Novel Fluorinated Hypoxia-targeted Compounds as Non-invasive Probes for Measuring Tumor-hypoxia by ^{19}F-Magnetic Resonance Spectroscopy (^{19}F-MRS)

MARIA V. PAPADOPOULOU, MING JI and WILLIAM D. BLOOMER

Evanston Northwestern Healthcare, Department of Radiation Medicine Evanston, IL 60201, U.S.A.

Abstract. *Background: ^{19}F-labeled 2-nitroimidazoles bound to hypoxic cells in tumors are known to be useful probes for measuring hypoxia, since they can allow their non-invasive detection to be carried out by ^{19}F nuclear magnetic resonance. Materials and Methods: Seven different multifluorinated nitroimidazole-based compounds have been synthesized to furnish this aim: N,N-bis(m-trifluoromethylbenzyl)-3-(2-nitro-1-imidazolyl)propylamine hydrochloride (Bis-mTFN-1); N,N-bis(p-trifluoro-methylbenzyl)-3-(2-nitro-1-imidazolyl)propylamine hydrochloride (Bis-pTFN-1); N-3,5-di-trifluoro-methylbenzyl, 3-(2-nitro-1-imidazolyl)propylamine hydrochloride (DiCF3); N-(m-trifluoromethyl-benzyl)-3-(2-nitro-1-imidazolyl)-propylamine hydrochloride (mTFN-1); N-(p-trifluoromethylbenzyl)-3-(2-nitro-1-imidazolyl)propylamine hydrochloride (pTFN-1); N-(p-trifluoromethylbenzylcarbonyl)-3-(2-nitro-1-imidazolyl)-propylamine (pTFA-1) and 5,6-dimethyl-4-[3-(2-nitro-1-imidazolyl)propylamino]-2-trifluoromethylpyrimidine hydrochloride (CF3PM). The compounds were studied in V79 cells in vitro, whereas selected compounds were tested for systemic toxicity in BALB/c mice. Results: With the exception of pTFA-1, all compounds were soluble in water or saline. All compounds were stable at room temperature as solids, or at 0-5˚C as aqueous solutions. Very good uptake were obtained in aerobic V79 cells with selected compounds. Thus, intracellular vs. extracellular concentration ratios (Ci/Ce) were increasing with input concentration up to 200. All compounds behaved as hypoxia-selective cytotoxins in V79 cells, in vitro, with selectivity ranging between 2.0 (DiCF3) to 15.5 (CF3PM). No lethality or body weight loss was observed with all tested compounds. Some signs of neurotoxicity were seen with bis-pTFN-1, mTFN-1 and pTFN-1 at the higher tested i.p. doses. No adverse effects were observed with CF3PM at any tested dose. Conclusion: These results suggest that some of the above compounds could be utilized as ^{19}F-MRS probes for measuring tumor-hypoxia, by accumulation and binding into the hypoxic regions of tumors.*

Evidence from clinical investigations strongly suggests that tumor hypoxia is a serious detriment to curative anticancer therapies in at least some human cancer types (1). The identification and quantification of hypoxic cells in the tumors of patients undergoing treatment, therefore, is of considerable importance. Bioreductively activated nitroimidazoles have been used as probes for measuring tumor-hypoxia by immunohistochemistry (2). However, non-invasive techniques for measuring hypoxia in tumors, such as with magnetic resonance spectroscopy (MRS), are highly desirable.

^{19}F-MRS probe molecules are particularly useful in biology because of the relatively high sensitivity for detection (0.83% that of ^{1}H) and low endogenous background. Therefore, appropriately fluorinated 2-nitroimidazoles have been synthesized in the past for the non-invasive detection of hypoxia in human cancers (3-5).

Nitroimidazoles undergo a hypoxia-dependent, one electron reduction catalyzed by cellular reductases, resulting in reactive intermediates that form covalent adducts with cellular components (6, 7). Since the adducts are cleared at a slower rate compared to the parent 2-nitroimidazole, the degree of hypoxia within the tumors can be assessed by measurement of residual (metabolized) drug after "washout" of the original compound. However, only those probes with multiple fluorine substitution or which can be given in relatively high doses (in the order of 0.1 mmol/kg of magnetically equivalent fluorine atoms) are suitable for MRS/magnetic resonance imaging studies (8). Other important design criteria for these probes include their chemical/biological stability (other than due to hypoxic bioreduction), pharmacokinetic/toxicity considerations, such as tumor-to-plasma and brain-to-plasma partition coefficients (which are in turn related to lipophilicity/hydrophilicity), as

Correspondence to: Maria V. Papadopoulou, Ph.D., Evanston Northwestern Healthcare, Department of Radiation Medicine, 2650 Ridge Avenue, Evanston, IL 60201, U.S.A. Tel: (847)570-2262, Fax: (847)570-1878, e-mail: m-papadopoulou@northwestern.edu

Key Words: Fluorinated hypoxia markers, ^{19}F-magnetic resonance spectroscopy, non-invasive probes, nitroimidazoles.

well as normoxic toxicity. In addition to these considerations, signal attenuation due to macromolecular binding and its influence on the stoichiometry of the retention of these compounds is relevant to the quantitation of hypoxia in tumors (8).

