An approach to the evaluation of the activity of the DNA repair enzyme O^6-methylguanine-DNA-methyl-transferase in tumor tissue in vivo: syntheses of 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine

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Abstract

The resistance of tumor cells to the cytostatic activity of methylating and chloroethylating anticancer drugs is determined by the level of expression of the DNA repair protein O^6-methylguanine-DNA-methyl-transferase (MGMT). The synthesis of labelled 6-benzyloxy-9H-purin-2-ylamine derivatives should hence allow a quantification of the MGMT status of tumor and non-target tissue in vivo. 6-benzyloxy-9-(2-fluoroethyl)-9H-purin-2-yl-amine and 6-benzyloxy-7-(2-fluoroethyl)-7H-purin-2-yl-amine were synthesized and evaluated in vitro, both showing an affinity of 1.8 µM. 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine were synthesized by alkylation of 6-benzyloxy-9H-purin-2-ylamine with 1-[^{18}F]fluoro-2-tosylethane in optimized yields of 41% and 20%, respectively. Biodistribution studies were performed in nude mice, carrying mex + (MGMT expressing) and mex− tumors. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: MGMT; Alkyltransferase; 6-benzyloxy-9H-purin-2-ylamine

1. Introduction

The resistance of tumor cells to the cytostatic activity of methylating and chloroethylating anticancer drugs is determined by the level of the DNA repair protein O^6-methylguanine-DNA-methyl-transferase (MGMT) (for review see Pegg, 1990). The antineoplastic drugs BCNU (Carmustin), CCNU (Lomustin), MeCCNU (Elmustin), and ACNU (Nimustin) are genotoxic through their reaction with nuclear DNA. The main critical cellular target of the agents is the O^6-position of guanine, which is chloroethylated. The resulting O^6-chloroethylguanine is not stable, leading to intramolecular rearrangement and finally to the formation of interstrand guanine-cytosine crosslinks. The cytostatics procarbazine, dacarbazine, temozolomide and streptozotocin methylate the O^6-position of guanine, giving rise to GC→AT transition mutations (Eadie et al., 1984), and via the mediation of mismatched repair, to reproductive cell death and apoptosis (Hickman and Samson, 1999; Ochs and Kaina, 2000).

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The repair protein MGMT binds rapidly to both O^6-methylguanine and O^6-chloroethylguanine in the DNA and transfers the methyl or chloroethyl moiety to its own cysteine acceptor site. Because of the irreversible nature of the alkyl group transfer, the enzyme remains inactivated (Pegg, 1990; Kaina et al., 1991). Thus, the amount of MGMT per cell is a direct measure of the cell's capability to repair O^6-guanine alkylation damage in DNA.

Expression of MGMT varies greatly with the type of tissue, with colorectal and lung tissues being among the highest, the nervous system among the lowest. Some types of tumor tissues display no detectable MGMT activity (Preuss et al., 1995, 1996a; Hengstler et al., 1999). Especially those tumors are expected to respond to a therapy with the above-mentioned cytostatic drugs. (Preuss et al., 1996b). It would therefore be valuable to develop a method which permits determination of the MGMT status of a given tissue in vivo.

Previously, it was reported that 6-benzyloxy-9H-purin-2-ylamine (2) is a potent inhibitor of MGMT, successfully depleting the amount of MGMT in a cell, while being non-toxic at the level of biologically effective doses (Reinhard et al., 2001). The effective dose required to produce 50% inactivation in cells upon incubation for 4 h (ED_{50}, in μM) was 0.05. Alteration of the 9-position of guanine had little effect on the affinity to MGMT, provided that the group is not negatively charged (Moschel et al., 1992) (the ED_{50} for 2-amino-6-benzyloxy-9-cyanomethylpurine was 0.8 μM). The synthesis of a labelled 9-substituted 6-benzyloxy-9H-purin-2-ylamine should hence allow an in vivo quantification of the MGMT status of tumor and non-target tissue, thereby assisting in designing an individual, patient-oriented chemotherapy. The prerequisites required would be the synthesis of a ^{18}F-labelled 9- or 7-substituted compound, whose ED_{50} would be comparable to that of 6-benzyloxy-9H-purin-2-ylamine, and synthesis of the analogous ^{19}F-compound for further toxicological evaluation.

