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Investigations into the synthesis, radiofluorination and conjugation of a new [^{18}F]fluorocyclobutyl prosthetic group and its in vitro stability using a tyrosine model system

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ABSTRACT

The [^{18}F]fluorocyclobutyl group has the potential to be a metabolically stable prosthetic group for PET tracers. The synthesis of the radiolabeling precursor *cis*-cyclobutane-1,3-diyl bis(toluene-4-sulfonate) **8** was obtained from epibromohydrin in 7 steps (2% overall yield). The radiolabeling of this precursor **8** and its conjugation to L-tyrosine as a model system was successfully achieved to give the new non-natural amino acid 3-[^{18}F]fluorocyclobutyl-L-tyrosine (L-3-[^{18}F]FCBT) [^{18}F]**17** in 8% decay-corrected yield from the non-carrier-added [^{18}F]fluoride. L-3-[^{18}F]FCBT was investigated in vitro in different cancer cell lines to determine the uptake and stability. The tracer [^{18}F]**17** showed a time dependent uptake into different tumor cell lines (A549, NCI-H460, DU145) with the best uptake of 5.8% injected dose per 5×10^5 cells after 30 min in human lung carcinoma cells A549. The stability of L-3-[^{18}F]FCBT in human and rat plasma and the stability of the non-radioactive L-3-FCBT in rat hepatocytes were both found to be excellent. These results show that the non-natural amino acid L-3-[^{18}F]FCBT is a promising metabolically stable radiotracer for positron emission tomography.

1. Introduction

Metabolism plays a huge role in the development of new drugs both in the therapeutic and diagnostic field.[1] With therapeutics, the metabolism of the drug can lead to biologically active metabolites which could cause numerous side-effects.[2] For diagnostics, in particular positron emission tomography (PET) radiopharmaceuticals, the images obtained from the PET camera are only visualizing the radionuclide attached to the biologically active molecule. If this radiolabeled molecule is metabolically unstable then the camera cannot distinguish between both the intact molecule and any of its metabolites containing the PET radionuclide. This often leads to undesirable accumulation in non-target organs increasing background activity and giving misleading information. Therefore it is essential when synthesizing such radiolabeled biologically active molecules that they remain metabolically stable to ensure that the image obtained is due to the injected PET tracer and not due to one or more of its metabolites. In PET research when investigating new biologically active molecules, it is attractive to synthesize an analog with a radiolabeled alkyl group attached to a heteroatom, namely oxygen and nitrogen. However, it is reported in the literature that potential sites for metabolism are alkylated heteroatoms.[3,4] For ^{18}F-labeled PET tracers, this type of metabolism has been reported for different biological targets; for example. the dopamine transporter radiotracer [^{18}F]FECNT;[3] the vesicular acetylcholine transporter ligand [^{18}F]FEOBV[4] and the non-peptide endothelin type A receptor antagonist [^{18}F]PD 156707 (Fig. 1).[5]

For these tracers the noticeable similarity is the [^{18}F]fluoroethylalkyl chain attached to either an oxygen or nitrogen heteroatom. Radiotracers labeled with an *N*- or *O*-[^{18}F]fluororethyl group can be prone to dealkylation through enzymatic reactions in the body, particularly the monooxygenase cytochrome P450,[6] to give polar radiometabolites which can interfere in PET imaging. All radiometabolites originating from N- or O-dealkylation are a consequence of the Phase I biotransformation and are believed to be [^{18}F]fluoroethanol.[3,7] The radiometabolite [^{18}F]fluoroethanol can undergo an oxidative biotransformation to the aldehyde [^{18}F]fluoroethanol and can also be further oxidized to [^{18}F]fluoroacetic acid.[3,8] This radiometabolite can be further metabolized, mainly in the liver, to the [^{18}F]fluoride ion which accumulates in the bone, skull and joints,[9] thus leading altogether to a higher background signal and can influence the PET image quality.

We hypothesized that replacing the [^{18}F]fluoroalkyl chain with a [^{18}F]fluorocycloalkyl group could increase the metabolic stability of a PET tracer. In the literature numerous examples can be found

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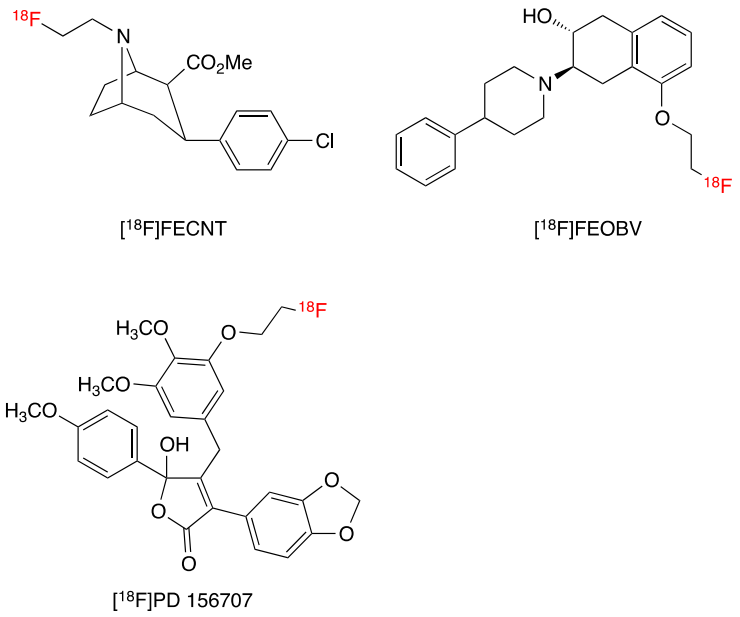


Figure 1. Selected examples of ^{18}F-labeled radiotracers based on the fluoroalkyl group with radiometabolites reported.

where pharmaceuticals with cycloalkyl rings attached to heteroatoms are more metabolically stable than their simple alkyl chain counterparts.[10–12] Radiolabeled cyclobutyl groups are not unknown and the Goodman group have explored different cyclobutyl unnatural amino acids for cancer imaging[13] and interestingly *anti*-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-3-[^{18}F]FACBC), a potential imaging agent for prostate cancer,[14–16] their were no visible metabolism in vivo.[17] The model system chosen to test our hypothesis was a tyrosine based amino acid, as a variety of *O*-alkylated tyrosines are reported, for example, *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET)[18] or DFMT.[19,20] These compounds are known to be transported into the cells via the well characterized and extensively documented large amino acid transporter (LAT).[21,22]

Herein, we describe the preparation of the novel tyrosine derivate *O*-(*cis*-3-[^{18}F]fluorocyclobutyl)-L-tyrosine (L-3-[^{18}F]FCBT) [^{18}F]**17** via an indirect radiolabeling method and the synthesis of the corresponding reference compounds (**11**, L-**17** and D-**17**). L-3-[^{18}F]FCBT was tested in vitro for both its uptake in different tumor cell lines (A549, NCI-H460, DU145) and its metabolic stability.

2. Results and discussion

2.1. Chemistry

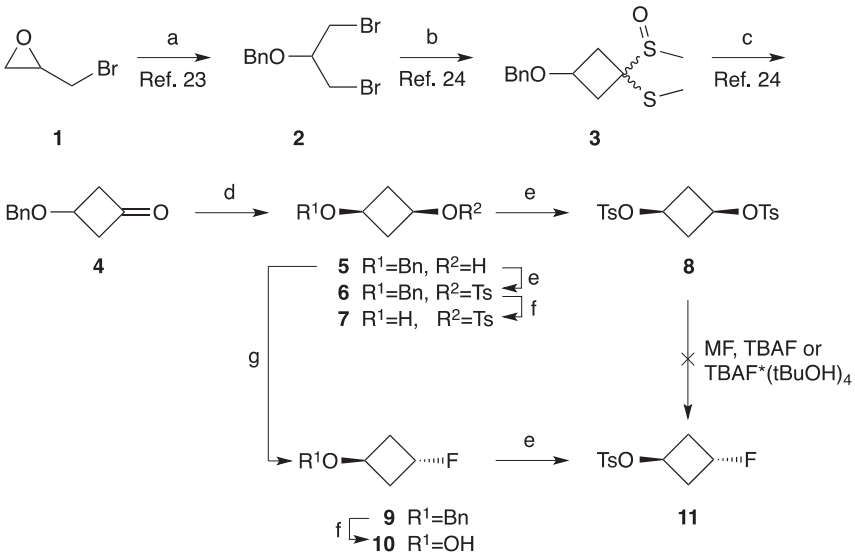
The precursor *cis*-cyclobutane-1,3-diyl bis(toluene-4-sulfonate) **8** for the indirect radiolabeling was prepared in a multistep synthesis starting from epibromohydrin **1** and benzyl bromide (Scheme 1). The first 3 steps are literature known and gave 3-(benzyloxy)cyclobutanone **4**.[15,23,24] The reduction of **4** with sodium borohydride ($NaBH_4$) gave the key intermediate *cis*-3-(benzyloxy)cyclobutanol **5** in 86% yield.

