Journal of Medicinal Chemistry

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b00652 • Publication Date (Web): 10 Jul 2015

Just Accepted

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Development of Fluorine-18 Labeled Metabolically Activated Tracers for Imaging of Drug Efflux Transporters with PET

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ABSTRACT

Increased activity of efflux transporters, *e.g.* P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), at the blood-brain barrier is a pathological hallmark of many neurological diseases, and the resulting multiple drug resistance represents a major clinical challenge. Non-invasive imaging of transporter activity can help to clarify the underlying mechanisms of drug resistance, and facilitate diagnosis, patient stratification and treatment monitoring. We have developed a metabolically activated radiotracer for functional imaging of P-gp/BCRP activity with positron emission tomography (PET). In preclinical studies, the tracer showed excellent initial brain uptake and clean conversion to the desired metabolite, although at a sluggish rate. Blocking with P-gp/BCRP modulators led to increased levels of brain radioactivity; however, dynamic PET did not show differential clearance rates between treatment and control groups. Our results provide proof-of-concept for development of pro-drug tracers for imaging of P-gp/BCRP function *in vivo*, but also highlight some challenges associated with this strategy.

INTRODUCTION

Membrane transporters at the blood-brain barrier (BBB) play an important role in maintaining this physiological barrier and hence, brain homeostasis: selective uptake transporters ensure the supply of essential nutrients like glucose, amino acids and nucleotides, whilst efflux transporters extrude toxic metabolites from the brain and prevent xenobiotics from reaching the central nervous system (CNS), thus providing a cellular detoxification system. The most prevalent efflux transporters at the BBB endothelium belong to the superfamily of adenosine triphosphate-binding cassette (ABC) transporters, and include P-glycoprotein (P-gp; ABCB1) and breast cancer resistance protein (BCRP; ABCG2).[1] P-gp and BCRP are located at the luminal membrane of the endothelial cells, where they extrude an array of structurally different compounds. Pharmacophore models developed for P-gp substrates suggest that compounds exhibiting two hydrogen bond acceptors, two aromatic rings and additional hydrophobic features are readily transported by the efflux pump.[2] Recent research shows considerable overlap in substrate scope of P-gp and BCRP,[3] and an increasing body of evidence suggests that they co-localize and cooperate in transporting compounds at the BBB.[4] Drug entry into the brain is therefore highly restricted, and is largely limited to compounds that are not recognized by efflux transporters and are able to cross the BBB by passive diffusion. In the course of many prevalent CNS disorders, especially refractory diseases like epilepsy, mood disorders and brain cancers, the delicate balance of transporter activity is distorted. The BBB becomes ‘leaky’, enabling toxic substances to temporarily reach the CNS, and, as a compensatory mechanism, activity and expression levels of efflux transporters increase.[5] As a consequence of transporter upregulation at the BBB, CNS drug concentrations in the brain become too low to achieve a therapeutic effect. The resulting multiple drug resistance (MDR) affects 20–40% of all patients suffering from

epilepsy, depression and schizophrenia, and is even more prevalent in drug refractory brain cancer and following human immunodeficiency virus infections.[6]

Quantitative assessment of efflux pump function by positron emission tomography (PET) can help to clarify the underlying mechanisms of drug resistance in individual patients, and facilitate stratification of patients for specific treatment strategies. As P-gp/BCRP mediates extrusion of the most commonly prescribed CNS drugs,[6c,7] a PET tracer that enables functional imaging of this transporter system can potentially be applicable to large patient cohorts spanning a range of diverse neurological diseases.

In recent years, a number of strategies for imaging of drug efflux transporters have been evaluated. Tracers that have been designed as inhibitors, e.g. [^{11}C]tariquidar and analogues, often act as substrates when administered in the low doses that are required for imaging with PET.[8] These P-gp/BCRP modulators, and P-gp substrates like [^{11}C]verapamil all have significant limitations: poor metabolic stability, low brain uptake (substrates), low binding affinity (inhibitors), and insufficient selectivity make quantitative imaging of drug efflux pumps challenging.[9] A more recent approach is the development of metabolically activated (pro-drug) tracers, as exemplified by carbon-11- and fluorine-18-labeled 6-halopurines for imaging of multidrug resistance protein 1.[10] The so-called metabolite extrusion method (MEM) relies on passive diffusion of a pro-drug tracer into the organ of interest, *e.g.* the brain, its subsequent trapping by metabolic conversion into an efflux pump substrate ('lock-in' mechanism), and transporter-mediated extrusion of the metabolite.[11] Provided that the physicochemical properties of the metabolite preclude passive diffusion across membranes, drug efflux transporter function can be directly correlated to the clearance rate of radioactivity from the target tissue, and hence readily be quantified by dynamic imaging. In order to allow MEM to be exploited for

quantitative imaging of P-gp/BCRP function *in vivo*, a pro-drug tracer should have a high initial brain uptake and be rapidly transformed into a single radioactive metabolite that acts as a dual P-gp/BCRP substrate, but does not interact with other transporters or targets in the brain. The radioactive metabolite should be unable to cross membranes by passive diffusion, so that in the absence of P-gp and BCRP, it is effectively trapped.[9b]