In the present work, the compounds 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4) were selected for this kind of study, because the [^{18}F]fluoroethyl moiety is easy to introduce in the 9- and

7-position of 6-benzyloxy-9H-purin-2-ylamine. Fig. 1 shows the 9- and 7-[^{18}F]fluoroethyl substituted 6-benzyloxy-9H-purin-2-ylamine. For the evaluation of the affinity of the tracers to MGMT, the respective ^{19}F-compounds were prepared via alkylation of O^6-benzyl-guanine and 2-bromo-1-fluoroethane.

2. Materials and methods

2.1. Materials

Unlabelled reagents were purchased from Merck (Darmstadt, Germany) and Lancaster (Mühlheim, Germany) and were used without further purification. Solvents were HPLC grade. HPLC was performed with an HPLC system from Sykam (Sykam Pump Model S1121, Gilching, Germany). UV detection was obtained using the UV detector Linear, UVIS 200 (254 nm) (Linear Instruments, San Jose, USA). Radioactivity detection was performed with a scintillation detector (MED Isomed 110, Nuklear-Medizintechnik Dresden GmbH, Germany). NMR spectra were recorded using a Bruker 200 MHz-FT-NMR spectrometer AC200 (Karlsruhe, Germany). Chemical shifts are quoted in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Elemental analyses were performed with an EL2 system (Elementar vario, Hannau, Germany). MS spectra were obtained on a MAT90 spectrometer (Finnigan, Bremen, Germany). Melting points were determined with an Electrothermal 9100 apparatus (Essex, UK) and are uncorrected. Reaction yields were determined using radio thin layer chromatography (plates by Merck, Darmstadt, Germany, Silicagel $60F_{254}$), and an Instant Imager (Packard Canberra, Dreieich, Germany) and are expressed as percent of radioactivity of (3) and (4) in relation to the total activity on the TLC plate. These data were confirmed via radio HPLC.

2.2. ED_{50} determination in vitro

ED_{50} doses of the derivatives 6-benzyloxy-9-(2-[^{18}F]-fluoroethyl)-9H-purin-2-yl-amine and 6-benzyloxy-7-(2-[^{18}F]-fluoroethyl-7H-purin-2-yl-amine were determined
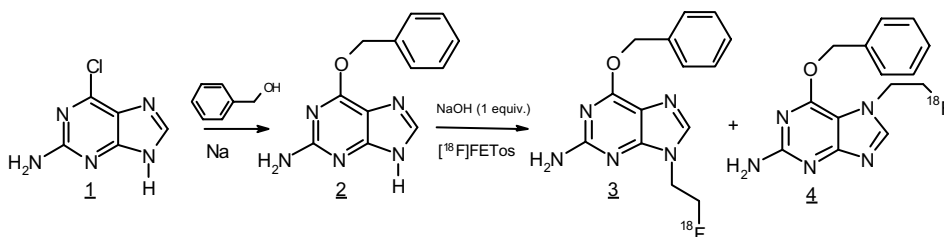

Fig. 1. ^{18}F-Fluoroalkylation of (2) to yield (3) and (4).