Taking into account the above considerations, we have synthesized a small series of fluorinated nitroimidazole-based compounds carrying three or six magnetically equivalent fluorines, as potentially useful hypoxia-markers by ^{19}F-MRS. The compounds were rationally designed to possess hydrophilicity through an amino functionality or an N-heteroaromatic chromophore, and multiple fluorine substitution for increased detection-sensitivity. Furthermore, the attachment of trifluoromethyl groups to aromatic rings offers improved chemical stability. In this work we describe the synthesis, characterization and *in vitro* evaluation of these compounds as hypoxia selective agents. In addition, toxicity evaluation of a subset of compounds was undertaken in BALB/c mice to select the best candidate(s) for further *in vivo* ^{19}F-MRS studies.

Materials and Methods

General Synthetic Procedure of bis-mTFN-1, mTFN-1 bis-pTFN-1, pTFN-1 and DiCF3. The appropriate trifluoromethyl-substituted benzyl bromide (1 eq) was added dropwise (15 min) to a solution of 2-nitro-1-imidazolyl-propylamine (1 eq) and potassium carbonate (9.2 eq) in acetonitrile (12-15 ml) and the reaction mixture was stirred under a nitrogen atmosphere, at room temperature for 48 h. The reaction mixture was then evaporated and re-dissolved in water-chloroform. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was separated by preparative TLC on alumina plates with ethyl acetate: petroleum ether mixture. Mono-alkylated and dialkylated products were obtained in the same reaction. The separated products were dissolved in ethyl acetate-ether and converted to their HCl salts by treating with HCl gas in dry ether (1 M solution).

N,N-bis(m-trifluoromethylbenzyl)-3-(2-nitro-1-imidazolyl)propylamine hydrochloride (Bis-mTFN-1). Separation on alumina plate with 40:60 ethyl acetate: petroleum ether mixture. R_f=0.76 (free amine). The hydrochloride salt was precipitated as a white solid; 187.7 mg (33%). ^{1}H NMR ($CDCl_3$) δ: [free amine] 7.60 (s, 2 H), 7.60-7.40 (m, 6 H), 7.0 (s, 1 H), 6.62 (s, 1 H), 4.33, (t, 2 H), 3.59, (s, 4 H), 2.52, (s, 2 H), 2.02 (m, 2 H), 1.6 (b, 1 H). ^{19}F NMR (CD_3OD) d: 11.5 (ref. TFA). UV-Vis (nm): 322 (NO_2). HRMS/FAB (m/z): Calcd for $C_{22}H_{21}F_6N_4O_2$: 487.42; Found (MeOH): 487.0 (100) $(M+H)^+$. Solubility in water 2.55 mM (~ 1.33 mg/ml)

N-(m-trifluoromethyl-benzyl)-3-(2-nitro-1-imidazolyl)-propylamine hydrochloride (mTFN-1). The free amine of mTFN-1 was separated from the above reaction as the second product; R_f=0.41. The hydrochloride salt was precipitated as a white solid; 59 mg (16%). ^{1}H NMR (CD_3OD) δ: 7.85 (s, 1 H), 7.81-7.62 (m, 3 H), 7.53 (s, 1 H), 7.17 (s, 1 H), 4.58 (t, 2 H), 4.31 (s, 2 H), 3.17 (t, 2 H), 2.30 (m, 2 H). ^{19}F NMR (CD_3OD) δ: 12.8 (ref. TFA). UV-Vis (nm): 322 (NO_2). HRMS/FAB (m/z): Calcd for $C_{14}H_{16}F_3N_4O_2$: 329.30; Found (MeOH): 329.1 (100) $(M+H)^+$. Solubility in water 4 mM (~ 1.46 mg/ml).