Histamine H_1 receptor (H_1R) antagonists are widely used for the treatment of allergies, and are often characterized by their ability to cross the BBB: compounds of the first generation readily enter the brain causing H_1R-mediated side effects, whereas substances of the second generation are avid P-gp substrates and hence give minimal exposure to the CNS.[12] Interestingly, several H_1R antagonists currently in clinical use are active metabolites of early generation drugs, e.g. cetirizine and fexofenadine are zwitterions formed by enzymatic oxidation of hydroxyzine and terfenadine, respectively.[13] These well-characterized metabolic pairs of H_1R antagonists were used as templates for the design of radiolabeled pro-drug tracers and metabolically activated dual P-gp/BCRP substrates (Figure 1).

RESULTS & DISCUSSION

Chemistry. Using the H_1R antagonists fexofenadine and cetirizine as templates, a library of compounds was prepared (Scheme 1 and Scheme 2) in order to identify (i) structural motifs required for P-gp substrate activity, (ii) functionalities that could be exploited as pro-drug moieties for metabolic activation, and (iii) potential positions for labeling with fluorine-18 (Figure 1). With the aim to circumvent the need for complex synthetic routes, 1-benzhydrylpiperazine (scaffold **A**, derived from cetirizine) and diphenyl(piperidin-4-yl)methanol (scaffold **B**, derived from fexofenadine) derivatives decorated with benzylic and aliphatic side

chains were initially evaluated. Scaffold **B** as well as its mono- and difluorinated analogues **C** and **D**, which were obtained by Grignard reactions with para-substituted piperidine derivatives (*vide infra*), were reacted with suitably functionalized benzylic halides by means of *N*-alkylation to give compounds **1**, **3**, **6**, **9**, **12**, **14**, and **16** (Scheme 1).[14] The alkylating reagents were either commercially available, or prepared from the respective tolyl derivatives by radical bromination with *N*-bromosuccinimide. The alcohol **1** was further modified by Swern oxidation to obtain aldehyde **2**,[15] whereas hydrolysis of the esters **3**, **6**, **9**, **12**, **14**, and **16** under basic conditions gave the corresponding carboxylic acids **4**, **7**, **10**, **13**, **15**, and **17**.

A fluorinated aliphatic side chain was prepared as depicted in Scheme 2. Treatment of α-hydroxy-γ-butyrolactone with DAST provided the fluorinated lactone **18**,[16] which after delactonization with methanol gave alcohol **19**, albeit in moderate yield (20%).[17] The alcohol **19** was converted into the corresponding tosylate **20**, which was used for subsequent *N*-alkylation of scaffolds **A** and **B**.[18] The resulting esters **21** and **25** were either reduced to give alcohols **22** and **26,**[19] or hydrolysed to yield the acids **24** and **28**.[20] Finally, treatment of the alcohols **22** and **26** with acetic anhydride provided the acyl esters **23** and **27**.

As biodistribution studies with [^{18}F]**23** and [^{18}F]**27** pointed to rapid defluorination of the aliphatic side chain *in vivo* (*vide infra*), a second set of compounds was designed consisting of fluorinated aromatic scaffolds and aliphatic side chains carrying pro-drug groups (alcohol, aldehyde and ester) as putative tracer candidates. To enable tuning of lipophilicity, additional scaffolds (**C**-**F**) were prepared (Scheme 3). Treatment of Boc-protected piperidine-4-carboxylic acid ethyl ester with 4-fluorophenylmagnesium bromide gave the difluorinated compound **33**.[21] The corresponding mono-fluorinated analogue **32** was prepared from Boc-protected 4-hydroxymethyl-piperidine using sequential oxidation and Grignard reactions.[22] Deprotection of

compounds **31**-**33** provided the mono- and difluorinated scaffolds **34, 35** and **37** (= scaffold **C, D** and **F**), whereas treatment of **32** with trifluoroacetic acid resulted in elimination of the tertiary alcohol to give compound **36** (= scaffold **E**). *N*-alkylation of scaffolds **C**, **E** and **F** with appropriately functionalized alkyl bromides provided the putative pro-drug tracers and non-radioactive reference compounds **38–47** (Figure 2).

In order to enable labeling of the putative tracers **23** and **27** with fluorine-18, the corresponding mesylate precursors **52** and **56** were prepared from a dioxolane building block (Scheme 4). The tosylate **48** was coupled to scaffolds **A** and **B**, and the resulting protected diols **49** and **53** were subsequently hydrolyzed under acidic conditions to give vicinal diols **50** and **54**. Selective acetylation of the primary alcohol in compounds **50** and **54** was achieved with acetic anhydride to give the acetates **51** and **55**, which upon mesylation gave the desired precursors **52** and **56**, respectively.