by incubation of HeLa S3 cells for a period of 4 h and referred to 6-benzyloxy-9H-purin-2-ylamine (O^6-BeG) as a reference compound 2 as previously described (Reinhard et al., 2001). HeLa S3 cells are MGMT proficient expressing MGMT at a level of 750 fmol/mg protein (Preuss et al., 1996b). Cells were cultivated in Dulbecco's MEM containing 5% fetal bovine serum. Data were obtained from plots of the amount of MGMT activity remaining upon incubation with different concentrations of the inhibitor versus the concentration of the test compound over a range of 0.1-200 μM. Testing of inhibition of MGMT activity by the drugs in vitro was performed using HeLa S3 cell extracts, with 150 μg cell extract protein per assay. Cell extracts were prepared as described (Reinhard et al., 2001) using a buffer containing 20 mM Tris-HCl, pH 8.5, 1 mM EDTA, 1 mM β-mercaptoethanol, 5% glycerol and a cocktail of protease inhibitors (10 μg/ml aprotinin, 10 μM bestatin, 10 μM leupeptin, 1 μM pepstatin and 0.1 μM PMSF). The sonication product was centrifuged to remove debris and the supernatant snap frozen in liquid nitrogen and stored in batches at −80°C. The extracts were incubated with the inhibitor for 45 min at 37°C. MGMT activity was determined essentially as previously described (Preuss et al., 1996b).

2.3. Biodistribution studies in mice

The animal experiments were carried out in compliance with the German animal experimentation laws. About 4 MBq of the respective isomers 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4) were injected intraperitoneal in 10 male nude mice, respectively. The mice weighed between 30 and 40 g, the tumors weighed between 0.2 and 0.7 g. The MGMT expressing tumors (designated as mex +) were grown as xenografts caused by the injection of 2×10^6 HeLa S3 cells. For raising MGMT deficient (mex–) tumors, 2×10^6 HeLa MR cells were injected. HeLa MR cells do not display detectable MGMT activity (Preuss et al., 1996b). The mice carried both tumors on the back, the mex + one on the left-hand side, the mex− one the right-hand side. For treatment with 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4) two animals were sacrificed at 10, 20, 30 and 60 min post-injection (p.i.), and in the case of 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) two animals were sacrificed at 30, 60, 90 and 120 min p.i. (plus two animals as control for each compound). The biodistribution of radioactivity was measured ex vivo for liver, kidney, lung and spleen and for both tumors, mex + and mex−. To this end, tumors and organs were removed immediately after sacrificing the animals and the radioactivity determined by PET analysis.

2.4. Statistical analysis

Due to the relatively small sample size, a statistical comparison of the data for each time point was not appropriate because the β-error would increase considerably. Therefore, the kinetic uptake data of mex + and mex− were analyzed using the entire time course and a paired *t*-test as well as the more robust Wilcoxon test for comparison between mex + and mex−. In order to assess the kinetic uptake correlation (as an indirect inverse parameter of the mex + /mex− difference) the correlation coefficient was calculated according to Pearson, assuming that a missing correlation would indicate a significant difference of the uptake in the mex + and mex− (Sachs, 1992).

2.5. Syntheses

2.5.1. 6-Benzyloxy-9H-purin-2-ylamine (2)

Sodium (3 eq.) (615 mg, 26.8 mmol) was dissolved in benzyl alcohol (37.5 ml) and 1 eq. 2-amino-6-chloropurine (1) (1.5 g, 8.9 mmol) was added. The mixture was stirred at 65°C for 16 h. After cooling to room temperature, 2 eq. sodium hydrogen phosphate were added. The reaction mixture was extracted with 2 N NaOH (20 ml). After adjusting pH to 7 with acetic acid, a white precipitate was collected which proved to be pure (TLC controlled) 6-benzyloxy-9H-purin-2-ylamine (500 mg, 25%).

- TLC conditions: chloroform/methanol 10 : 1; 6-benzyloxy-9H-purin-2-ylamine R_f of 0.32;
- mp: 197–199°C (Bowles et al., 1963), found: 200–201°C;
- ^{1}H NMR (DMSO) δ (ppm): 12.46 (s, 1H, NH-9), 7.9 (s, 1H, CH-8), 7.6–7.4 (m, 5H, ar-H), 6.2 (s, 2H, NH$_2$), 5.5 (s, 2H, CH$_2$-Bzl);
- MS (FD): m/z (% rel. int.) 242.25 (100, [M +]).

