi, iv) which were located on the right leg.

Biodistribution of [\(^{18}\text{F}\)]FETNIM at 1, 2 and 4 hours showed tumor/blood count density ratios of 2.29±0.599, 2.41±0.567 and 8.02±2.420; tumor/muscle ratios of 0.66±0.267, 2.11±0.347 and 5.92±2.240, respectively. Bone uptake did not alter significantly. Autoradiograms
indicated that $^{18}$F-PETNIM and $^{18}$F-FMISO could
differentiate hypoxic versus necrotic regions in the
tumor. The tumor oxygen tension, measured by an oxygen
needle probe, was 3.2-6.0 mmHg. PET imaging studies
indicated that the hypoxic tumor could be visualized at 1
hour postinjection.

The data disclosed herein indicate that $^{18}$F-PETNIM
and $^{18}$F-FMISO are selective to hypoxic cells. However,
$^{18}$F-PETNIM appears to be more hydrophilic than $^{18}$F-FMISO
as evidenced from the autoradiogram and chemistry.
Therefore, $^{18}$F-PETNIM has the potential to be used as a
diagnostic tool and as a follow-up for conventional
therapy or chemotherapy of hypoxic tumors. Other useful
applications of this compound would be in the diagnosis
of brain ischemia (acute stroke), nasopharyngeal cancer,
inflammation and cardiac infarction. Additionally,
because of the selectivity and specificity of
$^{18}$F-PETNIM, labeling erythronitroimidazole with a high
dose of $^{131}$I would allow for radionuclide therapy of
hypoxic tumors.

In summary, a simple and efficient synthetic method
to produce sufficient radioactivity of
$^{18}$F-fluoroerythronitroimidazole, a new PET agent for
imaging tumor hypoxia, has been prepared. PET imaging
studies, autoradiograms and biodistribution of this
compound indicate that $^{18}$F-fluoroerythronitroimidazole
is selective to hypoxic cells, chemically stable and more
hydrophilic than misonidazole. It is thus a useful
compound for the evaluation of hypoxic tumors. The
diagnostic information obtained from $^{18}$F-PETNIM would be
important for the follow-up therapy of patients with
hypoxic tumors.
EXAMPLE 1

Experimental Section (Applying to these Examples)

Nuclear magnetic resonance (NMR) spectra (\(^1\)H and \(^13\)C) were recorded at ambient temperature on an IBM-Bruker Model NR/200 AF spectrometer in the Fourier transform mode in CDC\(_3\) with tetramethylsilane as an internal reference. Chemical shifts (d) are reported in parts per million (ppm) and coupling constants (J) in hertz. Mass spectral analyses were conducted at the University of Texas, Health Science Center at Houston, Texas. The mass data was obtained by fast-atom bombardment on a Kratos MS50 instrument. The elemental analyses were conducted at Galbraith Laboratories Inc. (Knoxville, TN). High resolution mass spectroscopy (HRMS) was performed at the Midwest Center for Mass Spectrometry (Lincoln, NE). All chemical reactions were conducted in dry glassware and were protected from atmospheric moisture. Solvents were dried over freshly activated (300°C, 1h) molecular sieves (type 4A). The homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on silica-coated glass plates (silica gel 60 F 254, Merck) with mixtures of CH\(_3\)Cl-MeOH as the eluting solvent. Preparative separations were performed by column chromatography on silica gel (Merck, 230-400 mesh) with mixtures of CHCl\(_3\)-MeOH as eluant.

EXAMPLE 2

Synthesis of glycerol-1,3-ditosylate (Compound 2)

Compound 2 was synthesized according to the previous reported method with modifications (Benouzid et al., 1988). p-Toluenesulfonfyl chloride (10.29 g, 54 mmol) dissolved in dry pyridine was added to a stirred solution of anhydrous glycerol (2.48 g, 27 mmol) in dry pyridine (30 ml at 0°C). the solution was added slowly and left
to react for 44 hours in the refrigerator (0-3°C). The pink mixture was poured over crushed ice and acidified with concentrated HCl. The organic layer was separated and the aqueous layer was washed with methylene chloride (2 x 50 ml). The combined organic extracts were washed successively with 2N HCl (2 x 10 ml) and distilled water (2 x 10 ml) and dried over anhydrous Na₂SO₄. The filtrate was evaporated and the residual crude product was purified by column chromatography on silica gel to afford Compound 2 as an oil: 9.72 g (24.3 mmol) yield 90%. ¹H NMR [Chemical shift (δ), multiplicity, coupling constant (Hz), number of protons, atom]: 7.75 (d; J=8.2 Hz; 4H, Har.); 7.35 (d; J=8.2 Hz; 4H; Har.); 4.1 (bs; 4H; H₁,₃); 3.35 (bs; 1H; H₂); 2.4 (s; 6H; 2CH₃). ¹³C NMR (ppm): 145 (C ar.); 131.7 (C ar.); 129.7 (CH ar.); 127.6 (CH ar.); 69.3 (C₁₃); 66.7 (C₂); 21.2 (CH₃). Satisfactory ¹H and ¹³C NMR data as well as mass spectral data were recorded for this compound. These spectra were consistent with the structures shown in FIG. 1.