Whereas labeling of aliphatic groups with fluorine-18 is well established, aromatic [^{18}F]fluorination of small molecules bearing complex functional groups remains a challenge.[23] Limitations of existing labeling methods prompted us to explore the use of sulfonium salts[24] as leaving groups for aromatic [^{18}F]fluorination of the scaffolds described above.[25] For the synthesis of the sulfonium salts **62** and **64** bearing an electron deficient scaffold, the ketone **59** was prepared by a lithium-mediated reaction of bromoarene **57** and Weinreb amide **58** (Scheme 5). After Boc deprotection of **59**, the desired side chains were introduced by *N*-alkylation, and the ammonium salts of the resulting diaryl thioethers **61** and **63** were treated with diphenyliodonium triflate in the presence of copper(II) benzoate to give the triarylsulfonium salts **62** and **64**, respectively. Labeling of compounds with the electron neutral scaffold **E** required decoration of the sulfonium salts with anisole moieties in order to achieve regioselective

[^{18}F]fluorination. The thioether **65** was converted into ketone **66** as described above. Addition of phenylmagnesium bromide to **66** provided alcohol **67**, which upon treatment with trifluoroacetic acid yielded the deprotected 4-(methylene)piperidine **68**. Following the route outlined above, *N*-alkylation of **68** gave compounds **69**, **71** and **73**, which were converted into the corresponding triarylsulfonium salts **70**, **72** and **74** using bis-(4-methoxyphenyl)iodonium triflate **75**.[26]

Radiochemistry. Radiolabeling of the mesylate precursors **52** and **56** (Scheme 6) with [^{18}F]fluoride worked well in dimethyl sulfoxide under heating (20 min at 90 °C). Acetyl esters [^{18}F]**23** and [^{18}F]**27** were obtained in 40–50% analytical radiochemical yield (RCY) as determined by radio-HPLC. The isolated products were radiochemically pure but the specific activity was low (0.5 GBq/μmol) due to extensive decomposition of the respective precursors. As attempted hydrolysis of [^{18}F]**23** and [^{18}F]**27** to the corresponding alcohols [^{18}F]**22** and [^{18}F]**26** further reduced the specific activity, the acetyl esters [^{18}F]**23** and [^{18}F]**27** were used for an initial biological evaluation of compounds [^{18}F]**22** and [^{18}F]**26** assuming that the esters would be rapidly cleaved by esterases in the blood after intravenous injection (Scheme 6).

[^{18}F]Fluorination of triarylsulfonium salts gave the highest radiochemical yields when using a combination of potassium bicarbonate, kryptofix and DMSO with heating to 110 °C for 15 min.[25] In the case of substrates containing electron withdrawing groups on the aromatic moiety, *i.e.* the ketones **62** and **64** (Scheme 7), the radiochemical reaction was exceptionally clean and yielded [^{18}F]**45** (36–40% decay-corrected isolated RCY, n = 3) and [^{18}F]**47** (22% decay-corrected isolated RCY), respectively, without formation of any radioactive side products. Hydrolysis of [^{18}F]**45** afforded the alcohol [^{18}F]**46** (18% decay-corrected isolated RCY after two steps), whereas treatment of [^{18}F]**45** with phenylmagnesium bromide led to formation of the fexofenadine-like scaffold **C** with concurrent cleavage of the ester group to give the alcohol

[^{18}F]**39** (16% decay-corrected isolated RCY after two steps). Subsequent reaction of [^{18}F]**39** with acetyl chloride provided the acyl ester [^{18}F]**38**.

Compounds containing the non-activated ritanserin-like scaffold **E** were prepared by direct labeling of the respective sulfonium salts **70**, **72** and **74** with [^{18}F]fluoride (Scheme 8). The resulting esters [^{18}F]**40a** and [^{18}F]**43** as well as the acetal [^{18}F]**41** were hydrolyzed to give the alcohol [^{18}F]**40**, the carboxylic acid [^{18}F]**44** and the aldehyde [^{18}F]**43**, respectively. The radiochemical purity of the desired radiotracers ([^{18}F]**38**-[^{18}F]**40**, [^{18}F]**42**-[^{18}F]**44**, [^{18}F]**46** and [^{18}F]**47**) was > 96%, however, the use of multi-step synthesis resulted in moderate overall yields (2–18% decay-corrected isolated RCY after formulation in saline).