2.5.2. 6-Benzyloxy-9-(2-[^{19}F]fluoroethyl)-9H-purin-2-yl-amine and 6-benzyloxy-7-(2-[^{19}F]fluoroethyl)-7H-purin-2-yl-amine

6-benzyloxy-9H-purin-2-ylamine (2) [0.9 mmol (200 mg)] were suspended in 2.5 ml dry ethanol under argon and deprotonated by addition of 0.9 mmol (835 μl) 1 M ethanolic sodium ethanolate. After 20 min stirring, the ethanol was evaporated under reduced pressure, and the residue dissolved under argon in 1.7 ml dry DMF. Subsequently, 0.9 mmol (115 mg) 1-bromo-2-fluoroethane was added dropwise, and the mixture was stirred for 20 h. After evaporation of the solvent, the residue was dissolved in a minimum volume of chloroform and separated on a silica gel column with chloroform/methanol (10 : 1) to yield the 9-isomer (102 mg, 40%) and the 7-isomer (83 mg, 33%). The 7- and

9-isomers were identified via ^{13}C-NMR spectroscopy (Moschel et al., 1992).

TLC conditions: chloroform/methanol 10 : 1; 9-substituted isomer R_f of 0.7; 7-substituted isomer R_f of 0.45.

Analytical data of the 9-isomer

- ^{1}H NMR (DMSO) δ (ppm): 7.9 (s, 1H, CH-8), 7.6–7.4 (m, 5H, ar-H), 6.4 (s, 2H, NH$_2$), 5.5 (s, 2H, CH$_2$-Bzl), 4.9 (t, 1H, CH$_2$-F), 4.6 (t, 1H, CH$_2$-F), 4.4 (t, 1H, N-CH$_2$-), 4.3 (t, 1H, N-CH$_2$);
- ^{13}C NMR (DMSO) δ (ppm): 57.3 (N-CH$_2$), 66.8 (CH$_2$-benzyl), 85.7 (CH$_2$-F), 126.6 (aryl), 127.2 (aryl), 127.4 (aryl), 128.6 (C-5), 140.2 (C-8), 138.5 (C-4), 152.3 (C-6), 154.9 (C-2);
- MS (FD): m/z (% rel. int.) 287.25 (100, [M +]);
- Anal. (C$_{14}$H$_{14}$FN$_5$O) C, H, N calc.: C:58.53 H: 4.91 N: 24.58 found: C: 58.21 H: 4.35 N: 24.57.

Analytical data of the 7-isomer

- ^{1}H NMR (DMSO) δ (ppm): 8.2 (s, 1H, CH-8), 7.6–7.4 (m, 5H, ar-H), 6.4 (s, 2H, NH$_2$), 5.5 (s, 2H, CH$_2$-Bzl), 4.9 (t, 1H, CH$_2$-F), 4.6 (t, 1H, CH$_2$-F), 4.4 (t, 1H, N-CH$_2$-), 4.3 (t, 1H, N-CH$_2$)
- ^{13}C NMR (DMSO) δ (ppm): 59.4 (N-CH$_2$), 66.5 (CH$_2$-benzyl), 84.1 (CH$_2$-F), 126.3 (aryl), 127.4 (aryl), 127.6 (aryl), 130.6 (C-5), 144.2 (C-8), 145.5 (C-4), 152.9 (C-6), 156.9 (C-2);
- MS (FD): m/z (% rel. int.) 287.49 (100, [M +]);
- Anal. (C$_{14}$H$_{14}$FN$_5$O) C, H, N calc.: C:58.53 H: 4.91 N: 24.58 found: C: 58.47 H: 4.87 N: 24.49.