It was of interest to see if the basic reaction conditions of the reduction step would form another stereoisomer than the expected *cis*-alcohol **5**. The ^{1}H NMR- and ES-mass spectrum confirmed the structure of the alcohol **5**. Since the NMR data do not give information about the *cis*/*trans*-stereochemistry, NOE NMR experiment was performed and the *cis*-isomer **5** was confirmed as the major compound. Consequently, the observed stereoselective reduction suggests an axial attack of the nucleophile $NaBH_4$.

Standard tosylation methods were tested to synthesize **6**; using tosyl chloride (TsCl) with or without the catalyst 4-dimethylaminopyridine (DMAP) resulted in low yields (~30%). The use of *para*-toluenesulfonic anhydride (Ts_2O) in pyridine gave far superior yields (87%). The next step was the removal of the benzyl group with standard palladium on charcoal catalyzed hydrogenation conditions. Repeating the tosylation of the deprotected compound **7** with the Ts_2O method afforded the precursor for the indirect labeling *cis*-cyclobutane-1,3-diyl bis(toluene-4-sulfonate) **8** (65%, 3 steps).

The preparation of the ^{19}F-reference compound *trans*-3-fluorocyclobutyl toluene-4-sulfonate **11** was initially investigated via nucleophilic fluorinations of the bis-tosylate **8** using various different known methods; these included potassium fluoride (KF),[25] cesium fluoride (CsF),[26] silver fluoride (AgF)[27] or tetrabutylammonium fluoride (TBAF)[27] failed to give the desired reference compound **11**. Kim et al.[26] recently described TBAF to be hygroscopic which could have an influence on the TBAF reaction and they reported the significantly less hygroscopic fluorination agent TBAF*(tBuOH)$_4$ which was prepared simply by refluxing TBAF in tBuOH/hexane and recrystallization at room temperature in 55% yield. The fluorination of the bis-tosylate **8** was tried with the freshly prepared TBAF*(tBuOH)$_4$ refluxing in acetonitrile (1 h). Unfortunately, all attempts under a variety of conditions ranging from low to high precursor **8** concentrations failed to give the desired fluorotosylate **11**.

Alternatively the fluorinated compound **9** was then prepared from *cis*-3-(benzyloxy)cyclobutanol **5** by treatment with diethylaminosulfur trifluoride (DAST) at 0 °C in 37% yield. Fluorination was also successful with perfluoro-1-butanesulfonyl fluoride with a mixture of triethylamine trihydrofluoride in presence of triethylamine (TEA) at room temperature albeit in lower yield (25%) than the DAST method. Removal of the benzyl group using catalytic hydrogenation conditions with palladium on charcoal in methanol (Scheme 1) afforded the *trans*-3-fluorocyclobutanol **10**, which was confirmed by ^{1}H NMR spectroscopy. Unfortunately, the fluoroalcohol **10** was found to be volatile. A high quantity of product was lost during the solvent evaporation process and was avoided with careful evaporation of the solvent (40 °C, 200 mbar). The fluoroalcohol **10** was used without further purification in the tosylation step,



Scheme 1. Synthesis of the precursor for the indirect radiolabeling *cis*-cyclobutane-1,3-diyl bis(toluene-4-sulfonate) **8** and reference compound **11**. Reagents and conditions: (a) benzyl bromide, Hg_2Cl_2, 160 °C, (b) $CH_3S(O)CH_2SCH_3$, *n*-BuLi, THF, (c) HCl, THF, (d) $NaBH_4$, EtOH, 0 °C, (e) Ts_2O, pyridine, DCM, (f) H_2, 10% Pd/C, MeOH, (g) DAST, DCM. Notes: Bn = benzyl; M = K, Cs, Ag.

which gave the desired reference compound **11** in 14% yield (2 steps).

For the synthesis of both the D- and L-isomers of the ^{19}F reference 3-FCBT **17**, a different route was followed (Scheme 2). Due to the synthetic difficulties the reference compound was prepared via the phenolic alkylation of tyrosine with the mono-protected benzyl ether **5**. The route employed the Mitsunobu reaction[28] of the cyclobutanol derivate **5** with the commercially available Boc-L-Tyr-OMe **12** to give the desired product **13** in 88% yield (Scheme 2). After removal of the benzyl group with palladium on charcoal (Pd/C) under hydrogen (H_2), the hydroxyl group was fluorinated using DAST to give **15** in 38% yield. The protecting groups were removed sequentially, first the methyl ester with LiOH in methanol[29] and second the Boc group with HCl in dioxane to give the reference compound L-**17** (Scheme 2) with an enantiomeric purity of 94% (ee).[30] The D-isomer followed a similar procedure starting from Boc-D-Tyr-OMe where the desired D-**17** was obtained with an enantiomeric purity of 92% (ee).[30]

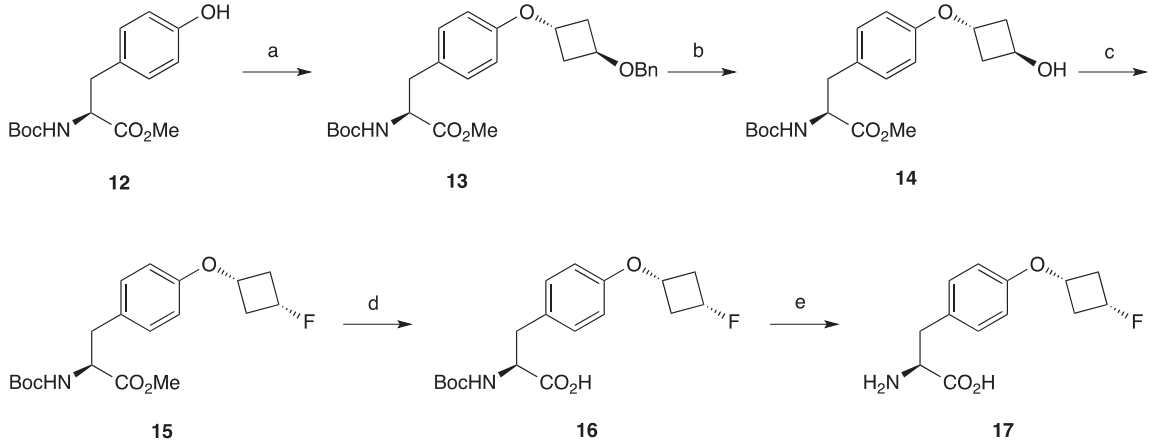
2.2. Radiochemistry

The radiofluorinated L-3-[^{18}F]FCBT [^{18}F]**17** was synthesized via a 2 step radiolabeling approach (indirect) with the first step being the radiofluorination of precursor **8** followed by its conjugation via an alkylation reaction with disodium salt of L-tyrosine generated in situ.[31,32] In the first step, *trans*-3-fluorocyclobutyl toluene-4-sulfonate [^{18}F]**11** was synthesized via non-carrier-added (n.c.a.) nucleophilic fluorination of *cis*-cyclobutane-1,3-diyl bis(toluene-4-sulfonate) **8** using dried [^{18}F]F^-, potassium carbonate and Kryptofix complex in acetonitrile (130 °C for 15 min) followed by C18 cartridge purification (Scheme 3).

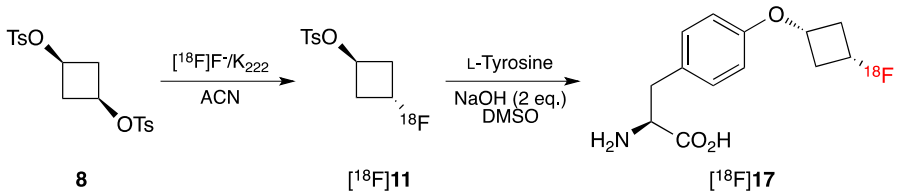
Then the intermediate prosthetic group [^{18}F]**11** was subsequently reacted with L-tyrosine in DMSO in presence of aqueous sodium hydroxide (150 °C for 15 min). After purification by semi-preparative HPLC and reformulation, L-3-[^{18}F]FCBT [^{18}F]**17** was obtained in 8% ± 5.5 yield (n = 14, decay corrected), radiochemical purity of greater than 99% (Fig. 2) and in a synthesis time of approximately 140 min. Analysis of the final product [^{18}F]**17** using chiral radio-HPLC showed that no racemization had occurred (Fig. 3).