In our hands, preparation of sulfonium salts from the corresponding thioethers provided a practical and versatile synthetic method that gave rapid access to a number of structurally diverse precursors for labeling. Conveniently, triarylsulfonium salts are highly stable, easy to handle, and can readily be purified using conventional techniques including extractive aqueous workup and column chromatography on silica gel. In labeling studies, the precursors showed a remarkable reactivity with [^{18}F]fluoride: activated substrates reacted at room temperature, and with heating the reaction proceeded in the presence of hydrogen bond donors. Furthermore, efficient and regioselective [^{18}F]fluorination of non-activated aromatic moieties was achieved using sulfonium salts decorated with electron rich spectator ligands. The ability to label a spectrum of electron neutral to electron deficient aromatic groups with ^{18}F in the presence of basic moieties, as well as hydrogen bond donors, makes the method broadly applicable to drug-like compounds, and hence opens up extensive pharmacological space for the design of small molecule PET tracers.

***In vitro* compound screening.** A luminescent P-gp ATPase assay was initially used to establish if the simplified fexofenadine derivatives retained the P-gp substrate activity of the

parent compounds, and to identify putative metabolic pairs that could be exploited for the design of a pro-drug tracer. The assay allows rapid screening of P-gp substrate affinity, but the accuracy is insufficient to determine IC_{50} values.[27] In order to determine structural elements that are prerequisite to efflux pump substrate activity, ATP turnover triggered by the carboxylic acids **4**, **7** and **10** was compared to that of parent compound fexofenadine (Figure 3A). Of the test compounds, the benzoic acid **4** evoked the highest ATPase activity, which was similar to that of fexofenadine, whereas ketone **7** and isobutyric acid **10** appeared to be poor P-gp substrates. To evaluate functional groups for the design of pro-drug tracers, the benzylic alcohol **1**, benzaldehyde **2**, and benzoic ester **3** were tested (Figure 3B). The results pointed to a gradual increase in P-gp affinity with increasing oxidation state, with alcohol **1** resulting in modest ATP consumption, whereas the aldehyde **2** and ester **3** appeared to be more avid substrates. Remarkably, incorporation of fluorine into the benzoic moiety of **4** to give compound **13** practically abolished the P-gp substrate affinity (Figure 3C). However, fluorine substituents on scaffold **B** were well tolerated, and the mono- and difluorinated derivatives **15** and **17** evoked ATP turnover comparable to that of compound **4**. The use of a fluorinated aliphatic side chain, as for alcohol **22** and carboxylic acid **24**, also appeared to be tolerated, and evoked ATP consumption to the same degree as their parent compounds hydroxyzine, and its metabolite cetirizine, respectively (Figure 3D). The results suggest that the zwitterions **4** and **24** are good P-gp substrates, whereas the corresponding alcohols **1** and **22** are likely to evade active efflux, and hence are suitable leads for pro-drug tracer development.

Tracer Evaluation *in Vivo*. The putative pro-drug tracers were evaluated using biodistribution studies in mice. The tissue distribution of radioactivity at 5 and 30 min post-injection (p.i.) was used to assess brain uptake, brain/blood ratios (Table 1), and the rate of clearance. Two

derivatives labeled at the aliphatic side chain, namely [^{18}F]**23** (cetirizine-like scaffold **A**) and [^{18}F]**27** (fexofenadine-like scaffold **B**), were initially investigated to guide the choice of scaffold. As discussed above, we envisaged that ester hydrolysis would lead to rapid formation of the resulting alcohols [^{18}F]**22** and [^{18}F]**26** *in vivo*. Administration of [^{18}F]**23** and [^{18}F]**27** resulted in good initial uptake of radioactivity in the brain (5.8 and 7.2% injected dose per gram tissue (ID/g) at 5 min p.i., respectively), however, high uptake (3–6% ID/g at 30 min p.i.) was also observed in bone tissue (femur and skull) suggesting rapid enzymatic defluorination *in vivo*. Hence, we abandoned further evaluation of derivatives containing fluorinated aliphatic side chains, and instead evaluated pro-drug tracers bearing fluorine-18 at aromatic moieties. Indeed, administration of the acetate ester [^{18}F]**38**, a structurally closely related analogue to [^{18}F]**27**, exhibited far superior C-F bond stability as judged by the low level of radioactivity in bone tissue (< 2% ID/g at 30 min p.i.). However, the brain uptake and brain/blood ratio of alcohol [^{18}F]**39**, as well as the corresponding acetyl ester [^{18}F]**38**, were low. To improve the initial uptake of the pro-drug tracer in the brain, we prepared an analogue of [^{18}F]**39** for which the hydroxyl group in scaffold **B** was replaced by an alkene to give scaffold **E**. The resulting pro-drug tracer [^{18}F]**40** exhibited excellent initial brain uptake (9.4% ID/g at 5 min p.i.) with high brain/blood ratios (10.4 and 7.3 at 5 and 30 min p.i., respectively). In contrast, administration of the corresponding carboxylic acid [^{18}F]**44** resulted in an initial brain uptake of 1.3% ID/g at 5 min p.i. with a brain/blood ratio of 0.4. In accordance with the results from the *in vitro* assay, replacement of the alcohol with an aldehyde ([^{18}F]**42**) or ester ([^{18}F]**43**) as the pro-drug group proved futile, and resulted in moderate brain uptake (3.1 and 2.9 ID/g at 5 min p.i., respectively). Alcohol [^{18}F]**46**, derived from scaffold **F**, showed good initial brain uptake (5.0% ID/g at 5 min p.i.), but brain clearance was rapid (1.8% ID/g at 30 min p.i.). The structurally related ester [^{18}F]**47** hardly

penetrated the brain, most likely due to the rapid hydrolysis *in vivo* to give the corresponding carboxylic acid (70% conversion at 5 min p.i.).