2.5.3. 1-[^{18}F]fluoro-2-tosylethane

^{18}F was produced via the ^{18}O(p,n)^{18}F reaction at a PETtrace cyclotron (GE Medical Systems, 17 MeV proton energy). The aqueous ^{18}F-fluoride (540–1100 MBq) was dried by addition of 15 μmol (2.0 mg) potassium carbonate and 15 μmol (5.6 mg) Kryptofix®222 in 2 ml acetonitrile and three subsequent azeotropic destillations. Afterwards, 15 μmol (5.5 mg) ethyleneglycol-1,2-ditosylate in 1 ml acetonitrile were added and brought to reaction at 85°C for 5 min. Addition of 20-fold excess of water was followed by loading onto a C-18 cartridge, on which both 1-[^{18}F]fluoro-2-tosylethane and ethylenglycol-1,2-di-p-tosylate were fixed. 1-[^{18}F]fluoro-2-tosylethane was selectively eluted with acetonitrile/water 50 : 50. Fixation on an EN-cartridge, drying with helium and elution with DMF yielded 1-[^{18}F]fluoro-2-tosylethane in 80% yield (decay corrected) (440–720 MBq).

2.5.4. 6-Benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4)

6-Benzyloxy-9H-purin-2-ylamine (2.4 mg, 10 μmol) dissolved in 1 ml dry DMF, and 0.9 eq. of a ethanolic

sodium ethanolate solution were added. After adding 1-[^{18}F]fluoro-2-tosylethane (440–720 MBq) in DMF, the reaction mixture was heated to 120°C for 15 min and subsequently diluted with water. The mixture was transferred onto a C-18 cartridge, and 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) as well as the simultaneously formed 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4) were eluted with ethanol. The solution was brought onto a LiChrospher 100 RP18EC (250 × 4 mm, 5 μm) column. Elution was performed using acetonitrile/water (45 : 55) at a flow rate of 1 ml/min. The effluent was monitored simultaneously by UV (Linear, UVIS 200, 254 nm) and radioactivity detection. The fractions containing 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) (R_T: 11.4 min) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4) (R_T: 14.3 min) were passed through different C-18 cartridges, eluted with 100 μl ethanol and diluted with 900 μl sterile water in order to obtain 1 ml of an injectable solution of (3) (276–454 MBq) and (4) (130–225 MBq).

Radiochemical yields were determined via radio thin layer chromatography on TLC aluminium sheets (silica gel 60 F$_{254}$) using chloroform/methanol (6 : 1) and via radio-HPLC. Both methods were in total accordance with each other. The specific activity was 5 GBq/μmol.

3. Results and discussion

3.1. Syntheses

Fig. 1 shows the synthesis of the precursor 6-benzyloxy-9H-purin-2-ylamine (2), which was carried out according to an analogous literature procedure (Bowles et al., 1963) starting from 2-amino-6-chloro-purine (1) and benzyl alcohol.

The standard compounds 6-benzyloxy-9-(2-[^{19}F]fluoroethyl)-9H-purin-2-yl-amine and 6-benzyloxy-7-(2-[^{19}F]fluoroethyl)-7H-purin-2-yl-amine were obtained by reacting 6-benzyloxy-9H-purin-2-ylamine with 1-bromo-2-fluoroethane. 1-[^{18}F]fluoro-2-tosylethane was synthesized according to an established procedure starting from ethylenglycol-1,2-ditosylate and dried [^{18}F]-fluoride/Kryptofix222 complex (Block et al., 1988). A systematic study of several influencing parameters of the ^{18}F-fluoroethylation (reaction time, temperature, solvent, amount of precursor, base) was performed. Fig. 1 also shows the reaction scheme for the labelling reaction with optimized parameters.

The reaction yield was greatest at a temperature of 120°C. At this temperature the yield, as a function of reaction time, reached a maximum between 5 and 15 min of 30 ± 3% for the 9-isomer and 60 ± 3% for the 7-isomer. Dry DMF was used as the reaction medium, and yields were constant for precursor amounts ranging

from 4 to 40 μmol. Provided a strong base was used (sodium ethanolate, sodium hydroxide or lithium hydroxide), yields were comparable, only weak bases (such as Hünig's base) led to lesser yields. These optimized reaction parameters led to radiochemical yields of 43% for the 9-isomer (3) and 20% for the 7-isomer (4) referring to [^{18}F]fluoride starting activity.