2.3. Determination of the lipophilicity of the non-radioactive L-3-FCBT

The lipophilicity values of L-3-FCBT and FET were calculated with ChemDraw,[33] MarvinSketch[33] and ACD ChemSketch[33] and



Scheme 2. Synthesis of the reference compound *O*-(*cis*-3-fluorocyclobutyl)-L-tyrosine (L-**17**). *O*-(*cis*-3-fluorocyclobutyl)-D-tyrosine (D-**17**) was synthesized accordingly, starting with Boc-D-Tyr-OMe. Reagents and conditions: (a) **5**, DEAD, Ph_3P, DMF, (b) H_2, 10% Pd/C, MeOH, (c) DAST, DCM, (d) LiOH, MeOH, (e) HCl in dioxane, DMF.



Scheme 3. Radiosynthesis of *O*-(*cis*-3-[^{18}F]fluorocyclobutyl)-L-tyrosine [^{18}F]**17** via the intermediate *trans*-3-[^{18}F]fluorocyclobutyl toluene-4-sulfonate [^{18}F]**11**.

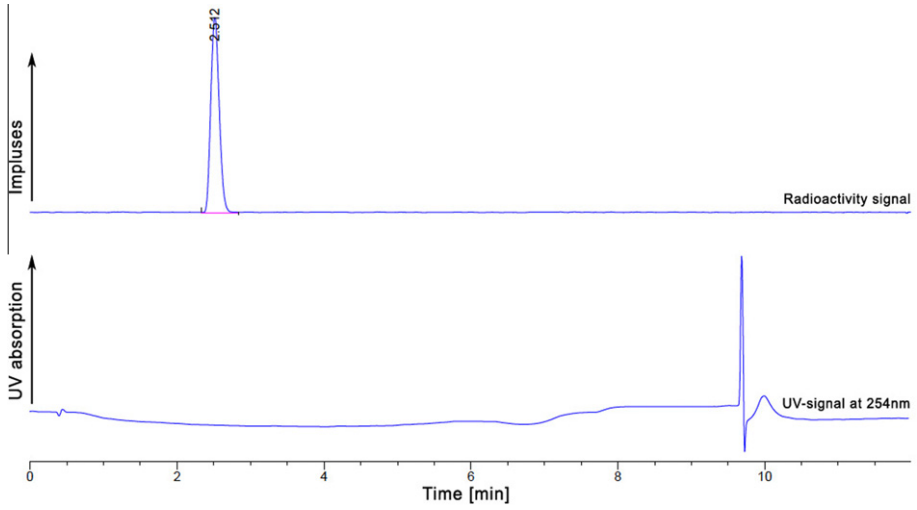


Figure 2. C18 radio-HPLC analysis of the final product L-3-[^{18}F]FCBT [^{18}F]**17** showed no impurities in the radio- as well as in the UV-trace.

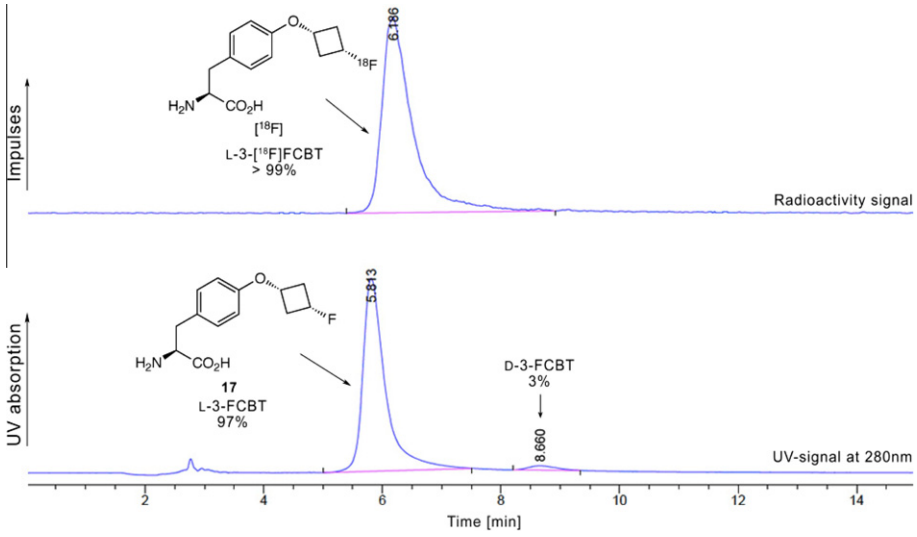


Figure 3. Chiral radio-HPLC analysis of final product L-3-[^{18}F]FCBT [^{18}F]**17** showed no impurities and only L-isomer to be present (top). Co-injection of the non-radioactive L-isomer of 3-FCBT L-**17** with traces of D-isomer visible (bottom).

experimentally determined by the extraction (Table 1). The calculated lipophilicity values of L-3-FCBT and FET are found to be in the similar range and indicating very hydrophilic character. These calculated trends were confirmed by the experimental determination of the log*D* of L-3-FCBT and was found to be −0.83. Comparing the experimental value of FET found in literature (−1.51), [18] L-3-FCBT showed a higher lipophilicity of −0.83 which could influence the pharmacokinetics of the tracer for future in vivo experiments.

Table 1
Calculated and experimental partition coefficient log*D* of L-3-FCBT and FET

Method	L-3-FCBT log*D*	FET log*D*
ChemDraw	−1.03	−1.39
MarvinSketch	−1.08	−1.14
ACD	−1.9	−2.2
Experimental	MarvinSketch	−1.51

Experimental log*D* value of FET according Ref. 18.

2.4. Cell uptake studies

The L-3-[^{18}F]FCBT was investigated in several cancer cell lines; human lung carcinoma cell lines (A549 and NCI-H460) and in human prostate cancer cell line (DU145). The time dependent uptake of L-3-[^{18}F]FCBT was tested by incubating the cells for different time periods (max. 60 min, Fig. 4). A time dependent uptake of L-3-[^{18}F]FCBT was observed in all the tumor cell lines tested. Human lung carcinoma cells A549 showed the fastest and highest uptake of the cell lines with an uptake of 2.8% applied dose/10^5 cells after 10 min reaching a plateau of 5.8% applied dose/10^5 cells at 30 min. In comparison, the human lung carcinoma cells (NCI-H460) and human prostate tumor cells (DU145) both showed comparable slower uptakes (0.7% applied dose/10^5 after 10 min) and increased to a value of 2.1% applied dose/10^5 after 60 min. However, these cells showed a linear uptake and did not reach a plateau after 60 min. The increased uptake of L-3-[^{18}F]FCBT into the A549 cells in comparison to the NCI-H460 and DU145 cells is due the higher expression of the LAT transporters on these cells (data not shown).

In addition a competitive uptake experiment using the non-radioactive *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) and non-radioactive L-3-FCBT was performed (Fig. 5). The L-3-[^{18}F]FCBT uptake could be essentially blocked (>82%) with the non-radioactive reference compounds (L-3-FCBT and FET) in all the tested cell lines.[34] The blocking of L-3-[^{18}F]FCBT displayed an interesting result as FET seems to block the uptake of L-3-[^{18}F]FCBT better than L-3-FCBT, an explanation for this could be that the FET is a better substrate for the LAT transporter and thus block the uptake of L-3-[^{18}F]FCBT more effectively.

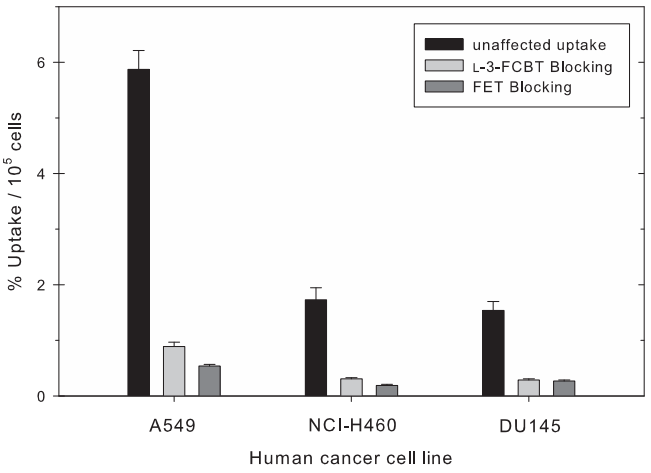


Figure 5. Blocking of L-3-[^{18}F]FCBT by adding 1 mM non-radioactive L-3-FCBT and FET over 30 min. Experiments were performed in triplicate. Error bars indicate standard deviation.