The 4-hydroxybutyl pro-drug tracer [^{18}F]**40** showed high initial brain uptake and a favorable clearance from the brain and was thus investigated further (Figure 4). The brain metabolism was exceptionally clean and gave the desired carboxylic acid [^{18}F]**44** as the only radioactive metabolite. The ratio of [^{18}F]**40** to [^{18}F]**44** in the brain decreased over time and was determined to be 6.1 at 30 min p.i., 1.5 at 60 min p.i., 0.6 at 90 min p.i. and 0.4 at 120 min p.i., respectively. However, as [^{18}F]**44** is extruded from the brain into the periphery, this ratio does not reflect the actual rate of metabolism. In the blood, oxidation of the alcohol was faster: whilst low amounts of [^{18}F]**40** were still detectable, [^{18}F]**44** constituted between 70 and 85% of total amount of radioactivity detected between 60 and 120 min p.i. (Figure 4C/D). Development of a MEM tracer requires that the radioactive metabolite does not cross the BBB to any significant extent by other mechanisms than extrusion by the targeted efflux pump. As the brain/blood ratios observed after administration of the carboxylic acid [^{18}F]**44** remained at or below 1.1 over the course of two hours after administration (Figure 4B), our results suggest that brain clearance from [^{18}F]**44** is largely mediated by active efflux. However, a faster metabolism of the pro-drug tracer [^{18}F]**40** would be desirable. The aldehyde [^{18}F]**42** and ester [^{18}F]**43** were designed as to accelerate formation of the carboxylic acid [^{18}F]**44** *in vivo*. Although metabolism to the zwitterions [^{18}F]**44** was completed within five (ester) and 60 (aldehyde) minutes, respectively, their low brain/blood ratios rendered them unsuitable as pro-drug tracers (*cf*. Table 1).

The pro-drug tracer [^{18}F]**40** was investigated further to determine if the corresponding metabolite [^{18}F]**44** acted as an efflux pump substrate *in vivo*. By injecting the dual P-gp/BCRP inhibitor tariquidar one to two hours prior to tracer administration at a dose of 15 mg/kg

bodyweight it was ensured that P-gp was fully blocked over the course of the biodistribution studies (longest time point 120 min p.i.).[28] The tariquidar dose is, however, not sufficiently high to fully inhibit BCRP. In a separate experiment, injection of elacridar (10 mg/kg bodyweight) was used to completely block both P-gp and BCRP.[8c] The comparison of the organ distribution without and with concomitant administration of tariquidar and elacridar, respectively (Figure 5A), showed a similar brain uptake at 30 min p.i. (ca. 8% ID/g) in all animals, but revealed a significant effect on efflux pump inhibition at 60–120 min p.i.: when blocking with tariquidar, tracer brain uptake (in %ID/g) at 60 and 90 min p.i. was 30 and 50% higher than in untreated animals. Using elacridar, the amount of tracer in the brain was doubled between 60 and 120 min p.i. Tracer levels in the blood remained constant between 1 and 2% ID/g in all animals. The results provide evidence for active transport of the metabolically activated tracer [^{18}F]**44** across the BBB by P-gp as well as BCRP.

Dynamic PET scans were performed in untreated mice, mice treated with either tariquidar or elacridar, and P-gp knockout mice (Figure 5B/C). Whole brain time-activity curves were fitted with the sum of a constant (irreversible compartment, representing the amount of tracer trapped in the brain) and a mono-exponential function (reversible compartment, representing the amount of tracer washed out from the brain and the efflux rate).[29] For analysis and determination of efflux rates, data from 10 min p.i. onwards was used as this gave the best match between the fitted curves and the experimental results. Focusing on the time from 60 min p.i. onwards, *i.e.* when increasing formation of the metabolite [^{18}F]**44** was observed, did not change the quantitative outcome measure.