It has not been possible to prevent the formation of the 7-substituted isomer. However, separation via HPLC was easy to perform so that the pure 9- and 7-substituted guanine derivatives could be obtained.

3.2. Evaluations

Fig. 2 shows the inhibition of MGMT by 6-benzyloxy-9H-purin-2-ylamine (2) which was used as a reference compound) and the derivatives 6-benzyloxy-9-(2-[^{19}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{19}F]fluoroethyl)-7H-purin-2-yl-amine (4). As shown by the dose-response curves, the fluoroethylpurine derivatives are active in inhibiting MGMT, albeit at a nearly 10-fold lower efficiency. The ED$_{50}$ values were 0.16 μM for 6-benzyloxy-9H-purin-2-ylamine and 1.8 μM for 6-benzyloxy-9-(2-[^{19}F]fluoroethyl)-9H-purin-2-yl-amine as well as for 6-benzyloxy-7-(2-[^{19}F]fluoroethyl)-7H-purin-2-yl-amine. The ED$_{50}$ experiments showed that the affinity of both isomers was high enough to allow biodistribution studies, directed at assessing the usefulness of these compounds for in vivo studies using PET.

The biodistribution of radioactivity of (3) and (4) was measured for liver, kidney, lung, spleen and both tumors, mex + and mex− . No significant differences have been observed for both the ^{18}F-labelled derivatives.
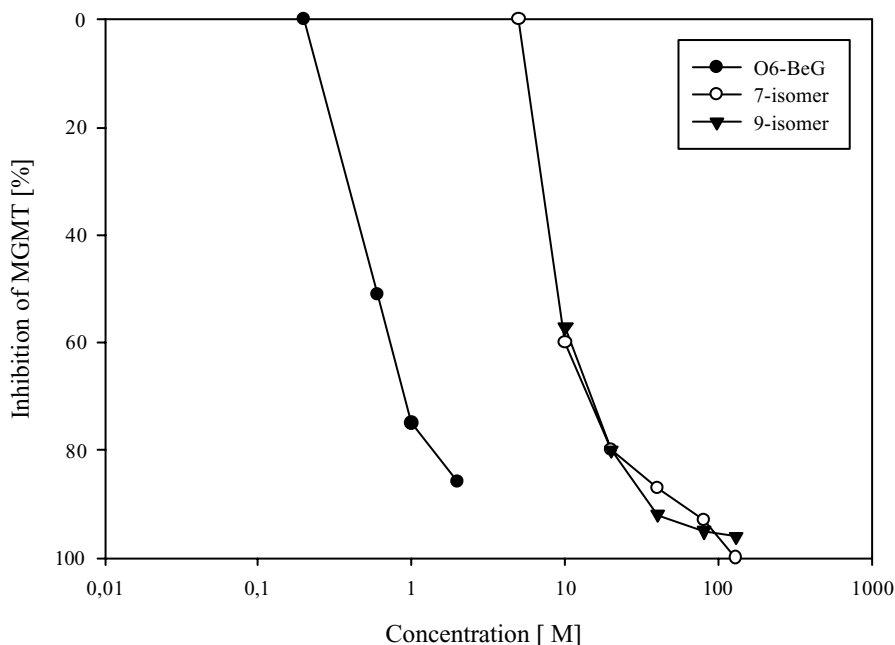

Fig. 2. Inhibition of MGMT at different concentrations of O^6-BeG (2) (reference compound), 9-isomer (3) and 7-isomer (4).

Table 1
Radioactivity uptake values (% inject. act./g) of 7- and 9-isomers in mex + −, mex− tumors in several organs at 30 and 60 min p.i.