2.5. Determination of in vitro plasma stability

The stability of L-3-[^{18}F]FCBT and [^{18}F]FET was determined in both human and rat plasma. L-3-[^{18}F]FCBT and [^{18}F]FET were incubated in human and rat plasma and in phosphate buffer solution at 37 °C for up to 120 min and analyzed by radio-HPLC and radio-TLC at different time points (0, 30, 60, 90 and 120 min), as shown in Figure 6.

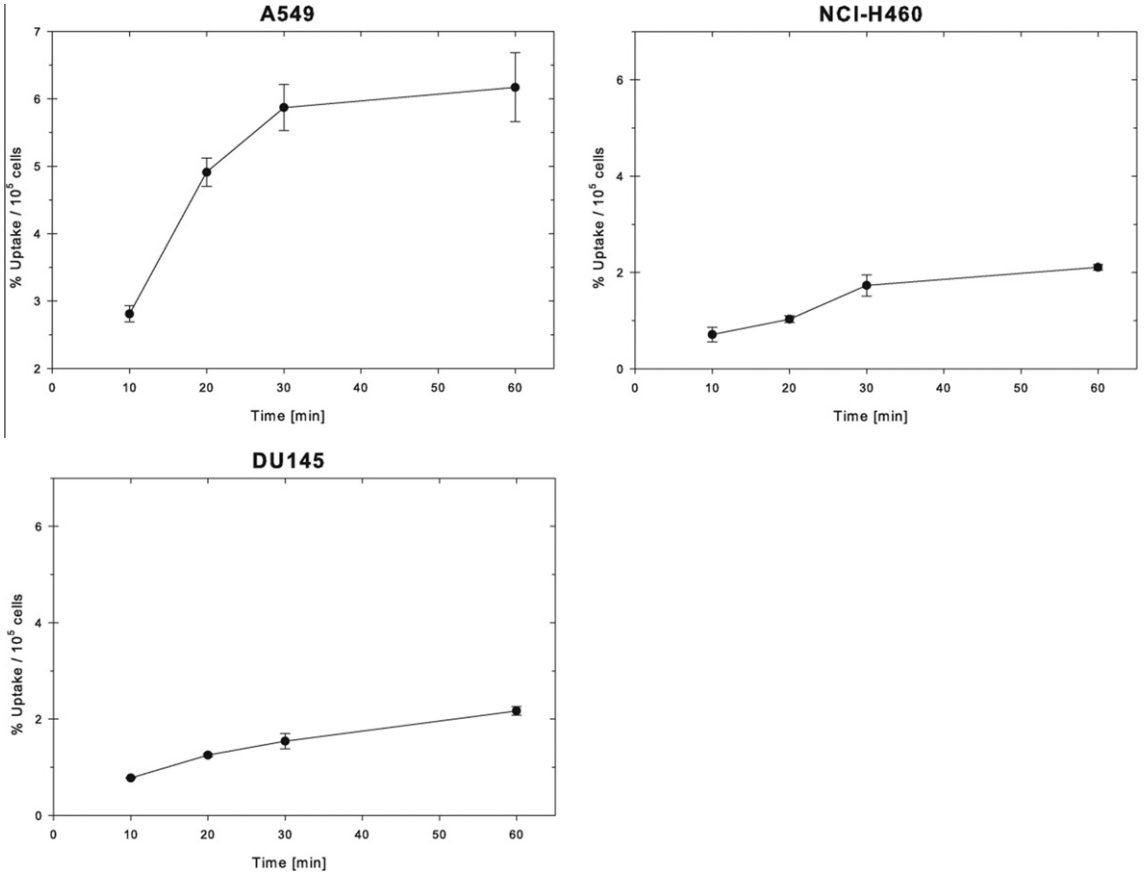


Figure 4. Uptake of L-3-[^{18}F]FCBT into human lung carcinoma cell lines (A549 and NCI-H460) and in human prostate cancer cell line (DU145) over 60 min in vitro. Experiments were performed in triplicate. Error bars indicate standard deviation.

L-3-[^{18}F]FCBT showed over 120 min an excellent stability (>99.9%) in human and rat plasma. [^{18}F]FET showed similar stability for 120 min (data not shown). The compounds were also stable in phosphate buffer solution over 120 min. With [^{18}F]FET, a very slight polar radio-peak was observed after 60 min in human and rat plasma, but was deemed to be insignificant as the amount was below 1%. In humans, [^{18}F]FET was reported to be relative metabolically stable with 95% and 87% intact tracer at 5 and 120 min, respectively.[35]

2.6. Determination of in vitro stability of L-3-FCBT in rat hepatocytes

The stability of the non-radioactive L-3-FCBT was tested in an in vitro experiment using hepatocytes isolated from Han Wister rats. This assay is important in the early preclinical phase of drug development as hepatocytes play a major role in a variety of metabolic processes in particular the cytochrome P450 (CP450) system. The metabolic stability was examined by incubating L-3-FCBT with the hepatocytes and analyzing at different time-points (0, 2, 8, 16, 30, 45 and 60 min) by HPLC-MS and the results are shown in Table 2. The results clearly show that the stability of L-3-FCBT in this assay is excellent and warrants further investigations in in vivo models.

3. Conclusion

The tyrosine derivative L-3-FCBT **17** with the novel fluorocyclobutyl moiety was successfully synthesized. The radiolabeled analog L-3-[^{18}F]FCBT [^{18}F]**17** was synthesized in low yields (8% ± 5.5, n = 14, decay corrected) and sufficient activity for biological evaluation via a 2 step method starting from bis-tosylate **8**. The L-[^{18}F]FCBT was shown to be transported into a number of different cancer cells and the uptake could be blocked essentially with either non-radioactive L-3-FCBT or FET, indicating that despite the bulkier cyclobutyl group the biological properties remain unchanged. In vitro stability of L-3-[^{18}F]FCBT in human and rat plasma were excellent over 120 and in an additional study using rat hepatocytes the stability of non-radioactive L-3-FCBT also showed excellent stability over 60 min. These results demonstrate that L-3-[^{18}F]FCBT is a promising stable PET imaging agent which warrants further evaluation to determine whether the ^{18}F-labeled fluorocyclobutyl moiety is a more metabolically stable group than the ^{18}F-labeled fluoroethyl moiety.

Table 2
Determination of in vitro metabolic stability in rat hepatocytes of non-radioactive L-3-FCBT

Incubation time (min)	(%) Intact FCBT
0	100
2	100
8	99
16	98
30	97
45	95
60	94

4. Experimental

4.1. Chemistry

All solvents and chemicals unless otherwise stated where purchased from Aldrich (Germany), Fluka (Switzerland), Acros Organics (Belgium), ABCR (Germany) or Merck (Germany) and used without further purification. The *O*-(2-fluoroethyl)-L-tyrosine (FET) reference compound was purchased from ABX GmbH (Germany). Silica chromatography was performed on Biotage Isolera Flash Purification System from Biotage® (Sweden) with the corresponding SNAP cartridges (10–340 g silica gel); solvent gradients were generated automatically, entering TLC values and were run in general from 5% of the weak solvent to 100% of the strong solvent in 10 column volumes. Thin-layer chromatography (TLC) was carried out with silica gel plates (TLC Silica gel 60 F254, 20 × 20 mm) from Merck (Germany) visualized under UV at 254 nm or with $KMnO_4$ staining. HPLC system used an Agilent 1100 and 1200 HPLC system with a binary pump, autosampler and diode array detector (DAD) and an attached gamma-detector (NaI) GABI from Raytest (Germany), using the Agilent 'ChemStation' software. Analytical HPLC column ACE C18 (50 × 4.6 mm) was obtained from ACE (Great Britain), the Astec Chirobiotic T Chiral HPLC column (250 × 4.6 mm) from Sigma–Aldrich (Germany). Semi-preparative Knauer HPLC system consisted of a Knauer pump, UV detector and an attached gamma-detector (NaI) GABI from Raytest using the Knauer 'ChromGate' software. Semi-preparative HPLC was performed with Phenomenex Synergi Hydro-RP 4 μ (250 × 10 mm) from Phenomonex (USA). Mass spectra (MS) were obtained on a ZQ 4000 LC and LCT TOF mass spectrometer from Waters/Micromass (USA) for electrospray ionization (ESI) and a DSQ CTC from Thermo Scientific (USA) for