In a first set of experiments (performed at AIT), untreated, tariquidar treated and P-gp knockout mice (Figure 5B) showed comparable initial brain uptake. The ratio of tracer that was

washed out from the brain to tracer that was trapped (washout/trapped ratio) was similar in untreated and knockout mice (2.9 and 2.8, respectively), whereas in tariquidar treated mice, the washout/trapped ratio was lower (1.5). This suggests that more tracer was trapped in the brains of the treated mice. In a second set of experiments (performed at UCL), elacridar treated mice were compared to untreated animals and showed a lower washout/trapped ratio (2.3 versus 4.1). The marginally higher peak brain uptake in elacridar treated mice compared to the control group may have contributed to the increased amounts of radioactivity at later time points (Figure 5C). It should be noted that discrepancies between the two control groups (washout/trapped ratio of 1.5 and 2.3, respectively) are most likely due to variations in the experimental setup. The difference in washout/trapped ratios provides evidence for [^{18}F]**44** acting as a dual P-gp/BCRP substrate, and the experiments in P-gp knockout mice suggest that BCRP can compensate for P-gp when extruding [^{18}F]**44**. However, minor differences in efflux rates when comparing untreated and treated mice raise the possibility that not only tracer [^{18}F]**44** but also the pro-drug tracer [^{18}F]**40** was washed out of the brain, either by passive diffusion or by active transport as an efflux pump substrate.

Due to the complimentary and partially overlapping substrate scope of efflux pumps belonging to the large family of ABC transporters it is possible that transporters other than P-gp/BCRP contribute to the efflux of [^{18}F]**40**/[^{18}F]**44**.[30] The tracer kinetics can also be affected by other factors such as off-target binding. Indeed, both the alcohol **40** and the carboxylic acid **44** showed high affinity to the human H_1R (2.3 ± 0.4 and 18 ± 4 nM, respectively) as determined in an *in vitro* ligand displacement assay. The affinity to H_1R may contribute to the retention of the pro-drug tracer [^{18}F]**40** in the brain and may affect the rate of oxidative metabolism. Moreover, the metabolic activation, *i.e.* the conversion of [^{18}F]**40** to [^{18}F]**44**, appeared to be slower than the

extrusion of the metabolite [^{18}F]**44**, and as a result there was no significant difference between the brain clearance rates of the control group and the two treatment groups. However, differences in the oxidizing rate of enzymes, as well as in efflux transporter distribution and function, may make [^{18}F]**40** more suitable as a pro-drug tracer in other species.[31]

SUMMARY & CONCLUSIONS

With the aim to develop a pro-drug tracer to enable dynamic imaging of P-gp/BCRP function *in vivo*, we used H_1R antagonists as lead compounds to synthesize a library of putative pro-drug tracers and their corresponding metabolites. In biodistribution studies, derivatives containing a fluorinated aliphatic side chain were found to undergo rapid defluorination *in vivo*. The results prompted us to develop triarylsulfonium salts as novel precursors for aromatic labeling of drug-like molecules with fluorine-18. This labeling strategy provided access to a range of putative pro-drug tracers and corresponding metabolites, and allowed identification of the alcohol [^{18}F]**40** as a lead pro-drug tracer candidate. [^{18}F]**40** fulfills many of the requirements of MEM: it has excellent initial brain uptake (9.4% ID/g 5 min p.i.) and undergoes clean metabolic conversion to the desired carboxylate [^{18}F]**44** in the brain. Blocking experiments with the dual P-gp/BCRP inhibitors tariquidar and elacridar led to significantly increased levels of radioactivity in the brain in a period between 60 and 120 minutes after tracer injection, suggesting that the metabolite [^{18}F]**44** is a substrate for both P-gp and BCRP. However, there was no significant difference in the brain efflux rates between control animals and treatment groups as measured by dynamic PET. Because of the sluggish metabolic conversion and cross-reactivity with H_1R we were not able to use [^{18}F]**40**/[^{18}F]**44** for quantification of the efflux rate; however, the tracer is still likely to provide a qualitative depiction of regional brain efflux rates.

Our results provide proof-of-concept for development of metabolically activated pro-drug tracers for imaging of P-gp/BCRP activity with PET. However, the results also highlight some of the challenges in achieving appropriate rates of enzymatic conversion and brain clearance.

EXPERIMENTAL SECTION

Chemistry. Reagents were purchased from Sigma-Aldrich, Acros Organics or Fluorochem and were used without further purification. Purification of non-labeled compounds by column chromatography was performed on silica unless otherwise specified. For characterization of non-labeled compounds, ^{1}H and ^{13}C NMR spectra were recorded at room temperature unless otherwise specified. The respective instruments, Bruker Avance 400, 500 or 600 were operated at a frequency of 400, 500 or 600 MHz for ^{1}H and 125 or 150 MHz for ^{13}C, respectively. Proton decoupled ^{19}F NMR spectra were recorded on a Bruker Avance 300 instrument at a frequency of 282 MHz. All spectra were internally referenced to the respective deuterated solvents. Chemical shifts are reported in ppm and coupling constants (*J*) are given in Hertz (Hz). Full NMR assignment was performed with the aid of multidimensional and long range experiments. High resolution mass data were recorded on either a Thermo Finnigan MAT900xp (CI, EI) or a MALDI microMX (TOF) mass spectrometer. Melting points were taken on a Gallenkamp heating block and are uncorrected. Elemental analysis (C, H, N) was performed on a CE440 analyser (Exeter Analytical).