EXAMPLE 3

Synthesis of Glycerol-1,3-ditosylate-2-0-acetylate (compound 3)

Glycerol-1,3-ditosylate (5 g, 12.5 mmol) was added dropwise, while stirring 5 minutes at about 0°C, to a solution of acetic anhydride (2 ml, 20 mmol) and BF₃·etherate (1.0 ml) in anhydrous ether (50 ml). The reaction mixture was stirred for 10 minutes, washed successively with 25% sodium acetate solution (10 ml) and water (2 x 15 ml), and then dried over anhydrous sodium sulfate. The solvent was evaporated to yield glycerol-1,3-ditosylate-2-0-acetylate as a white solid (5.48 g, 12.4 mmol). The structure of the product was confirmed by ¹H-NMR ¹³C-NMR, mass spectral data and elemental analysis. These spectra were consistent with the structure of glycerol-1,3-ditosylate-2-0-acetylate shown
in FIG. 1. \(^1\)H NMR: 7.8 (d; J=8.0 Hz; 4H; Har.); 7.3 (d; J=8.0 Hz; 4H; Har.); 5.05 (t; J=4.8 Hz; 1H; H\(_2\)); 4.1 (d; J=4.8 Hz; 4H; H\(_{1,3}\)); 2.4 (s; 6H; 2CH3ar.); 1.8 (s; 3H; CH\(_3\) ac.). \(^{13}\)C NMR (ppm): 169.6 (COCH\(_3\)); 145.3 (C ar.); 132.2 (C ar.); 130 (CH ar.); 127.9 (CHar.); 68.1 (C\(_2\)); 66.5 (C\(_{1,3}\)); 21.6 (CH\(_3\) ar.); 20.5 (OCOCH\(_3\)). mass [FAB, (C\(_{19}H_{22}S_2O_9\))\(^+\)] m/z: 441 (M\(^+\), 5%); 383 (M\(^+\)-OCOCH\(_3\), 4%); 271 (M\(^+\)-tosylate, 100%), 229 (M\(^+\)-1-tosylate -COCH\(_3\); 3%).

Anal. Calc. (C\(_{19}H_{22}S_2O_9\)): C: 51.58, H:4.97 Found: C:51.50 H:4.93.

EXAMPLE 4

Synthesis of 1-(2'-Nitro-1'-imidazolyl)-
2-0-acetyl-3-0-tosylpropanol (Compound 4)

A mixture of glycerol-1,3-ditosylate-2-0-acetylate (0.44 g, 1 mmol), 2-nitroimidazole (0.1 g, 0.9 mmol) and cesium carbonate (0.29 g, 0.9 mmol) in 10 ml of dry DMF, through which argon was bubbled for 10 minutes. The mixture was heated at 50°C for 1 hour. The mixture was then cooled and DMF was carefully removed under reduced pressure. The residue was taken up in ethyl acetate and filtered. Removal of ethyl acetate in vacuo gave a yellow oil which was chromatographed on silica gel eluted with 50-67% ethyl acetate/petroleum ether to afford 1-(2'-Nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol as a white solid (0.17 g, 0.43 mmol), yield 48%. This was dried in vacuo and stored at 0°C. The structure of compound 4 was confirmed by \(^1\)H-NMR, \(^{13}\)C-NMR, and mass spectral data. The spectra were consistent with the structure of 1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol shown in FIG. 1. HPLC showed the retention time of this compound to be 9.83 minutes (UV 310 nm, C-18 reverse phase column, eluted with water/methanol 0-80% at a flow rate of 1.5 ml/min). However, if the reaction was heated at 100°C for 16 hours, a major byproduct (Compound 5) was isolated.
Compound 4: IR (Nujol) (cm⁻¹): 1760; 1610; 1560; 1500; 1480; 1300; 1240; 1200. ¹H NMR: 7.8 (d; 2HJ=8.1 Hz; 2Har), 7.4 (d; 2H; J=8.1 Hz; 2Har), 7.1 (d; 2H; J=2.9; H-imidazolyl), 5.3 (m; 1H; H₂), 4.85 (dd; 1H; J=14.4, 3.5 Hz; H₃a), 4.5 (dd; 1H; J=14.4, 8.4 Hz; H₃b), 4.2 (dd; 2H; J=5.4 Hz; H₃), 2.45 (s; 3H; CH₃tosyl), 1.95 (s; 3H; CH₃ac). ¹³C NMR (50 MHz), δ (ppm): 169.3 (C=O), 145.6 (Car), 132 (Car), 130.1 (Char), 129.9 (C-imidazolyl), 128.3 (CH-imidazolyl), 127.9 (Char), 126.5 (CH₂imidazolyl), 68.8 (C₂), 67.2 (C₁), 49.3 (C₃), 21.6 (CH₂tosyl), 20.3 (CH₃ac). HRMS calc'd for C₁₅H₁₆N₃SO₇ 384.08655, Found 384.0869.