N,N-bis(p-trifluoromethyl-benzyl)-3-(2-nitro-1-imidazolyl)propylamine hydrochloride (Bis-pTFN-1). Separation on alumina plate with 45:55 ethyl acetate: petroleum ether mixture. R_f=0.68 (free amine). The hydrochloride salt was precipitated as a white solid; 112 mg (18%). ^{1}H NMR (CD_3OD) d: 7.79 (d, 4 H), 7.69 (d, 4 H), 7.44 (s, 1 H), 7.13 (s, 1 H), 4.50 (s, 4 H), 4.46 (t, 2 H), 3.20 (t, 2 H), 2.42 (m, 2 H). ^{19}F NMR (CD_3OD) δ: 11.3 (ref. TFA). UV-Vis (nm): 320 (NO_2). HRMS/FAB (m/z): Calcd for $C_{22}H_{21}F_6N_4O_2$: 487.42; Found (MeOH): 487.0 (100) $(M+H)^+$. Solubility in water 2.00 mM (~ 1.05 mg/ml).

N-(p-trifluoromethylbenzyl)-3-(2-nitro-1-imidazolyl)propylamine hydrochloride (pTFN-1). The free amine of pTFN-1 was separated from the same as above reaction as the second product; R_f=0.32. The hydrochloride salt was precipitated as a white solid; 132.5 mg (31%). ^{1}H NMR (D_2O) δ: 7.86 (d, 2 H), 7,68 (d, 2 H), 7.51 (s, 1 H), 7.26 (s, 1 H), 4.61 (t, 2 H), 4.39 (s, 2 H), 3.23 (t, 2 H), 2.37 (m, 2 H). ^{19}F NMR (D_2O) δ: 12.37 (ref. TFA). UV-Vis (nm): 320 (NO_2). HRMS/FAB (m/z): Calcd for $C_{14}H_{16}F_3N_4O_2$: 329.30; Found (MeOH): 329.0 (100) $(M+H)^+$. Solubility in water 4 mM (~ 1.46 mg/ml).

N-3,5-di-trifluoromethylbenzyl, 3-(2-nitro-1-imidazolyl)propylamine hydrochloride (DiCF3). The corresponding reaction with 3,5-bis-trifluoromethyl-benzyl bromide provided four products on silica gel TLC (55:45 ethyl acetate : petroleum ether). However, only the 4th product with an R_f of 0.40 was isolated and converted to its hydrochloride salt as above; white solid, 147.2 mg (37%). ^{1}H NMR (CD_3OD) δ: 8.15 (s, 2 H), 8.12 (s, 1 H), 7.55 (s, 1 H), 7.17 (s, 1 H), 4.58 (t, 2 H), 4.40 (s, 2 H), 3.20 (t, 2 H), 2.31 (m, 2 H). ^{19}F NMR (CD_3OD) δ: 11.18 (ref. TFA). UV-Vis (nm): 323 (NO_2). HRMS (m/z): Calcd for $C_{15}H_{14}F_6N_4O_2$: 396.2923; Found (MeOH): 396.10208 (100) $(M)^+$. Solubility in water 7 mM (~ 3 mg/ml).

The synthesis of N-(p-trifluoromethylbenzylcarbonyl)-3-(2-nitro-1-imidazolyl)-propylamine (pTFA-1). 2-Nitro-1-imidazolyl propylamine (193 mg, 1.135 mmol) was dissolved in dichloromethane (3 ml) with 5-fold excess of triethylamine (790 μl) and a dichloromethane solution (2 ml) of *p*-trifluoromethylbenzoyl chloride (244 mg, 1.135 mmol) was added dropwise under an inert atmosphere. Then, the reaction mixture was stirred overnight at room temperature, evaporated and the residue was chromatographed on silica gel with ethyl acetate to give a white solid, 293 mg (75.5%), m.p. 110-112˚C. ^{1}H NMR (CD_3OD) δ: 7.98 (d, 2 H), 7.78 (d, 2 H), 7.58 (s, 1 H), 7.17 (s, 1 H), 4.57 (t, 2 H), 3.50 (t, 2 H), 2.20 (m, 2 H). ^{19}F NMR (CD_3OD) d: 11.23 (ref. TFA). UV-Vis (nm): 320 (NO_2). IR (nujol, cm^{-1}): 3320 (N*H*CO), 1640 (NH*C*O). HRMS (m/z): Calcd for $C_{15}H_{15}F_3N_4O_3$: 356.30; Found (MeOH): 357.1173 (100) $(M+1)^+$. Soluble in DMSO (58.5 mM).