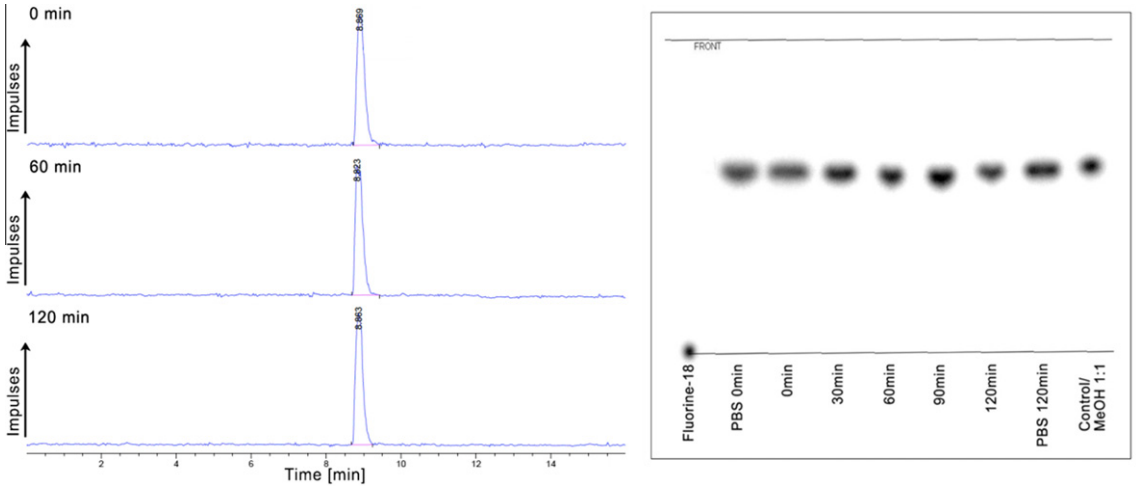


Figure 6. In vitro stability of L-3-[^{18}F]FCBT in human plasma over 120 min; radioactivity signal of radio-HPLC (left) and radio-TLC (right). Rat data identical—not shown.

chemical ionization (CI). ^{1}H and ^{19}F nuclear magnetic resonance (NMR) spectra were recorded with the Bruker AVANCE-300 and 400 MHz and the Bruker DRX-600 NMR spectrometer.

4.2. *cis*-3-(Benzyloxy)cyclobutanol (5)

3-(Benzyloxy)cyclobutanone **4** was prepared according to the method described earlier.[15,23,24] To a suspension of **4** (10.4 g; 59.0 mmol) in ethanol (100 mL) was added sodium borohydride (2.45 g; 64.9 mmol) in portions over 30 min. The mixture was stirred at 0 °C for 3 h and monitored by TLC upon reaction completion (ethyl acetate/hexane 1:1). The pale yellow mixture was passed through a pad of Celite®, concentrated and the residue was re-dissolved in methanol (CARE: gas evolution), then neutralized with 3 M HCl and concentrated in vacuo. To the residue was added water (80 mL) and extracted with diethyl ether (80 mL). The combined organic phases were dried with anhydrous sodium sulfate (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by silica chromatography with a gradient of ethyl acetate and hexane. Product fractions were combined and concentrated to give **5** (8.97 g, 86%) as a clear colorless oil. TLC (ethyl acetate/hexane 1:1, R_f ~0.28). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.87 (d, J = 6.32 Hz, OH) 1.90–2.00 (m, 2H, CH) 2.68–2.79 (m, 2H, CH) 3.64 (quin, J = 6.95 Hz, 1H, CH) 3.92 (sxt, J = 6.97 Hz, 1H, CH) 4.43 (s, 2H, CH_2) 7.28–7.40 (m, 5H, CH_{arom}). ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 39.6 (CH_2) 41.4 (CH_2) 59.8 (CH) 64.4 (CH) 70.4 (CH_2) 127.7 (CH) 127.9 (CH) 127.9 (CH) 128.5 (CH) 138.1 (C). ESI-MS: *m/e* 179 $[M+H]^+$.

4.3. *cis*-3-(Benzyloxy)cyclobutyl toluene-4-sulfonate (6)

A solution of *cis*-3-(benzyloxy)cyclobutanol **5** (1.10 g; 6.2 mmol) in dichloromethane (40 mL) was cooled to 0 °C and pyridine (1.0 mL; 12.3 mmol) was added followed by addition of *p*-toluenesulfonic anhydride (3.02 g; 9.3 mmol) under nitrogen. The mixture was stirred at 0 °C for 5 h and an additional 24 h at room temperature. The mixture was concentrated in vacuo, suspended in diethyl ether (50 mL) washed with 1 M HCl, saturated sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate and concentrated in vacuo. The resulting yellow oil was purified by silica chromatography, eluting with a gradient of ethyl acetate/hexane to afford **6** (1.87 g, 87%) as a clear pale yellow oil. TLC (ethyl acetate/hexane 1:2, R_f ~0.43). ^{1}H NMR (600 MHz, $CDCl_3$): δ ppm 2.11–2.23 (m, 2H, CH) 2.45 (s, 3H, CH_3) 2.58–2.65 (m, 2H, CH) 3.63 (quin, J = 6.95 Hz, 1H, CH) 4.37 (s, 2H, CH_2) 4.46 (quin, J = 7.26 Hz, 1H, CH) 7.28–7.37 (m, 7H, CH_{arom}) 7.78 (d, J = 8.34 Hz, 2H, CH_{arom}). ESI-MS: *m/e* 355 $[M+Na]^+$.

4.4. *cis*-3-Hydroxycyclobutyl toluene-4-sulfonate (7)

A solution of *cis*-3-(benzyloxy)cyclobutyl toluene-4-sulfonate **6** (1.8 g; 5.42 mmol) in absolute ethanol (35 mL) was stirred at room temperature with palladium on charcoal 10% (1.02 g; 0.96 mmol) and a positive pressure of hydrogen (balloon) for 4 h. After reaction completion, the catalyst was removed by filtration over Celite® and concentrated in vacuo to give the desired product **7** (1.3 g, 96%) as a yellow oil which was used without further purification. TLC (ethyl acetate/hexane 1:2, R_f ~0.11). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.73 (br s, OH) 2.06–2.19 (m, 2H, CH) 2.46 (s, 3H, CH_3) 2.64–2.78 (m, 2H, CH) 3.92 (quin, J = 7.02 Hz, 1H, CH) 4.42 (quin, J = 7.06 Hz, 1H, CH) 7.35 (d, J = 7.91 Hz, 2H, CH_{arom}) 7.79 (d, J = 8.10 Hz, 2H, CH_{arom}).

4.5. *cis*-Cyclobutane-1,3-diyl bis(toluene-4-sulfonate) (8)

A solution of *cis*-3-hydroxycyclobutyl toluene-4-sulfonate **7** (4.29 g; 17.7 mmol) in dichloromethane was cooled to 0 °C. Pyridine (2.9 mL) was added, followed by the addition of *p*-toluenesulfonic anhydride (8.67 g; 26.6 mmol). The mixture was stirred 72 h at room temperature. The reaction mixture was concentrated to dryness and re-suspended in diethyl ether (75 mL). The suspension was washed with 0.5 M HCl, saturated sodium bicarbonate solution and brine. The mixture was dried over sodium sulfate, filtered and concentrated in vacuo. The crude product was purified by silica chromatography eluting with ethyl acetate/hexane (1:6) to give pure **8** (5.35 g, 64%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ ppm 2.29–2.37 (m, 2H, CH), 2.45 (s, 6H, CH_3), 2.60–2.68 (m, 2H, CH), 4.40 (quin, 2H, CH), 7.33 (d, 4H, CH_{arom}), 7.71–7.75 (m, 4H, CH_{arom}). ^{13}C NMR (151 MHz, $CDCl_3$): δ ppm 21.7 (CH_3), 39.1 (CH_2), 39.1 (CH), 127.8 (CH), 130.0 (CH), 133.4 (C) 145.2 (C). CI-MS: *m/e* 396 $[M]^+$.

4.6. *trans*-3-(Fluorocyclobutyl) benzyl ether (9)

To an ice-cooled solution of *cis*-3-(benzyloxy)cyclobutanol **5** (0.9 g; 5.54 mmol) in dry dichloromethane (25 mL) was added diethylaminosulfur trifluoride (0.86 mL; 6.54 mmol). The mixture was stirred for 2 h at 0 °C under nitrogen and was allowed to warm to room temperature overnight. The yellow–brown reaction mixture was washed with water (20 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 20 mL). The organic layers were combined, dried with sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica chromatography with a gradient of ethyl acetate/hexane to give pure **9** (306 mg, 37%) as a clear colorless oil. TLC (ethyl acetate/hexane1:2, R_f ~0.67). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.34–2.63 (m, 4H, CH), 4.31–4.41 (m, 1H, CH), 4.43 (s, 1H, CH), 5.14–5.19 (m, 0.5H, CH–F), 5.28–5.38 (m, 0.5H, CH–F), 7.28–7.58 (m, 5H, CH_{arom}). ^{19}F NMR (400 MHz, $CDCl_3$): δ ppm −176.4. ESI-MS: *m/e* 254 $[M+H]^+$.