Compound 5: ¹H NMR: 7.15 (2H, H-imidazolyl); 4.9 (S, 1H, H-imidazolyl); 4.8 (m, 1H, H₂); 4.2 (d, 4H, J=3.5 Hz); H₁,₃; 2.1 (S, 3H, CH₃). ¹³C NMR: 171.1 (C=O), 127.7 (CH-imidazolyl), 68.2 (CH-NO₂), 65.4 (C₂), 52.8 (C₁,₃), 21 (CH₃ac). HRMS calc'd for C₈H₁₂N₃O₄ 214.08278, found 214.0827.

EXAMPLE 5
Radiosynthesis of [¹⁸F]FMISO (Compound 1)

[¹⁸F]Fluoride was obtained from the cyclotron facility of the University of Texas M.D. Anderson Cancer Center as produced by proton irradiation of enriched [¹⁸O]water in a small-volume silver target. An aliquot containing 250-500 mCi of ¹⁸F activity was combined with 26 mg of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (KRYPTOFIX® 222) and anhydrous potassium carbonate (4.6 mg), heated under reduced pressure to remove [¹⁸O]water, and dried by azeotropic distillation with acetonitrile (3 x 1.5 ml). The tosyl analogue of misonidazole (compound 4) (5 mg), prepared from 1,3-ditosyl-2-0-acetyl glycerol (compound 3) was dissolved in acetonitrile (1.5 ml), and the KRYPTOFIX® 222-fluoride complex added. The mixture was
then warmed at 95°C for 10 min. After cooling, the reaction mixture was passed through a silica gel Sep-Pak column (Whatman Lab, Clifton, NJ) and eluted with ether (2 x 2.5 ml). The solvent was evaporated and the resulting mixture hydrolyzed with 1N HCl (2 ml) at 90°C for 5 min. The resultant mixture was cooled under N₂ and neutralized with 2N NaOH (0.8 ml) and 1N NaHCO₃ (1 ml). The mixture was then processed by passage through a short alumina C-18 (Sep-Pak) column and a 0.22 μm Millipore filter, followed by 5 ml of 10% ethanol saline.

Comparison with an ³H-NMR spectrum of the unlabeled fluoromisonidazole (FMISO) confirmed the structure. A yield of 20-40 mCi of pure product was isolated (12-18% yield, decay corrected) with end-of-bombardment (EOB) at 60-70 min. HPLC was performed on a C-18 Radial-Pak column, 8 x 100 mm, with pure water, using a flow rate of 1 ml/min. The no-carrier-added product corresponded to the retention time (4.8 min) of the unlabeled fluoromisonidazole under similar conditions. The radiochemical purity was greater than 95%. Under UV detection (310 nm), there are no other impurities. A radio-TLC scanner (Bioscan, Washington, DC) showed the retardation factor of 0.5 for the final product, using a silica gel plate G/UV 254, 5 x 20 cm (Whatman, Anaspec, MI) chloroform: methanol (7:3) eluant, which corresponded to unlabeled FMISO. In addition, KRYPTOFIX® 222 was not visualized (developed in iodine chamber) on the silica gel-coated plate using 0.1% v/v triethylamine in methanol as an eluant. The specific activity of FMISO was determined to be in the range of 1-2 Ci/μmol based upon UV and radioactivity detection of a sample of known mass and radioactivity. The identity and purity of [¹⁸F]FMISO were confirmed by HPLC.
EXAMPLE 6

Half-life Test of $[^{18}F]$fluoromisonidazole

After the $[^{18}F]$fluoromisonidazole was prepared, an aliquot (10 $\mu$Ci) of this radioactive product was counted on a gamma counter at various time intervals (30, 60, 120 min.). To assure the correct isotope ($^{18}F$) is incorporated into the nitroimidazole analogue, the radioactivity of the final product at different time intervals is counted and decay is corrected.

EXAMPLE 7

Sterility and Pyrogenicity

To demonstrate sterility, each batch of product is tested using Bactec culture vials with aerobic and anaerobic materials (NR6 and NR7) (Towson, MD). An aliquot (0.3 ml) of the final solution is incubated in vials for 7 days at 37°C. Sterility is examined every day and assayed by visualizing the cloudiness of the solution. The ten samples tested have consistently been shown to be sterile.

To assure low or absent pyrogenicity, a LAL kit (Whittaker Bioproduct, Walkersville, MD) is used. To prove drug solution does not enhance or inhibit the LAL test, we used three 1:1 dilutions (0.25 ml of drug solution to 0.25 ml of sterile water) to test pyrogenicity of each batch of product. Compared to standard solutions (12.5 $\text{EU} / \text{ml}$, 1.25 $\text{EU} / \text{ml}$ and negative), the tested product solutions (ten samples) proved to be pyrogen-free ($0 \text{EU} / \text{ml}$) and did not interfere with the assay sensitivity.
EXAMPLE 8

Pharmacology and Toxicology Data

Pharmacology

Fluoromisonidazole (FMISO) has been shown to bind selectively to hypoxic cells in vitro and in vivo at radiobiologically significant oxygen levels. In patient studies, the hypoxic elements within a tumor volume was defined as regions with a threshold regional tumor to plasma \(^{18}\text{F}-\text{FMISO}\) ratio of \(\geq1.4\) by 2 or more hours after injection.