The synthesis of 5,6-dimethyl-4-[3-(2-nitro-1-imidazolyl)propylamino]-2-trifluoro-methylpyrimidine hydrochloride (CF3PM). 2-Nitro-1-imidazolyl propylamine (163 mg, 0.96 mmol) and 4-chloro-5,6-dimethyl-2-trifluoromethyl-1,3-pyrimidine (200 mg, 0.92 mmol) were refluxed in propanol (3 ml) for 14 h. The reaction mixture was cooled down and a salt was precipitated, which was identified as the HCl of 2-nitro-1-imidazolyl propylamine (89 mg). The filtrate was

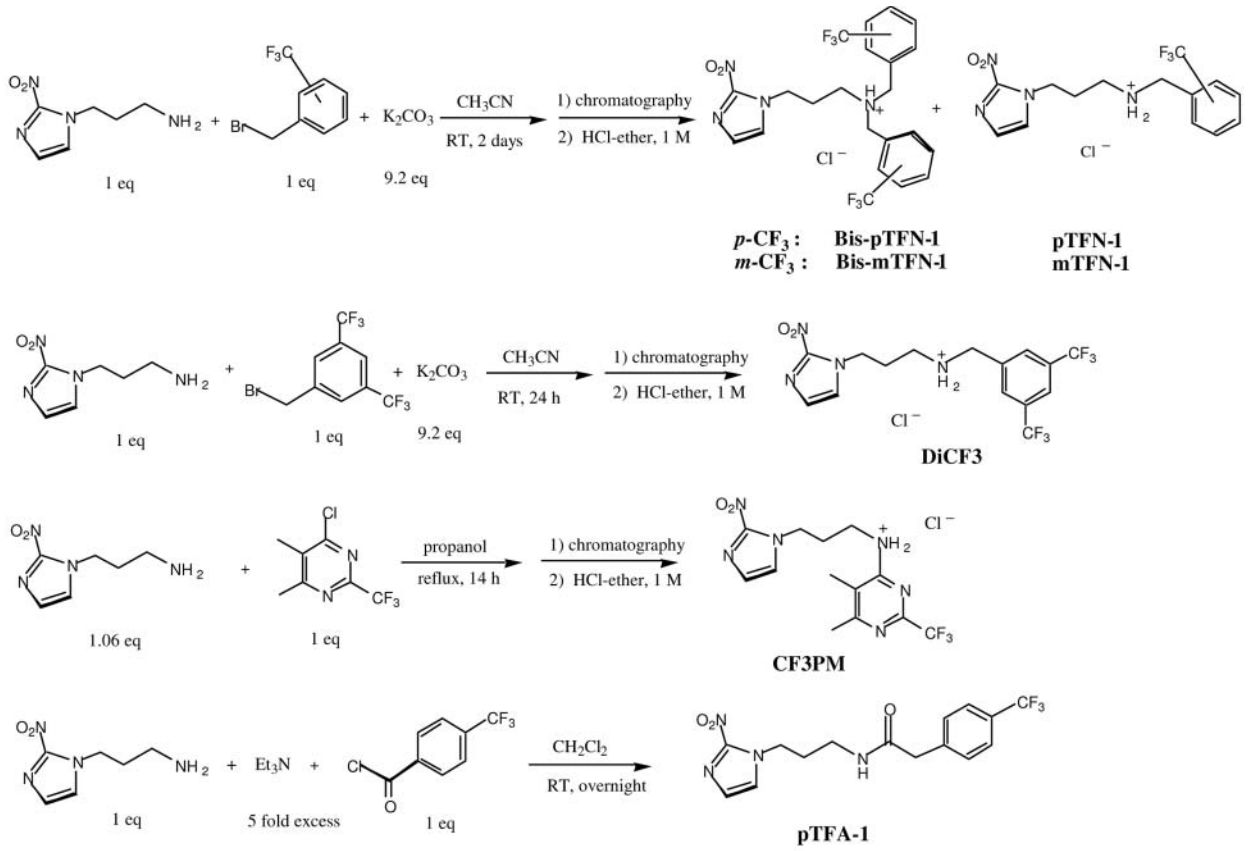

Figure 1. *Synthesis of bis-mTFN-1, mTFN-1, bis-pTFN-1, pTFN-1, DiCF3, CF3PM and pTFA-1.*

evaporated and the residue was re-dissolved in methanol and chromatographed on alumina with 50:50 ethyl acetate : petroleum ether. Unreacted 4-chloro-pyrimidine was isolated as the first band (R_f=0.96) and it was 84 mg (42 % of the initial amount). A yellowish crystalline product was isolated as the second major band (R_f=0.59) which was identified by ^{1}H NMR as the free amine of CF3PM. This free amine was finally converted to its HCl salt by precipitation with HCl gas in dry ether from a dry acetone solution. White salt, 105 mg (30 %). ^{1}H NMR (D_2O; 4.80 ppm) δ: 7.48 (s, 1 H), 7.16 (s, 1 H), 4.56 (t, 2 H), 3.77 (t, 2 H), 2.50 (s, 3 H), 2.31 (m, 2 H), 2.10 (s, 3 H). ^{19}F NMR (D_2O) δ: 4.98 (ref. TFA); 4.75 (in $CDCl_3$ as free amine). UV-Vis (nm): 330 (NO_2). HRMS (m/z): Calcd for $C_{13}H_{16}F_3N_6O_2$: 345.2130; Found (AcCN): 345.1229 (100) $(M+H)^+$. Solubility in water 10 mM (~3.8 mg/ml).