4.7. *trans*-3-Fluorocyclobutyl toluene-4-sulfonate (11)

A solution of *trans*-3-(fluorocyclobutyl) benzyl ether **9** (152 mg; 0.84 mmol) in methanol (10 mL) was stirred with 10% palladium on charcoal (140 mg) and with a positive pressure of hydrogen (balloon) at room temperature. The mixture was filtered and the solvent was partly evaporated in vacuo (40 °C/200 mbar, volatile!) to give **10** as a clear colorless oil with traces of methanol. The product was used without further purification due to the low boiling point. TLC (ethyl acetate/hexane 1:2, R_f ~0.26; stained with $KMnO_4$). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.22–2.39 (m, 2H, CH), 2.48–2.63 (m, 2H, CH), 4.59–4.72 (m, 1H, CH), 5.15–5.39 (m, 1H, CH), OH not visible; ^{19}F NMR (376 MHz, $CDCl_3$): δ ppm −178.3.

A solution of *trans*-3-fluorocyclobutanol **10** (50 mg; 0.56 mmol) in dry dichloromethane (5 mL) was cooled to 0 °C and pyridine (82 μl; 1.0 mmol) was added, followed by *p*-toluenesulfonic anhydride (201 mg; 0.62 mmol). The mixture was stirred for 5 h at 0 °C under nitrogen and was allowed to warm to room temperature overnight. The yellow solution was concentrated in vacuo. The resulting residue was diluted with HCl 0.5 M (5 mL), extracted with diethyl ether (10 mL) and the organic layer was washed with saturated sodium bicarbonate and brine. The mixture was dried with sodium sulfate, filtered and concentrated in vacuo. The crude oil was purified by silica chromatography, eluting with a gradient of ethyl acetate/hexane to give **11** (59 mg, 14%, over 2 steps) as a clear colorless oil. TLC (ethyl acetate/hexane 1:2, R_f ~0.52). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.47 (s, CH_3), 2.48–2.62 (m, 4H, CH), 5.03–5.08 (m, 1H, CH), 5.10–5.19 (m, 0.5H, CH–F), 5.24–5.33 (m, 0.5H, CH–F), 7.36 (d, J = 8.10 Hz, 2H, CH_{arom}), 7.79 (d, J = 8.29 Hz, 2H, CH_{arom}). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 21.7 (CH_3), 38.8 (CH_2), 72.3 (CH), 85.7 (CH), 127.9 (CH), 129.9 (CH), 133.5 (C), 145.1 (C). ^{19}F NMR (376 MHz, $CDCl_3$): δ ppm −178.8. ESI-MS: *m/e* 245 $[M+H]^+$.

4.8. Methyl *O*-[*trans*-3-(benzyloxy)cyclobutyl]-*N*-(*tert*-butoxycarbonyl)-L-tyrosine (13)

To a solution of Boc-Tyr-OMe L-**12** (1.02 g; 3.35 mmol) and *cis*-3-(benzyloxy)cyclobutanol **5** (1.33 g; 7.37 mmol) in dry DMF (25 mL) was added diethyl diazocarboxylate (1.20 mL; 7.37 mmol). The yellow solution was stirred under nitrogen and triphenylphosphine (1.98 g; 7.37 mmol) was added. The mixture was stirred at room temperature for 23 h and concentrated in vacuo. The crude oil was dissolved in chloroform (50 mL) and washed with water (3 × 30 mL) to remove dimethylformamide. The organic layer was dried with sodium sulfate, filtered and concentrated in vacuo to give a brown oil. The crude product was purified by silica chromatography, eluting with a gradient of ethyl acetate/hexane to give the L-**13** (1.35 g, 88%) as a clear colorless oil. TLC (ethyl acetate/hexane 1:2, R_f ~0.46). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.43 (s, 9H, CH_3), 2.38–2.56 (m, 2H, CH), 2.94–3.11 (m, 2H, CH), 3.72 (s, 3H, CH_3), 4.30–4.39 (m, 1H, CH), 4.46 (s, 2H, CH_2), 4.50–4.60 (m, 1H, CH), 4.78–4.88 (m, 1H, CH), 4.90–5.00 (m, 1H, NH), 6.71 (d, 2H, CH_{arom}), 7.02 (d, 2H, CH_{arom}), 7.29–7.42 (m, 5H, CH_{arom}). CI-MS: *m/e* 356 $[M-Boc]^+$.

4.9. Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*trans*-3-hydroxycyclobutyl)-L-tyrosine (14)

A solution of methyl *O*-[*trans*-3-(benzyloxy)cyclobutyl]-*N*-(*tert*-butoxycarbonyl)-L-tyrosine **13** (1.35 g; 2.96 mmol) in methanol (20 mL) was stirred with 10% palladium on charcoal (500 mg, 50% wet). The mixture was stirred under a positive pressure of hydrogen (balloon) at room temperature for 2 h. The mixture was filtered and the solvent concentrated in vacuo. The crude oil was dissolved in dichloromethane, filtered through Celite®, washed with dichloromethane and concentrated in vacuo to give **14** (1.02 g, 94%) as a clear colorless oil which was used without further purification. TLC (ethyl acetate, R_f ~0.54). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.42 (s, 9H, CH_3), 1.80 (br s, 1H, OH), 2.34–2.59 (m, 2H, CH), 3.02 (m, 2H, CH), 3.72 (s, 3H, CH_3), 4.47–4.59 (m, 1H, CH), 4.60–4.70 (m, 1H, CH), 4.79–4.90 (m, 1H, CH), 4.96 (d, *J* = 7.91 Hz, 1H, NH), 6.71 (d, 2H, CH_{arom}), 7.02 (d, 2H, CH_{arom}). ESI-MS: *m/e* 366 $[M+H]^+$.

4.10. Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*cis*-3-fluorocyclobutyl)-L-tyrosine (15)

To an ice-cooled solution of methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*trans*-3-hydroxycyclobutyl)-L-tyrosine **14** (658 mg; 1.80 mmol) in dry dichloromethane (25 mL) was added DAST (358 µL; 2.70 mmol). The mixture was stirred at 0 °C for 3 h and was allowed to reach room temperature overnight. The crude product was concentrated in vacuo and purified by column chromatography with a gradient of ethyl acetate/hexane to give **15** (257 mg, 38%) as white crystals. TLC (ethyl acetate/hexane 1:2, R_f ~0.62). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.42 (s, 9H, CH_3), 2.36–2.53 (m, 2H, CH), 2.95–3.08 (m, 2H, CH), 3.72 (s, CH_3), 4.17–4.27 (m, 1H, CH), 4.50–4.60 (m, 1H, CH), 4.81 (quin, 0.5H, CH-F), 4.94 (quin, 0.5H, CH-F), 4.96 (d, *J* = 7.91 Hz, 1H, NH), 6.73 (d, *J* = 8.59 Hz, 2H, CH_{arom}), 7.03 (d, *J* = 8.59 Hz, 2H, CH_{arom}). ^{19}F NMR (376 MHz, $CDCl_3$): δ ppm −169.3. ESI-MS: *m/e* 368 $[M+H]^+$.

4.11. *N*-(*tert*-Butoxycarbonyl)-*O*-(*cis*-3-fluorocyclobutyl)-L-tyrosine (16)

To a solution of methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*cis*-3-fluorocyclobutyl)-L-tyrosine **15** (85 mg; 23 µmol) in methanol (3.5 mL) was added 1 M lithium hydroxide (650 µL; 65 µmol). The clear mixture was stirred for 6 h at room temperature. After TLC showed full conversion, the pH of the mixture was adjusted to pH 5 with 1 M HCl and concentrated in vacuo. The resulting oil was dissolved in ethyl acetate, washed with brine and evaporated to dryness, was then re-dissolved in ethyl acetate, dried with sodium sulfate, filtered and concentrated in vacuo to give **16** (80 mg, 97%) as a white solid. TLC (ethyl acetate/hexane 1:1, spot at start). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.41 (s, 9H, 3 CH_3), 2.33–2.51 (m, 2H, CH), 2.75 (d, 1H, CH), 2.90 (dd, 2H, CH), 3.12 (d, 2H, CH), 4.16–4.27 (m, 1H, CH), 4.52 (d, *J* = 5.56 Hz, 1H, CH), 4.76 (quin, 0.5H, CH–F), 4.90 (quin, 0.6H, CH–F), 4.98 (d, *J* = 7.58 Hz, 1H, NH), 6.05 (br s, COOH), 6.74 (d, *J* = 8.34 Hz, 2H, CH_{arom}), 7.09 (d, *J* = 8.34 Hz, 2H, CH_{arom}). ^{19}F NMR (376 MHz, $CDCl_3$): δ ppm −169.2. ESI-MS: *m/e* 354 $[M+H]^+$.