The following Table summarizes the results of dosimetry calculation for radiation exposure to several hormonal tissues.

<table>
<thead>
<tr>
<th>Dosimetry:</th>
<th>Normal Tissue Dosimetry for (^{18}\text{F}-\text{FMISO})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>mrad/mCi±SD</td>
</tr>
<tr>
<td>Liver</td>
<td>26.8±9.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>25.8±5.5</td>
</tr>
<tr>
<td>Muscle</td>
<td>16.8±7.9</td>
</tr>
<tr>
<td>Bladder</td>
<td>81.5±47</td>
</tr>
</tbody>
</table>

Muscle is typical of most normal tissues; the total body musculature receives 120 mrem in a routine study for a 70 Kg male given the dose of 0.1 mCi/Kg. The liver and kidneys receive nearly twice the muscle dose, and the bladder wall about five times as much.

The data obtained from this study demonstrate the feasibility of using \(^{18}\text{F}-\text{FMISO}\) to detect hypoxia in human head and neck tumors. Clinical trials will determine whether a relationship exists between \(^{18}\text{F}-\text{FMISO}\) uptake and tumor radiation response.
Toxicity

Nitroimidazoles are chemotherapeutically important as antiprotozoal and antibacterial agents (Hoffer et al.). The most common side effect of MISO toxicity is peripheral sensory neuropathy of the hands and feet, characterized by mild to moderate paresthesias and loss of vibration sensitivity (McNeill et al.). Others have reported convulsions after large single doses, and the development of toxicity, and encephalopathies, following small multiple doses (McNeill et al.). The dose which affects the neurotransmitter system was in the range of 0.76 mg/g (in mice).

The specific activity of $^{18}$F-FMISO prepared is in the range of 1-3Ci/μmol. The physical material will be in the range of 2-6μg per patient. This radiotracer dose is far below the toxicity dose. In addition, animal acute toxicity studies have been performed, rats (n=3) were administered 2mCi/Kg as a single dose. All rats tolerated this dose without toxicity on longer follow-up periods (5 days). This dose is ten-fold higher than the dose thus far studied.

**EXAMPLE 9**

**PET Imaging Studies Using $^{18}$F-FMISO**

PET imaging studies were performed on two patients with nasopharyngeal cancer (NPC). Each patient was positioned supine in the scanner so that the detector rings would span the entire tumor region. A 20-min attenuation scan was performed with a 4 mCi $^{68}$Ge-ring source prior to tracer injection. After each patient had received 10 mCi of $^{18}$F-FMISO, eight consecutive 15-min scans were taken. The total number of counts collected per scan was in the range of 15-30 million. Each patient
was also administered \(^{18}\text{F}\)FDG to ascertain the tumor region.

In the first patient, \(^{18}\text{F}\)FMISO showed tumor uptake in the lymph node region (see FIG. 3) suggesting the tumor metastasized to lymph node is hypoxic. In the second patient, the recurrent primary tumor is hypoxic (see FIG. 4). This study demonstrates that \(^{18}\text{F}\)FMISO is able to detect primary or metastatic hypoxic tumors.

**EXAMPLE 10**

*Synthesis of L-Threitol-1,4-di-p-tosylate-2,3-O-diacetylate*  
*(Compound 6)*

A solution of L-threitol 1,4-di-p-tosylate (1g, 2.3 mmole) was added dropwise with stirring over 5 min. at room temperature, to a solution of acetic anhydride (3 ml, 31 mmole) and BF$_3$etherate (0.2 ml) in anhydrous ether (20 ml). The reaction mixture was stirred for 10 min., washed successively with 25% sodium acetate solution (10 ml) and water (25 ml x 2), and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was distilled under reduced pressure to give the acetylated compound. Yield 94%. The structure of the desired compound was confirmed by \(^1\text{H}\)NMR, \(^{13}\text{C}\)NMR and mass spectral data. \(^1\text{H}\)NMR: 7.75 (d; J=8.3 Hz; 4H; Har.); 7.35 (d; J=8.3 Hz; 4H; Har.); 5.2 (t; J=3.2 Hz; 2H; H2, 3H); 4.2 (t; J=5 Hz; 4H; H1,4); 2.5 (s; 6H; 2CH$_3$ar.); 2.0 (s; 6H; 2CH$_3$ac.). \(^{13}\text{C}\)NMR: 169.5(OOCOCH$_3$); 145.3 (C ar.); 132.2(C ar.); 129.9 (CH ar.); 127.9(Ch ar.); 68.3 (C2,3); 66.4 (Cl,4); 21.6 (CH$_3$ ar.); 20.4 (OOCOCH$_3$). MS [FAB, C22H26S2010] m/z: 515(M$^+$+1, 55); 455 (M$^+$-OOCOCH$_3$, 8%); 342 (M$^+$-tosylate, 100%).
EXAMPLE 11