Cells. V79 exponentially growing cells as monolayer cultures in RPMI 1640 medium supplemented with 10 μg/ml insulin and 10% fetal calf serum were trypsinized, centrifuged (750 g) for 5 min, harvested and suspended in 25 ml Erlenmeyer flasks fitted with rubber caps at 5x10^5 cells/ml (5 ml). The flasks were shaken (100 rpm) at 37˚C under aerobic conditions or made hypoxic by gassing with 95% N_2 plus 5% CO_2 humidified gas mixture for 1 h.

Toxicity in vitro. To determine the acute toxicity of each compound, cells were exposed for 1 h under hypoxic or aerobic conditions to various compound concentrations and then processed for clonogenicity (9). Colonies of 50 cells or greater were counted.

Drug uptake measurements. Intracellular (Ci) and extracellular (Ce) drug concentrations were determined as has been described by us before (10). Briefly, 10^7 cells/flask were exposed to various drug concentrations or no drug for 30 min, under aerobic conditions, at 37˚C. Afterwards, the cells were pelleted, lysed with 90 μl distilled water and deproteinized with 0.9 ml acetonitrile. Similarly, a small volume of the supernatant (200 μl) was combined with 9 equal volumes (1.8 ml) of acetonitrile and the remaining supernatant was discarded. After centrifugation and filtration all samples were stored at –70˚C until UV spectroscopic analysis at 330 nm (absorption of the nitro group) for Ci and Ce determination. The efficiency of drug-recovery from cell lysates or supernatant was determined in parallel studies in which lysates and supernatant of untreated cells were spiked with known compound concentrations. Mean intracellular concentration of drug was calculated using a value of 810 fl as the intracellular water content of log-phase cells and taking in account the efficiency of recovery. The recovery from supernatants and lysates was ca. 74% and was used to correct Ce and Ci values.

Toxicity in vivo. A subset of compounds were also tested for systemic toxicity in female BALB/c mice, by injecting various *i.p.* doses in saline and observing the animals for lethality or adverse effects, up to 30 days.

Table I. *Toxicity parameters in V79 cells, in vitro.*

Compound	IC_{50}(A) (μM)	IC_{50}(H) (μM)	HS
Bis-mTFN-1	1464	460	3.2
mTFN-1	2179	414	5.3
Bis-pTFN-1	2406	570	4.2
pTFN-1	3891	396	9.8
DiCF3	1186	588	2.0
CF3PM	1719	109	15.5
pTFA-1	ND	ND	ND

IC_{50} values under hypoxia (H) or air (A) were calculated by multiplying the concentration of each compound and the time needed in hours for the compound to cause 50% inhibition in clonogenicity of V79 cells. Hypoxic selectivity (HS) is the ratio $IC_{50(A)}$ /$IC_{50(H)}$. ND: not determined.

Table II. *Toxicity in BALB/c mice.*

Compound	Highest tested IP dose (mg/kg)	% Median weight change on day 4	Other symptoms
Saline	–	+3.2	
Bis-pTFN-1	100	–2.9	At 50 and 80 mg/kg: sedative effects; At 100 mg/kg: leg tremor and spasms* 20 min post injection.
pTFN-1/ *m-TFN-1*	150	+3.1/+3.2	At 50 and 80 mg/kg: sedative effects; At 120 and 150 mg/kg: leg tremor and spasms 20 min post injection.
CF3PM	150	+3.4	No apparent signs of toxicity

*These symptoms last 30 to 45 min.

Results

The synthesis of all fluorinated compounds is outlined in Figure 1 and described analytically in Materials and Methods. Even though the yields obtained were moderate in most of the cases, the synthetic procedure is straightforward, without any unexpected twists and it can be therefore further optimized. Bis-mTFN-1 and mTFN-I, as well as bis-pTFN-1 and pTFN-1, are separated from the same corresponding reaction and the yield of the mono- or bis-substituted derivative depends on the reaction conditions. All compounds were purified chromatographically and characterized with ^{1}H NMR, ^{19}F NMR, UV-Vis and HRMS/FAB spectroscopy. All compounds demonstrated a single peak in their ^{19}F NMR spectra and therefore, they can be used as ^{19}F MRS probes.