Organ	30 min p.i.		60 min p.i.	
	7-isomer [% inject. act./g]	9-isomer [% inject. act./g]	7-isomer [% inject. act./g]	9-isomer [% inject. act./g]
Liver	2.2 ± 0.5	2.3 ± 0.7	1.3 ± 0.2	2.0 ± 0.6
Kidney	1.8 ± 0.4	1.1 ± 0.2	0.5 ± 0.2	1.2 ± 0.1
Lung	0.9 ± 0.2	1.1 ± 0.1	0.5 ± 0.1	1.1 ± 0.1
Mex +	0.7 ± 0.2	0.5 ± 0.1	1.0 ± 0.1	0.5 ± 0.1
Mex−	0.8 ± 0.2	0.8 ± 0.7	1.1 ± 0.1	0.6 ± 0.1

The excretion was primarily via the kidneys, and there was a significant uptake in the liver reaching 0.5%ID/g and 1.4%ID/g, respectively at 120 min p.i. The uptake of the ^{18}F-labelled isomers was similar in mex+ and mex− tumors. At 30 and 60 min after injection, its accumulation was 0.7 ± 0.2%ID/g and 1.0 ± 0.1%ID/g for mex+ tumors compared to 0.8 ± 0.2%ID/g and 1.1 ± 0.1%ID/g for mex− tumors, respectively. The uptake of the 9-isomer was somewhat lower, but still comparable for mex+ tumors (0.5 ± 0.1%ID/g and 0.5 ± 0.1%ID/g) and mex− tumors (0.8 ± 0.7%ID/g and 0.6 ± 0.1%ID/g) at 30 and 60 min p.i., respectively. The calculated statistical correlation for (4) were $r = 0.886$, $p < 0.005$ and $r = 0.903$, $p < 0.005$ for (3) (Pearson correlation coefficient). According to t-test $p = 0.103$, Wilcoxon test $p = 0.161$ for (4) and t-test $p = 0.261$, Wilcoxon test $p = 0.204$ for (3), there was no significant difference between the uptake in mex+ and mex− tumors for both the isomers. There was no significant accumulation of the drugs in the MGMT expressing tumor (see Table 1), probably due to the fast kinetics of the benzylether cleavage.

4. Conclusion

The ^{18}F-fluoroethyl-derivatives of 6-benzyloxy-9H-purin-2-ylamine, 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4), were synthesized from 6-benzyloxy-9H-purin-2-ylamine using 1-[^{18}F]fluoro-2-tosylethane. The best reaction system proved to be dry DMF as solvent, a reaction temperature of 120°C, and sodium ethanolate as base. These reaction parameters resulted in radiochemical yields of 42% for the 9- (3) and 20% for the 7-isomer (4) referring to [^{18}F]fluoride starting activity.

The ED_{50} experiments showed that the affinities of 6-benzyloxy-9-(2-[^{19}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{19}F]fluoroethyl)-7H-purin-2-yl-amine (4) were high enough to allow biodistribution studies, directed at assessing the usefulness of these compounds for in vivo studies using PET. However, there was no significant accumulation of radioactivity in the MGMT expressing tumor, probably due to the fast diffusion of the compounds into the tumors and the fast kinetics of the benzylether cleavage. Therefore, 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-7H-purin-2-yl-amine (4) are obviously unsuitable for PET studies of the MGMT status of tumors in situ. Based on our initial studies (Nesseler et al., 1999), 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine was recently proven to provide better potential in vivo (Vaidyanathan et al., 2000) compared to 6-benzyloxy-9-(2-[^{18}F]fluoroethyl)-9H-purin-2-yl-amine (3) and 6-benzyloxy-7-(2-[^{18}F]fluoroethyl)-

7H-purin-2-yl-amine (4). Due to the rather low yield of 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine, the syntheses of new molecules with a higher affinity to MGMT than 6-benzyloxy-9H-purin-2-ylamine is in progress, which will bear the radioactive tag at the aromatic group that is irreversibly transferred to MGMT, and which will be available in higher radio-chemical yields.

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