Synthesis of 1-(2'-Nitro-1'-imidazolyl)-2,3
-0-acetyl-4-0-tosylbutanol
(Compound 8)

2-Nitroimidazole (100 mg, 0.88 mmole) was dissolved in DMF (1 ml), cesium carbonate (289 mg) was added and the reaction stirred for 5 min at room temperature. Threitol 1,4-di-p-tosylate 2,3-diacetate (1.1 eq., 431.5 mg, 0.97 mmole) was added last, and the reaction was stirred at 80°C for 1 hour. The reaction was filtered and the DMF removed in vacuo. The residue was chromatographed on alumina eluted with ethyl acetate/petroleum ether. The structure of resultant 1-(2'-Nitro-1'-imidazolyl)-2,3-O-acetyl-4-O-tosylbutanol, the fluoromisonidazole butanol analog, was confirmed by $^1$H NMR and $^{13}$C NMR. $^1$H NMR: 7.85(d, J=8.2 Hz, 2Har.); 7.4(d, 2H; J=8.2 Hz, 2Har.); 7.15(d, 2H; J=2.9; H-imidazolyl); 5.4 (m, 1H; H2); 5.2 (m, 1H; H3); 4.95(dd; 1H; J=14.5, 3.5 Hz; H4a); 4.5(dd; 1H; J=14.5, 3.5 Hz; H4b) 4.3 (m; 2H; h1); 2.45(s; 3H; CH$_3$tosyl); 1.98(s; 6H; 2CH$_3$ac.). $^{13}$C NMR 169.4 (OOCOCH3); 146(Car.); 132(Car.); 130.1(CHar); 128.3(C-imidazolyl); 127.9(CH-imidazolyl); 127.4(CHar); 126.7(CH-imidazolyl); 69.3(C2,3); 67.1(C1); 49.3(C4); 21.6(CH3tosyl); 20.5(CH3ac.).

EXAMPLE 12

Synthesis of ($^{18}$F)Fluoromisonidazole butanol analogue
(Compound 1B)

This compound can be prepared from 1-(2'-Nitro-1'-imidazolyl)-2,3-O-acetyl-4-O-tosylbutanol, KRYPTOFIX® 222-fluoride complex in acetonitrile as described for ($^{18}$F)FMISO synthesis. The ($^{18}$F) fluoromisonidazole butanol analog is usable for PET tumor imaging.
EXAMPLE 13
Radiosynthesis of $^{131}$I Iodomisonidazole (Compound 1C)

5 mg of tosylmisonidazole was dissolved in 1 mL of acetone. Na$^{131}$I (1 mCi in borate buffer) was added. The reaction mixture was heated at 80°C for 1 hour. Radio-thin layer chromatographic (TLC) analysis showed two peaks with Rf values of 0.01 and 0.72. The first peak was free Na$^{131}$I and the second peak was $^{131}$I-iodomisonidazole. Radio-TLC indicated the desired product has 45% yield. The pure product was obtained after hydrolysis and then passed through a silica gel column and eluted with ether:petroleum ether:triethylamine (1:1:10%). This compound is useful therapeutically.

EXAMPLE 14
Synthesis of (2’-nitro-1’-imidazolyl)-2,3-isopropylidene-4-tosylbutanol (Compound 10)

A mixture of 1,4-ditosyl-2,3-isopropylidene-D-threitol (ET) (0.47 g, 1 mmol), 2-nitroimidazole (0.1 g, 0.9 mmol), and cesium carbonate (0.29 g, 0.9 mmol) in 10 ml of dry dimethylformamide (DMF) was heated at 60°C for 1 h. The reaction was then cooled and DMF was removed under reduced pressure. The residue was taken up in ethyl acetate and filtered. Removal of ethyl acetate in vacuo produced a yellow oil which was chromatographed on silica gel, eluted with petroleum ether/ethyl acetate (gradient form 10% to 70%) to afford a white solid (0.27 g, 0.63 mmol, 70% yield). The structure of the product was determined by $^1$H and $^{13}$C NMR and mass spectral data. HPLC showed the retention times of the title compound to be 12.45 min, and the ET compound to be 14.8 min (UV 254 nm, Brownlee C-18 5µ reverse phase column, 4.6x100 mm eluted with water/methanol, 0-80%, at a flow rate of 1.0 ml/min).
EXAMPLE 15

Radiosynthesis of $[^{18}\text{F}]$fluoroerythro-2-nitroimidazole
(FETNIM) (COMPOUND 9)