All compounds but pTFA-1 were evaluated for their ability to be metabolized under hypoxic conditions in V79 cells. The results are summarized in Table I and representative graphs are presented in Figure 2. All tested compounds are more toxic under hypoxic rather than aerobic conditions with selectivity indexes (SI) ranging from 2.0 to 15.5. CF3PM demonstrated the greatest hypoxic selectivity and DiCF3 the lowest (Table I). These data suggest that the tested compounds are preferably metabolized under hypoxic conditions and, by binding to macromolecules, can be accumulated in the hypoxic tumor tissues. Thus, some of these compounds, depending on their *in vivo* toxicity and pharmacological profile, can be potentially used as hypoxia markers with ^{19}F MRS.

Selected compounds were tested for intracellular uptake under aerobic conditions in V79 cells. Figure 3 demonstrates such an example with bis-mTFN-1. In general, a very good uptake was observed and the obtained intracellular concentrations were ca. 100 fold greater than the input concentrations. In addition, intracellular peak concentrations (Ci) were ca. 200 fold greater than the corresponding extracellular concentrations (Ce) (Figure 3B).

Based on the *in vitro* selectivity data, four representative compounds, Bis-pTFN-1, pTFN-1, mTFN-1 and CF3PM, were evaluated for *in vivo* toxicity in BALB/c mice by injecting various single *i.p.* doses of each compound in saline (Table II). No lethality was observed with all compounds and at all tested levels. In addition, no weight loss was observed with pTFN-1, mTFN-1 or CF3PM at all tested doses. A minimal 2.9% loss in the median weight was observed with bis-pTFN-1 at the highest tested dose of 100 mg/kg, on day 4 post injection. At 50 and 80 mg/kg, bis-pTFN-1 caused sedative effects, whereas at 100 mg/kg, leg tremor and spasms were observed 20 min post injection. The latter symptoms lasted 30 to 45 min. pTFN-1 or mTFN-1 also demonstrated sedative effects in BALB/c mice at the lower tested doses of 50 and 80 mg/kg, whereas at 120 and 150 mg/kg leg tremor and spasms were observed 20 min post injection. CF3PM was well tolerated up to 150 mg/kg without apparent signs of neurotoxicity, presumably because it does not cross the blood brain barrier (Table II).

From the above *in vitro* and *in vivo* evaluation, mTFN-1 and CF3PM were selected as the best candidates for their *in vivo* evaluation as ^{19}F-MRS probes for measuring hypoxia in tumors. The results of these studies are discussed in the accompanying paper.

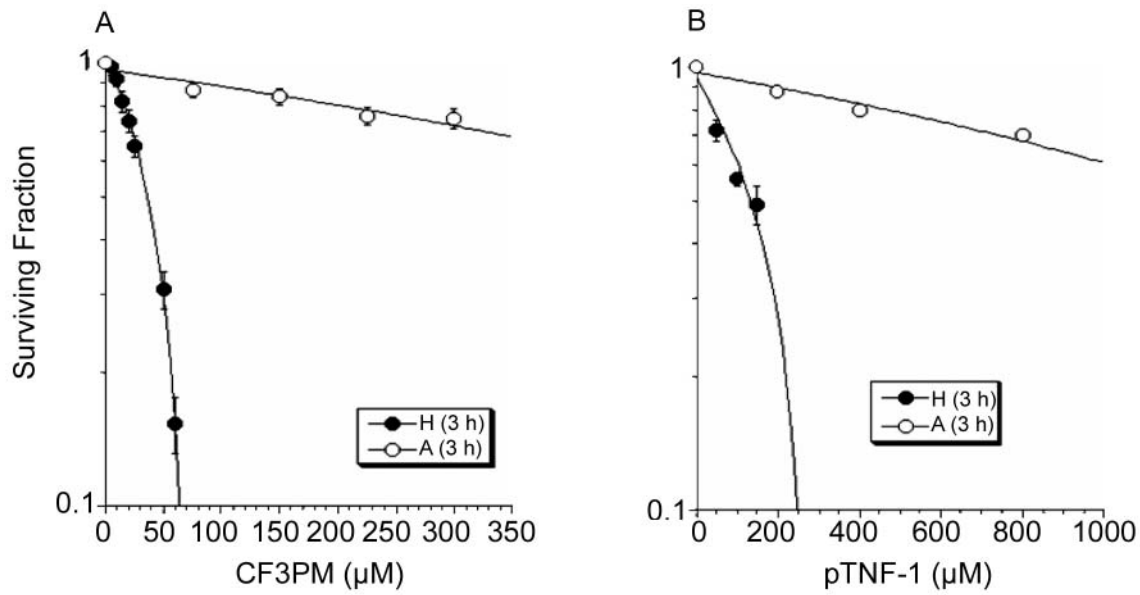

Figure 2. *Concentration dependent cytotoxicity of CF3PM (A) and pTFN-1 (B) in V79 cells, exposed for 3 h (37˚C) to each compound under hypoxic (solid circles) or aerobic conditions (open circles).*