We have recently described the synthesis of compounds **29–32**, **36**, **37**, **40**, **43**, **45**, **46**, **57–62**, **65–72** and **75**.[25] Compounds **18**,[16] **34**,[32] **35**[32] and **48**[33] have been described by others, and analytical data are in accordance with the published data.

(1-(4-(Hydroxymethyl)benzyl)piperidin-4-yl)diphenylmethanol (1). Under inert atmosphere, diphenyl(piperidin-4-yl)methanol hydrochloride (2.1 g, 7.0 mmol), (4-(chloromethyl)phenyl)methanol (1.0 g, 6.4 mmol), potassium carbonate (2.9 g, 21.0 mmol) and potassium iodide (0.1 g, 0.64 mmol) were heated in acetone (25 ml) for 6 h. After cooling the inorganic components were filtered off and the filtrate was purified by column chromatography (TEA 0.5%; DCM: methanol = 100: 0 → 98: 2). The resulting colorless oil was triturated with diethyl ether to give a white solid (0.3 g, 13%). Mp: 119 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.48 (d, J = 7.4 Hz, 4H, ph'-2,6*H*), 7.29-7.22 (m, 8H, ph-2,3,5,6*H*, ph'-3,5*H*), 7.10 (t, J = 7.3 Hz, 2H, ph'-4*H*), 5.24 (s, 1H, CO*H*), 5.11 (t, J = 4.9 Hz, 1H, CH_2O*H*), 4.46 (d, J = 5.5 Hz, 2H, CH_2OH), 3.41 (br s, 2H, NCH_2), 2.81 (br s, 2H, pip-2,6H^{eq}), 2.53-2.49 (m, 1H, pip-4*H*), 2.06 (br s, 2H, pip-2,6H^{ax}), 1.49-1.47 (m, 2H, pip-3,5H^{ax}), 1.24 (d, J = 11.1 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 147.3 (ph'-1*C*), 128.7 (ph-1*C*), 127.8 (ph'-2,6*C*), 126.3 (ph'-4*C*), 125.8 (ph-4*C*), 125.7 (ph'-3,5*C*, ph-2,3,5,6*C*), 78.5 (*C*OH), 62.7 (*C*H_2OH), 62.3 (N*C*H_2), 53.3 (pip-2,6*C*), 43.3 (pip-4*C*), 25.9 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{29}NO_2$, 386.2120; found, 386.2129. Elemental analysis: calcd. for $C_{26}H_{29}NO_2 \cdot 0.75\ H_2O$, C 77.87, H 7.67, N 3.49; found, C 77.94, H 7.39, N 3.49.

4-((4-(Hydroxydiphenylmethyl)piperidin-1-yl)methyl)benzaldehyde (2). Oxalyl chloride (0.22 ml, 2.6 mmol) was dissolved in DCM (5 ml) and the solution was cooled to < -50 °C. Under inert conditions, DMSO (0.22 ml, 3.1 mmol) dissolved in DCM (2 ml) was added dropwise and the solution was stirred for 15 min. Alcohol **1** (0.25 g, 0.65 mmol) dissolved in DCM (5 ml) was added and the solution was stirred for further 15 min. The reaction was quenched with TEA (0.8 ml, 5.7 mmol) and allowed to warm to room temperature. Water (30 ml) was added and the solution extracted with DCM. The organic phase was dried ($MgSO_4$),

filtered, concentrated and crystallized using a mixture of petroleum ether: ethyl acetate = 3: 2 to give the product as a white solid (0.09 g, 34%). Mp: 149 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.98 (s, 1H, C*H*O), 7.82 (d, J = 8.2 Hz, 4H, ph-2,6*H*), 7.53-7.48 (m, 6H, ph-3,5*H*, ph'-2,6*H*), 7.23 (t, J = 7.8 Hz, 4H, ph'-3,5*H*), 7.10 (t, J = 7.3 Hz, 2H, ph'-4*H*), 5.23 (s, 1H, CO*H*), 3.51 (s, 2H, NCH_2), 2.76 (d, J = 11.2 Hz, 2H, pip-2,6H^{eq}), 2.53-2.45 (m, 1H, pip-4*H*), 1.97 (t, J = 11.0 Hz, 2H, pip-2,6H^{ax}), 1.53-1.44 (m, 2H, pip-3,5H^{ax}), 1.23 (d, J = 12.8 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 192.8 (*C*HO), 147.3 (ph'-1*C*), 146.2 (ph-4*C*), 135.1 (ph-1*C*), 129.5 (ph-2,6*C*), 129.2 (ph-3,5*C*), 127.8 (ph'-3,5*C*), 125.8 (ph'-4*C*), 125.8 (ph'-2,6*C*), 78.5 (*C*OH), 62.0 (N*C*H$_2$), 53.6 (pip-2,6*C*), 43.3 (pip-4*C*), 26.0 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{27}NO_2$, 386.2120; found, 386.2106. Elemental analysis: calcd. for $C_{26}H_{27}NO_2 \cdot 0.5$ H_2O, C 79.16, H 7.15, N 3.55; found, C 78.95, H 6.90, N 3.48.