5 $[^{18}\text{F}]$Fluoride was produced in the cyclotron facility
(42 MeV, Cyclotron Corp., Berkeley, California) at the
University of Texas M.D. Anderson Cancer Center by proton
irradiation of enriched $^{18}$O-water in a small-volume
titanium target. The target was filled with 2 ml of
$[^{18}\text{O}]$ water. Aliquots containing 400-500 mCi of $^{18}$F
activity after 1 hour beam time (12 $\mu$A current) was
combined with KRYPTOFIX®-2,2,2 (26 mg) and anhydrous
potassium carbonate (4.6 mg), heated under reduced
pressure to remove $[^{18}\text{O}]$ water, and dried by azeotropic
distillation with acetonitrile (3x1.5 mL). The tosyl
analogue of 2-nitroimidazole (Compound 10, 20 mg),
prepared from (Compound 10) was dissolved in acetonitrile
(1.5 mL), added to the KRYPTOFIX®-fluoride complex, and
then warmed at 95°C for 10 min. After cooling, the
reaction mixture was passed through a silica gel Sep-pak
column (Whatman Lab, Clifton, NJ) and eluted with ether
(2 x 2.5 mL). The solvent was evaporated and the
resulting mixture was hydrolyzed with 2 N HCl (1 mL) at
105°C for 10 min. The mixture was cooled under N$_2$ and
neutralized with 2 N NaOH (0.8 mL) and 1 N NaHCO$_3$ (1 mL).
The mixture was passed through a short alumina column, a
C-18 Sep-Pak column, and a 0.22-$\mu$m Millipore filter,
followed by eluting 6 mL of 10% ethanol/saline. A yield
of 70-80 mCi of pure product was isolated (20-30% yield,
decay corrected) with the end of bombardment (EOB) at 70
min. HPLC was performed on a Brownlee (5$\mu$ reverse phase)
column, 4.6x100 mm, with water/methanol, 0-80%, using a
flow rate of 1 mL/min. The no-carrier-added product
corresponded to the retention time (3.11 min) of the
unlabeled FETNIM under similar conditions. The retention
time before hydrolysis of compound 9 was 8.94 min. The
radiochemical purity was greater than 99%. Under the UV
detector (254 nm), there were no other impurities. A radio-TLC scanner (Bioscan, Washington, DC) showed a retardation factor of 0.3 for the final product using a silica gel plate G/UV 254, 5 x 20 cm (Whatman, Anaspec, MI), eluted with chloroform:methanol (7:3), which corresponds to the unlabeled FETNIM. In addition, KRYPTOFIX®-2,2,2 was not visualized (developed in iodine chamber) on the silica gel-coated plate using 0.1% (v/v) triethylamine in methanol as an eluant. The specific activity of $^{18}$F FETNIM ranged from 1 to 2 Ci/µmol based upon UV and radioactivity detection of a sample of known mass and radioactivity.

**EXAMPLE 16**

**PET Imaging Studies Using $^{18}$F FETNIM**

PET imaging was performed with a positron camera (Positron Corporation, Houston, TX). The tomograph has a field-of-view of 42 cm on transverse and 12 cm on coronal planes. The axial resolution on the reconstructed plane is 1.2 cm. Twenty-one transaxial slices separated by 5.2 mm were reconstructed and displayed in standard uptake value (SUV) which measures the ratio of tissue radiotracer uptake to that of the whole body uptake for each scan.

Three male New Zealand white rabbits each weighing 3 kg were inoculated at a single site in the right buttock area with a 0.5 ml suspension of minced VX2 tumor fragments ($10^6$ cells/rabbit). The tumors are maintained through serial animal passage and are available from the Department of Veterinary Medicine (The University of Texas M.D. Anderson Cancer Center, Houston, TX). When the tumor size reached 2 cm by two weeks after inoculation, each rabbit was administered 4 mCi of $^{18}$F FETNIM. A 20 minute attenuation scan was performed with a 4 mCi $^{68}$Ge-ring source prior to tracer injection.
Each rabbit was supine in the scanner so that the
detector rings would span the entire lumbar region.
Eight consecutive 10 minute scans were acquired. There
was a 5 minute wait between scans for data transfer. The
total number of counts collected per scan was in the
range of 3-6 million.

The coronal, transaxial and sagittal views of a PET
image of a tumor-bearing rabbit 1 hour after
administration of \(^{18}\text{F}\)FETNIM is shown in FIG. 7. The
rabbit was scanned from cranial to caudal direction. The
tumor can be visualized at one hour postinjection of
\(^{18}\text{F}\)FETNIM. The SUV value of the tumor was greater than
10.

**EXAMPLE 17**

**Autoradiographic studies of \(^{18}\text{F}\)FETNIM**

in tumor-bearing rats

Female Fischer 344 mammary tumor-bearing rats (n=3)
after receiving \(^{18}\text{F}\)FETNIM (2 mCi, iv) were sacrificed at
1 h. To ascertain the metabolic and hypoxic character of
the tumor, one rat was administered
\(^{18}\text{F}\)fluorodeoxyglucose and another rat was given
\(^{18}\text{F}\)fluoromisonidazole (2 mCi, iv). The rats were
sacrificed at 1 hour postinjection. The rat body was
fixed in a carboxymethyl cellulose (4%) block. The
frozen body in a block was mounted to a cryostat (LKB
2250 cryo-microtome, Ijamsville, MD) and 40 \(\mu\)m coronal
sections were made. The section was freeze dried, then
mounted on a x-ray film (X-Omat AR, Kodak, Rochester, NY)
for 24 hours.