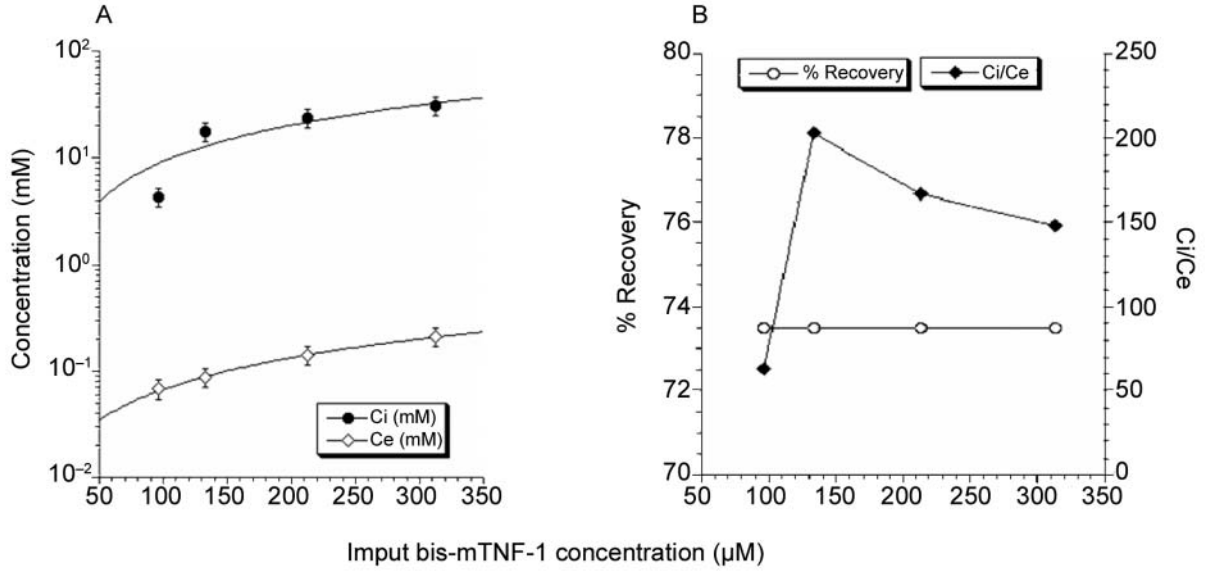

Figure 3. *Cellular uptake of bis-mTFN-1 by aerobic V79 cells. Intracellular (Ci) and extracellular (Ce) concentrations (A), as well as uptake factors (Ci/Ce) and recovery (B) were plotted versus bis-mTFN-1 input concentration.*

Discussion

Hypoxia occurs in a vast majority of rodent and human solid tumors as the result of the highly disorganized and inadequate tumor vasculature, which leads to an impaired oxygen delivery (1). A non-invasive detection of tumor hypoxia by ^{19}F-MRS could find important applications in selecting cancer patients for various therapies, including antiangiogenic, antivascular, hypoxia-targeted gene therapy or therapy with bioreductive prodrugs, and could predict the treatment outcome following radio/chemotherapy. However, hypoxia is also associated with several other disorders (*e.g.,* ischemic stroke, ischemic heart disease, arthritis), in the detection of which a ^{19}F-MRS probe could be envisaged.

The only ^{19}F-MRS probe for non-invasive detection of tumor-hypoxia in clinical trials is the compound SR-4554 (3). SR-4554 is a 2-nitroimidazolyl acetamide with 3 magnetically equivalent fluorine atoms in the side acetamidic moiety, attached to the nitroimidazole ring. As an acetamide, SR-4554 demonstrates limited solubility. In addition, it is lipophilic enough to cross the blood brain barrier, since it can be detected in the mouse brain at 1.08 h post administration (11), and therefore it may cause chronic toxicity.

We have synthesized several multifluorinated 2-nitroimidazole amine derivatives, which contain one or two magnetically equivalent trifluoromethyl groups attached to an aromatic ring. This attachment offers stability, necessary for their detection sensitivity, whereas the amino functionality allows their conversion to hydrochloride salts and guarantees hydrophilicity and hence reduced systemic toxicity. Indeed, all the amino-compounds are quite soluble in saline (1.25-3.5 mg/ml). Despite their hydrophilicity, a very good uptake in cells was observed (Figure 3).

All tested compounds target hypoxia *in vitro* since metabolism was observed only under hypoxic conditions in V79 cells. It is well established that nitroimidazoles, upon reductive metabolism are accumulated in the hypoxic cells by covalent binding to various macromolecules (6, 7). We have also shown previously by ^{19}F-NMR efflux studies that another multifluorinated hypoxia-probe, NLTQ-1, is accumulated in hypoxic V79 cells by binding to cellular macromolecules (4).