4.12. *O*-(*cis*-3-Fluorocyclobutyl)-L-tyrosine hydrochloride salt (L-17)

A solution of *N*-(*tert*-butoxycarbonyl)-*O*-(*cis*-3-fluorocyclobutyl)-L-tyrosine **16** (79 mg; 0.22 mmol) in DMF (0.6 mL) was treated with 4 M HCl in dioxane (0.5 mL; 2.24 mmol) for 1 h at room temperature. The mixture was concentrated in vacuo, re-suspended in ethanol/water (1:1) and extracted with a small amount of dichloromethane. To reduce the organic solvent, the aqueous phase was concentrated and gave after freeze–drying the product **17** (25 mg, 44%) as a white solid. TLC (DCM/MeOH 9:1, spot at start). ^{1}H NMR (300 MHz, CD_3OD): δ ppm 2.19–2.36 (m, 2H, CH), 2.95–3.12 (m, 2H, CH), 3.24–4.16 (m, 1H, CH), 4.27–4.37 (m, 1H, CH), 4.84 (quin, 1H, CH–F), 4.92 (quin, 1H, CH–F), 6.85 (d, 2H, CH_{arom}), 7.20 (d, 2H, CH_{arom}), COOH and NH_2 not visible. ^{13}C NMR (151 MHz, CD_3OD): δ ppm 36.7 (CH_2), 40.3 (CH_2), 40.5 (CH_2), 55.8 (CH), 63.6 (CH), 63.8 (CH), 81.0 (CH), 82.4 (CH), 116.6 (CH), 128.1 (CH), 131.7 (CH), 158.3 (C), 171.8 (C). ESI-MS: *m/e* 254 $[M+H]^+$. HPLC (chiral): ratio D/L 3%: 97%, rt = 3.84 min.[30]

4.13. *O*-(*cis*-3-Fluorocyclobutyl)-D-tyrosine hydrochloride salt (D-17)

O-(*cis*-3-Fluorocyclobutyl)-D-tyrosine hydrochloride salt D-**17** was synthesized according to the procedure described for its L-isomer L-**17**.

4.13.1. Methyl *O*-[*trans*-3-(benzyloxy)cyclobutyl]-*N*-(*tert*-butoxycarbonyl)-D-tyrosine (D-13)

Clear oil, 1.29 g (3.81 mmol) of D-**12** gave D-**13** (1.16 g, 72%). TLC (ethyl acetate/hexane 1:2, R_f ~0.39). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.43 (s, 9H, CH_3), 2.37–2.58 (m, 4H, CH), 2.92–3.11 (m, 3H, CH_3), 3.72 (s, 3H, CH_3), 4.30–4.39 (m, 1H, CH), 46 (s, 2H, CH_2), 4.54 (m, *J* = 7.16 Hz, 1H, CH), 4.79–4.88 (m, 1H, CH), 4.96 (d, *J* = 7.91 Hz, 1H, NH), 6.71 (d, *J* = 8.48 Hz, 2H, CH_{arom}), 7.02 (d, *J* = 8.48 Hz, 2H, CH_{arom}), 7.28–7.41 (m, 5H, CH_{arom}). ESI-MS: *m/e* 456 $[M+H]^+$.

4.13.2. Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*trans*-3-hydroxycyclobutyl)-D-tyrosine (D-14)

Clear oil, 1.52 g (2.96 mmol) of D-**13** gave D-**14** (1.35 g, 97%). TLC (DCM/MeOH 9:1, Rf ~0.59). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 1.42 (s, 9H, CH_3), 1.63 (br s, 1H, OH), 2.34–2.57 (m, 4H, CH), 2.93–3.11 (m, 2H, CH_2), 3.72 (s, 3H, CH_3), 4.48–4.59 (m, 1H, CH), 4.59–4.70 (m, 1H, CH), 4.80–4.89 (m, 1H, CH), 4.97 (d, *J* = 8.10 Hz, 1H, NH), 6.71 (d, *J* = 8.67 Hz, 2H, CH_{arom}) 7.02 (d, *J* = 8.48 Hz, 2H, CH_{arom}). ESI-MS: *m/e* 366 $[M+H]^+$.

4.13.3. Methyl *N*-(*tert*-butoxycarbonyl)-*O*-(*cis*-3-fluorocyclobutyl)-D-tyrosine (D-15)

White solid, 147 mg (4.16 mmol) of D-**14** gave of D-**15** (1.52 mg, 8%). TLC (ethyl acetate/hexane 1:2, R_f ~0.62). ^{1}H NMR (300 MHz,

$CDCl_3$): δ ppm 1.43 (s, 9H, CH_3) 2.37–2.52 (m, 4H, CH) 2.94–3.10 (m, 2H, CH_2) 3.72 (s, 3H, CH_3) 4.17–4.26 (m, 1H, CH) 4.51–4.59 (m, 1H, CH) 4.72–4.93 (m, 1H, CH) 4.94–4.99 (m, 1H, NH) 6.73 (d, J = 8.59 Hz, 2H, CH_{arom}) 7.03 (d, J = 8.34 Hz, 2H, CH_{arom}). ^{19}F NMR (376 MHz, $CDCl_3$): δ ppm −169.3. ESI-MS: *m/e* 368 $[M+H]^+$.

4.13.4. *N*-(*tert*-Butoxycarbonyl)-*O*-(*cis*-3-fluorocyclobutyl)-D-tyrosine (D-16)

White solid, 19 mg (56 μmol) of D-**15** gave D-**16** (21 mg, 95%). TLC (ethyl acetate/hexane 1:1, spot at start). ^1H NMR (300 MHz, CD_3OD): δ ppm 1.38 (s, 9H, CH_3), 2.17–2.37 (m, 2H, CH), 2.76–2.88 (m, 1H, CH), 2.93–3.04 (m, 1H, CH), 3.04–3.12 (m, 1H, CH), 4.28 (d, J = 4.04 Hz, 2H, CH_2), 4.71–4.81 (m, 1H, NH), 6.76 (d, J = 8.59 Hz, 2H, CH_{arom}), 7.13 (d, J = 8.59 Hz, 2H, CH_{arom}). ^{19}F NMR (376 MHz, CD_3OD): δ ppm −170.5.

4.13.5. *O*-(*cis*-3-Fluorocyclobutyl)-D-tyrosine hydrochloride salt (D-17)

White solid, 14 mg (48 μmol) of D-**16** gave D-**17** (17 mg, 92%). TLC (DCM/MeOH 9:1, spot at start). ^1H NMR (400 MHz, CD_3OD): δ ppm 2.17–2.36 (m, 2H, CH), 2.94–3.15 (m, 2H, CH), 3.19–3.28 (m, 1H, CH), 4.17 (dd, J = 7.58, 5.31 Hz, 1H, CH), 4.32 (m, 1H, CH), 4.77 (quin, J = 6.57 Hz, 1H, CH), 6.85 (d, J = 8.59 Hz, 2H, CH_{arom}), 7.20 (d, J = 8.59 Hz, 2H, CH_{arom}). ^{19}F NMR (376 MHz, CD_3OD): δ ppm −170.5. ESI-MS: *m/e* 254 $[M+H]^+$. HPLC (chiral): ratio D/L 96%: 4%, rt = 5.43 min.[30]