In vivo autoradiographic studies in mammary tumor-
bearing rats indicated that the tumor could be visualized
1 hour postinjection. However, in the center region of
the tumor, there was a lack of uptake of \(^{18}\text{F}\)FETNIM,
suggesting tissue necrosis (FIG. 8). The necrotic region can be verified from the photograph. The autoradiogram of $[^{18}F]$FDG indicated that the tumor was metabolically active. The distribution of $[^{18}F]$FDG in a tumor-bearing rat was different from $[^{18}F]$FETNIM and $[^{18}F]$FMISO. The intestines and colon showed high uptake in $[^{18}F]$FETNIM and $[^{18}F]$FMISO. However, the medullary of the kidney area had higher uptake in the $[^{18}F]$FETNIM specimen.

EXAMPLE 18
Polar Graphic Oxygen Needle Probe Measurements

To confirm hypoxic tumors detected by imaging, intratumoral pO$_2$ measurements were performed using the Eppendorf computerized histographic system. Twenty to twenty-five pO$_2$ measurements along each of two to three linear tracks were performed at 0.4 mm intervals on each tumor (40-75 measurements in total). Tumor pO$_2$ measurements were conducted on 3 tumor-bearing rats and rabbits. Using an on-line computer system, the pO$_2$ measurements of each track were expressed as absolute values relative to the location of the measuring point along the track, and as the relative frequencies within a pO$_2$ histogram between 0 and 100 mmHg with a class width of 2.5 mm.

Intratumoral pO$_2$ measurement of the mammary tumors and VX2 tumors indicated that both tumor oxygen tensions ranged from 3.2 to 6.0 mmHg; the normal muscle oxygen tension was 30-40 mmHg. Both tumors are hypervascular in their viable region and hypovascular in their central region. Both tumor-bearing animal models are suitable for the evaluation of tumor hypoxia.
EXAMPLE 19

In Vivo Biodistribution of [18F]FETNIM

in Tumor-Bearing Rats

Female Fischer 344 rats (250-275 g) (Harlan, Inc., Indianapolis, IN) were inoculated with mammary tumor cells in the lumbar area using a 13762 tumor cell line (s.c. 10^5 cells/rat). After 14 days, a tumor size of 1-2 cm was observed. Three groups of rats (N=3/group) were anesthetized with ketamine (10-15 mg/rat). The [18F]FETNIM reconstituted in 5% ethanol/saline was given to rats (10 μCi/rat, iv) and tissue distribution was conducted at 1h, 2h and 4h intervals. The tissues were excised, weighed and counted for radioactivity. The percent of injected dose per gram of tissue weight was determined.

The tissue distribution of [18F]FETNIM in the tumor-bearing rats is shown in Table 2. Bone had high affinity for ionic fluoride, the bone uptake value did not alter, suggesting the in vivo stability of [18F]FETNIM. The tumor/muscle, tumor/blood and tumor/liver count density ratios showed increases with time (FIG. 9), the tumor-to-blood count density being 8.0±2.42 at 4 hour postinjection of [18F]FETNIM. This increased tumor-to-background ratio suggests that [18F]FETNIM has the potential to detect tumor hypoxia.
**TABLE 2**

Biodistribution of $[^{18}F]$Fluoroerythronitroimidazole in Tumor-Bearing Rats$^1$
(Percent of Injected Dose/Gram Weight; N=3/Time Interval)

<table>
<thead>
<tr>
<th>TIME</th>
<th>1 HOUR</th>
<th>2 HOUR</th>
<th>4 HOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLOOD</td>
<td>0.3493 ± 0.02173</td>
<td>0.1950 ± 0.02828</td>
<td>0.0993 ± 0.01266</td>
</tr>
<tr>
<td>LUNG</td>
<td>0.3577 ± 0.00723</td>
<td>0.2107 ± 0.05713</td>
<td>0.1027 ± 0.02122</td>
</tr>
<tr>
<td>LIVER</td>
<td>0.4530 ± 0.01493</td>
<td>0.3123 ± 0.08225</td>
<td>0.1843 ± 0.02458</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>0.3183 ± 0.02011</td>
<td>0.2013 ± 0.05918</td>
<td>0.0953 ± 0.01401</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.8860 ± 0.00424</td>
<td>0.5145 ± 0.02334</td>
<td>0.4663 ± 0.06866</td>
</tr>
<tr>
<td>BONE</td>
<td>0.2570 ± 0.14001</td>
<td>0.0810 ± 0.00424</td>
<td>0.0753 ± 0.01570</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>1.2600 ± 0.08627</td>
<td>0.2670 ± 0.09036</td>
<td>0.1377 ± 0.01893</td>
</tr>
<tr>
<td>TUMOR</td>
<td>0.7957 ± 0.20358</td>
<td>0.5516 ± 0.15818</td>
<td>0.8113 ± 0.33773</td>
</tr>
<tr>
<td>TUMOR/BLOOD</td>
<td>2.2900 ± 0.59940</td>
<td>2.4100 ± 0.56719</td>
<td>8.0200 ± 2.42000</td>
</tr>
<tr>
<td>TUMOR/MUSCLE</td>
<td>0.6602 ± 0.26659</td>
<td>2.1100 ± 0.34679</td>
<td>5.9200 ± 2.24000</td>
</tr>
<tr>
<td>TUMOR/LIVER</td>
<td>1.7600 ± 0.44151</td>
<td>1.7700 ± 0.25608</td>
<td>4.3300 ± 1.31000</td>
</tr>
</tbody>
</table>