For a non-invasive probe of tumor hypoxia by ^{19}F-MRS detection, minimal or manageable toxicity is a very important factor besides sensitivity. We have proved that four of our new compounds demonstrate a relatively safe toxicity profile (Table II). CF3PM appears to be the most promising compound as a hypoxia probe by ^{19}F MRS because it is the most hydrophilic of all derivatives (solubility in saline 3.5 mg/ml), it demonstrates the highest hypoxic selectivity (Table I) and no apparent *in vivo* toxicity up to the tested dose of 150 mg/ml (Table II). This dose represents 1.18 mmol/kg of magnetically equivalent fluorine atoms, namely 12-fold the amount necessary for detection by ^{19}F-MRS.

In the accompanying paper we further discuss the utility of selected derivatives as hypoxia probes by ^{19}F-MRS *in vivo*.

Acknowledgements

The authors thank Dr. Howard Rosenzweig for reviewing this manuscript as well as for his intellectual input. This work was presented in part at the 94th Annual AACR Meeting, Washington, DC, 2003.

References

1 Bussink J, Kaanders JHAM and van der Kogel AJ: Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. Radioth Oncol *67*: 3-15, 2003.

2 Hodkiss RJ: Use of 2-nitroimidazoles as bioreductive markers for tumor hypoxia. Anticancer Drug Des *13*: 687-702, 1998.

3 Seddon BM, Payne GS, Simmons L, Ruddle R, Grimshaw R, Tan S, Turner A, Raynaud F, Halbert G, Leach MO, Judson I and Workman P: A phase I study of SR-4554 *via* intravenous administration for non-invasive investigation of tumor hypoxia by magnetic resonance spectroscopy in patients with malignancy. Clin Cancer Res *9*: 5101-5112, 2003.

4 Papadopoulou MV, Pouremad R, Rao MK, Ji M and Bloomer W: *In vitro* evaluation of 4-[3-(2-nitro-1-imidazolyl)-propylamino]-7-trifluoromethyl-quinoline hydrochloride (NLTQ-1), a new bioreductive agent as a hypoxia marker by ^{19}F-Magnetic Resonance Spectroscopy (MRS). In Vivo *15*: 365-372, 2001.

5 Aboagye E, Lewis AD, Graham MA, Tracy M, Kelson AB, Ryan K and Workman P: Pharmacokinetics, bioavailability and biodistribution in mice of a rationally designed fluorinated 2-nitroimidazole probe SR-4554 for the detection of hypoxia by magnetic resonance spectroscopy. Anti-Cancer Drug Des *11*: 231-242, 1996.

6 Urtasun RC, Koch CJ, Franko AJ, Raleigh JA and Chapman JD: A novel technique for measuring human pO_2 at the cellular level. Br J Cancer *54*: 453-457, 1986.

7 Aboagye E, Lewis AD, Johnson A, Workman P, Tracy M and Huxham IM: The novel fluorinated 2-nitroimidazole hypoxia probe SR-4554: reductive metabolism and semi-quantitative localization in human ovarian cancer multicellular spheroids as measured by electron energy loss spectroscopic analysis. Br J Cancer *72*: 312-318, 1995.

8 Workman P, Maxwell RJ and Griffiths JR: Non-invasive MRS in new anticancer drug development. NMR Biomed *5*: 270-272, 1992.

9 Papadopoulou M, Ji M, Rao MK and Bloomer WD: 4-[3-(2-Nitro-1-imidazolyl)-propylamino]-7-chloroquinoline hydrochloride (NLCQ-1), a novel bioreductive compound as a hypoxia-selective cytotoxin. Oncol Res *12*: 185-192, 2000.

10 Papadopoulou MV, Ji M, Rao MK and Bloomer WD: 9-[3-(2-Nitro-1-imidazolyl)-propylamino]-cyclopenteno[b]quinoline hydrochloride (NLCPQ-1). A novel DNA-affinic bioreductive agent as cytotoxin and radiosensitizer. Oncol Res *8*: 425-434, 1996.

11 Aboagye E, Maxwell RJ, Kelson AB, Tracy M, Lewis AD, Graham MA, Horsman MR, Griffiths JR and Workman P: Preclinical evaluation of the fluorinated 2-nitroimidazole N-(2-hydroxy-3,3,3,-trifluoropropyl)-2-)-2-nitro-1-imidazolyl)acetamide (SR-4554) as a probe for the measurement of tumor hypoxia. Cancer Res *57*: 3314-3318, 1997.

Received May 29, 2006
Accepted June 6, 2006