4.14. Radiosynthesis

The aqueous n.c.a. [^{18}F]fluoride from the cyclotron target (Eckert & Ziegler in Adlershof, Berlin, Germany) was separated from the [^{18}O]water with a QMA Sep-Pak® cartridge and collected into a 5 mL Wheaton glass V-vial (Wheaton Industries Inc., Millville, USA) containing a solution of Kryptofix® 2.2.2 (5 mg; 13 μmol) and K_2CO_3 (1 mg; 15 μmol) in acetonitrile (0.95 mL) and water (0.05 mL). The solvents were evaporated and the residue dried at 110 °C under a light nitrogen stream, more anhydrous acetonitrile (ACN) was added, and the drying process was repeated. The precursor *cis*-cyclobutane-1,3-diyl bis(toluene-4-sulfonate) **8** (5 mg; 13 μmol) in anhydrous ACN (0.5 mL) was added to the reaction vial containing the dry [^{18}F]fluoride-cryptate complex and the reaction mixture was stirred at 130 °C for 15 min in a sealed vial. The crude intermediate was purified by passing through a Waters C18 light Sep-Pak® (equilibrated with 5 mL ethanol followed by 5 mL water and dried by passing 20 mL air through), washing with water (5 mL) and eluted with ACN (0.8 mL) and the solvent was evaporated at 110 °C with a nitrogen stream. The *trans*-3-[^{18}F]fluorocyclobutyl toluene-4-sulfonate [^{18}F]**11** was diluted with DMSO (1 mL) and added to L-tyrosine (~5 mg; 28 μmol) with aqueous 10% sodium hydroxide (2 equiv, 22.1 μL). The reaction was heated at 150 °C for 10 min, cooled to 50 °C and quenched by addition of water acidified with 1 M HCl to pH 2 (4 mL). The crude product was purified by semi-preparative HPLC (Synergi Hydro RP 4μ, 250 × 10 mm; 15% acetonitrile in water acidified with 1 M HCl to pH 2; flow 3 mL/min). The product peak was collected, diluted with water acidified with 1 M HCl to pH 2 (25 mL) and trapped on a Waters C18 environmental cartridge (equilibrated with 10 mL ethanol, 10 mL acidified water and the cartridge dried by passing 20 mL air through). The cartridge was washed with water acidified with 1 M HCl to pH 2 (5 mL) and the product was eluted with fractions (0.5 ml) of 1:1 mixture of ethanol and phosphate buffered saline (total volume: 1.5 mL). Starting from 881 MBq [^{18}F]fluoride, 44 MBq (12%, decay corrected) of the desired product ([^{18}F]**17**) was obtained in excellent radiochemical purity (>99%) and within 144 min. Specific activity was found to be 25 GBq/μmol. Radiochemical and chemical purity were determined by analytical radio-HPLC (UV at 280 nm) using a ACE C18 column (50 × 4.6 mm) at a flow rate of 2 mL/min using a gradient (0–7 min) from ACN +0.1% TFA/water +0.1% TFA 5:95 (v/v) to 95:5 (v/v). The retention time of [^{18}F]**17** was 2.4–2.7 min.

[^{18}F]FET was synthesized via a 2 step reaction. In the first step, 2-bromoethyl 4-nitrobenzenesulfonate (10 mg; 49 μmol) in *o*-dichlorobenzene (0.5 mL) was reacted with the dry [^{18}F]fluoride–cryptate complex (preparation mentioned above) for 10 min at 120 °C in a sealed 5 mL Wheaton glass V-vial followed by distillation of the product 1-bromo-2-[^{18}F]fluoroethane at 120 °C for about 20 min into a receiver vial containing DMSO (0.5 mL).[36] The 2-bromoethyl 4-nitrobenzenesulfonate was synthesized according to literature[37] using 4-nitrobenzenesulfonyl chloride in presence of 1,2,2,6,6-pentamethylpiperidine. L-Tyrosine (5 mg; 28 μmol) dissolved in anhydrous DMSO (0.2 mL) and 2 M NaOH (27.6 μL; 55 μmol) were added to the receiver vial and heated at 120 °C for 15 min in a sealed vial. The reaction mixture was diluted with water (4 mL) and purified by semi-preparative HPLC (ACE 5 C18, 250 × 4.6 mm; 15% acetonitrile +0.1% TFA in water +0.1% TFA; flow 3 mL/min). The product peak (retention time ~15 min) was collected, diluted with water acidified with 1 M HCl to pH 2 (35 mL) and trapped on a Waters C18 plus cartridge (equilibrated with 5 mL ethanol, 10 mL acidified water and the cartridge dried by passing 20 mL air through). The cartridge was washed with water acidified with 1 M HCl to pH 2 (5 mL) and the product was eluted with fractions (0.5 ml) of 1:1 mixture of ethanol and water acidified with 1 M HCl to pH 2. Starting from 2889 MBq [^{18}F]fluoride, 136 MBq (12%, decay corrected) of the desired product was obtained in excellent radiochemical purity (>99%) and within 160 min.

4.15. Determination of the lipophilicity of the non-radioactive L-3-FCBT

The log*D* assay was performed manually in two single vials. L-3-FCBT (0.1 mg) was dissolved in 600 μL octanol saturated with 10 mM phosphate buffer pH 7.4 (with the help of sonication for 3 min) and equilibrated by shaking for 4 min at a constant temperature (20 °C). Phases were separated by centrifugation at 10 min (20 °C, 3000 rpm). The sample was analyzed by a Waters Alliance 2695 HPLC with a Xterra MS C18 2.5 μm column (4.6 × 30 mm) with a gradient of 5–65% ACN +0.01% TFA in water +0.01% TFA in 3 min, hold for 2 min and a flow rate of 1.5 mL/min. The samples were quantified at 254 nm by area integration.

4.16. Cell uptake studies

The A549 and DU145 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (invitrogen) supplemented with 10% fetal bovine serum (FBS) and the NCI-H460 were cultured in DMEM/F-12 (1:1) (invitrogen) supplemented with 10% FBS. The cells were seeded 1–2 days prior to the assay and grown until sub-confluency in 48 well plates. Prior to the assay, the cell culture medium was removed and the cells were washed with 500 μL phosphate buffered saline (PBS). After adding 250 μL of PBS +0.1% Bovine serum albumin (BSA) containing 0.2 MBq of L-3-[^{18}F]FCBT in 8% EtOH per well, the cells were incubated at 37 °C in a humidified atmosphere (5% CO_2) for 10, 20, 30 and 60 min. For the blocking experiment, the cells were co-incubated with non-radioactive FET (1 mM) and non-radioactive L-3-FCBT (1 mM) for 30 min to monitor radioactivity uptake. To stop tracer uptake after the time point, the incubation buffer was removed, the cells were washed with 500 μL PBS and lysed with 500 μL 1 M NaOH. Subsequently, the amount of radioactivity in the cell lysate was determined in a Perkin Elmer 1480 Wizard 3″ Automated Gamma Counter. Aliquots of the applied tracer amount were measured in a gamma counter to

determine the total amount in counts per minute (cpm) together with the samples to correct for radioactivity decay. Cell numbers per well were determined after detaching cells by trypsinization in 3 wells prior to start of the assay and counted with the Invitrogen Automated Cell Counter. The mean number of cells was calculated and normalized to 10^5 cells. The assay was performed in triplicates.

4.17. Determination of in vitro plasma stability

The tracers L-3-[^{18}F]FCBT [^{18}F]**17** and [^{18}F]FET were incubated in plasma of human and rats at 37 °C for different time periods (0, 30, 60, 90 and 120 min) at a concentration of 22 MBq/mL. Samples were precipitated with MeOH and subsequently centrifuged for 15 min at 3000 rpm and the supernatant was analyzed with an Agilent 1200 HPLC-system with a Raytest radio-detection with the column Phenomenex Luna 5 μm C18 100 Å LC column (250 × 4.6 mm) with a gradient of 5–95% ACN in water in 10 min and a flow rate of 1 mL/min and by TLC using the solvent Butanol/AcOH/H_2O 12:3:5 for developing the TLC plate. The stability of the test compound was quantified by comparison of the remaining amount at the different time points with the amount of the 0 min sample (control) and is expressed in% of initial concentration.

4.18. Determination of in vitro metabolic stability in rat hepatocytes

Hepatocytes from Han Wistar rats were isolated via a 2 step perfusion method. After perfusion, the liver was carefully removed from the rat: the liver capsule was opened and the hepatocytes were gently shaken out into a Petri dish with ice-cold Williams' medium E (WME). The resulting cell suspension was filtered through sterile gaze in 50 ml falcon tubes and centrifuged at 50×*g* for 3 min at room temperature. The cell pellet was re-suspended in 30 mL WME and centrifuged through a Percoll gradient for 2 times at 100×*g*. The hepatocytes were washed again with WME and resuspended in medium containing 5% Fetal Calf Serum (FCS). Cell viability was determined by trypan blue exclusion.

For the metabolic stability assay liver cells were distributed in WME containing 5% FCS to glass vials at a density of 0.5 × 10^6 vital cells/mL. The non-radioactive L-3-FCBT was added to a final concentration of 1 μM. During incubation, the hepatocyte suspensions were continuously shaken and aliquots were taken at 2, 8, 16, 30, 45 and 60 min, to which equal volumes of cold methanol were immediately added. Samples were frozen at −20 °C overnight, subsequently centrifuged for 15 min at 3000 rpm and the supernatant was analyzed with an Agilent 1200 HPLC-system with LC-MS/MS detection.

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