---

1. 13762 cell line was inoculated to rats (s.c. $10^5$ cells/rat). When tumor size reached 1-2cm, each rat was administered 10μCi tracer.
REFERENCES

The following references are incorporated by reference herein in pertinent part for the reasons cited in the above text.


It is understood that equivalents of the following claims involve simple substitution of analogous chemicals and methods well known to those of skill in the art.
CLAIMS:

1. 1-(2'-Nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol.

2. Glycerol-1,3-ditosylate-2-0-acetylate.

3. 1-(2'-Nitro-1'-imidazolyl)-2,3-0-diacytel-4-0-tosylbutanol.

4. Threitol 1,4-di-tosylate-2,3-0-diacytelate.

5. A method for preparing 1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol comprising reacting glycerol-1,3-ditosylate-2-0-acetylate with 2-nitroimidazole.

6. The method of claim 5 where the reacting is in dimethylformamide.

7. The method of claim 5 where the reacting is in the presence of cesium carbonate.

8. The method of claim 5 where the reacting is at about 50°C for about 1 hour.

9. A method for preparing $^{18}$F-fluoromisonidazol comprising reacting 1-(2'-nitro-1'-imidazolyl)-2-
propanol with $^{18}\text{F}$ complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane.

10. The method of claim 9 where the reacting is in acetonitrile.

11. The method of claim 9 where the reacting is at from about 90°C to about 100°C.

12. A method for preparing 1-(2′-nitro-1′-imidazolyl)-2,3-acetyl-4-0-tosylbutanol comprising reacting Threitol-1,4-di-tosylate-2,3-0-acetylrate with 2-nitroimidazole.

13. A method for preparing a $^{18}\text{F}$-fluoromisonidazole butanol analogue comprising reacting 1-(2′-nitro-1′-imidazolyl)-2,3-acetyl-4-0-tosylbutanol with $^{18}\text{F}$ complexed with 4,7,13,16,21,24-hexaoxio-1,10-diazabicyclo(8,8,8)hexacosane.


15. (2′-nitro-1′-imidazolyl)-2,3-isopropylidene-4-tosylbutanol.

17. A method for preparing (2'-nitro-1'-imidazolyl)-2,3-isopropylidene-4-tosylbutanol comprising reacting 1,4-ditosyl-2,3-isopropylidene-D-threitol with 2-nitroimidazole.

18. The method of claim 17 where the reacting is in dimethylformamide.

19. The method of claim 17 wherein the reacting is in the presence of cesium carbonate.

20. The method of claim 17 where the reacting is at about 60°C for about 1 hour.

21. A method for preparing $[^{18}F]$fluoroerythro-2-nitroimidazole comprising reacting (2'-nitro-1'-imidazolyl)-2,3-isopropylidene-4-tosylbutanol with $^{18}F$ complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane.

22. The method of claim 21 wherein the reacting is in acetonitrile.

FIG. 1
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FIG. 5

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\[
\text{2-NIM} + \text{Tso-CH}_2\text{-CH-CH-CH}_2\text{-OTs} \rightarrow \text{ET}
\]

\[
\text{CsCO}_3 \underset{\text{DMF}}{\rightarrow} \text{NO}_2\text{-CH}_2\text{-CH-CH-CH}_2\text{-OTs} \rightarrow \text{[^{18}F]FETNIM}
\]

FIG. 6
FIG. 7
FIG. 9

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D233/91 C07D405/06 C07B59/00 A61K51/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search 10 February 1995

Date of mailing of the international search report 22.02.95

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<tr>
<td>P, X</td>
<td>CHEMICAL ABSTRACTS, vol. 121, no. 1, 4 July 1994, Columbus, Ohio, US; abstract no. 9252h, A. CHERIF ET AL. 'Rapid synthesis of 3-[18 F]fluoro-1-(2'-nitro-1'-imidazolyl)-2-prop anol ([18F]fluoromisonidazole).' page 982 ;column 2 ; see abstract &amp; PHARM. RES., vol.11, no.3, 1994 pages 466 - 469</td>
<td>1, 2, 5, 9</td>
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