Methyl 4-((4-(hydroxydiphenylmethyl)piperidin-1-yl)methyl)benzoate (3). Under inert atmosphere, diphenyl(piperidin-4-yl)methanol hydrochloride (2.0 g, 6.6 mmol), methyl 4-(bromomethyl)benzoate (1.26 g, 5.5 mmol), potassium carbonate (2.73 g, 19.8 mmol) and potassium iodide were suspended in acetonitrile (50 ml) and refluxed overnight. After cooling to room temperature the inorganic components were filtered off and the filtrate was purified by column chromatography (TEA; DCM: methanol = 100: 0 → 98: 2). The product was obtained as a white solid (1.4 g, 61%). Mp: 116 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.87 (d, J = 8.2 Hz, 2H, ph-2,6*H*), 7.48 (d, J = 7.6 Hz, 4H, ph'-2,6*H*), 7.41 (d, J = 8.2 Hz, 2H, ph-3,5*H*), 7.23 (t, J = 7.7 Hz, 4H, ph'-3,5*H*), 7.09 (t, J = 7.3, 2H, ph'-4*H*), 5.25 (s, 1H, O*H*), 3.81 (s, 3H, OCH_3), 3.47 (s, 2H, NCH_2), 2.74 (d, J = 10.8 Hz, 2H, pip-2,6H^{eq}), 2.50-2.43 (m, 1H, pip-4*H*), 1.94 (t, J = 11.0 Hz, 2H, pip-2,6H^{ax}), 1.54-1.43 (m, 2H, pip-3,5H^{ax}), 1.23 (d, J = 12.1 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 166.1 (*C*=O), 147.2 (ph'-1*C*), 144.6 (ph-4*C*), 129.0 (ph-2,6*C*),

128.7 (ph-3,5*C*), 128.1 (ph-1*C*), 127.7 (ph'-3,5*C*), 125.7 (ph'-4*C*), 125.7 (ph'-2,6*C*), 78.4 (*C*OH), 61.8 (N*C*H_2), 53.5 (pip-2,6*C*), 52.0 (O*C*H_3), 43.3 (pip-4*C*), 25.8 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{27}H_{29}NO_3$, 415.2142; found, 415.2131. Elemental analysis: calcd. for $C_{27}H_{29}NO_3$, C 78.04, H 7.03, N 3.37; found, C 77.79, H 7.04, N 3.32.

4-((4-(Hydroxydiphenylmethyl)piperidin-1-yl)methyl)benzoic acid (4). Compound **3** (1.0 g, 2.4 mmol) was dissolved in methanol (2 ml) and THF (2 ml). Aqueous potassium hydroxide (2 M, 4 ml) was added and the mixture was heated in the microwave oven for 15 min at 70 °C. After cooling the organic solvents were removed under reduced pressure and the aqueous solution neutralized with HCl. The product precipitated as white solid that was recrystallized from 2-propanol under addition of a drop of water (0.71 g, 74%). Mp: > 250 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.91 (d, J = 8.1 Hz, 2H, ph-2,6*H*), 7.53-7.48 (m, 6H, ph-3,5*H*, ph'-2,6*H*), 7.24 (t, J = 7.7 Hz, 4H, ph'-3,5*H*), 7.11 (t, J = 7.3 Hz, 2H, ph'-4*H*), 5.54 (s, 1H, O*H*), 3.77 (br s, 2H, NCH_2), 2.95 (br s, 2H, pip-2,6H^{eq}), 2.58 (br s, 1H, pip-4*H*), 2.30 (br s, 2H, pip-2,6H^{ax}), 1.63-1.59 (m, 2H, pip-3,5H^{ax}), 1.28 (d, J = 12.6 Hz, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.2 (*C*=O), 147.0 (ph'-1*C*), 139.8 (ph-4*C*), 130.3 (ph-1*C*), 129.8 (ph-3,5*C*), 129.3 (ph-2,6*C*), 127.8 (ph'-3,5*C*), 125.9 (ph'-4*C*), 125.7 (ph'-2,6*C*), 78.4 (*C*OH), 60.5 (N*C*H_2), 52.7 (pip-2,6*C*), 43.4 (pip-4*C*), 25.0 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{27}NO_3$, 402.2069; found, 402.2085. Elemental analysis: calcd. for $C_{26}H_{27}NO_3 \cdot H_2O$, C 74.44, H 6.97, N 3.34; found, C 74.28, H 6.64, N 3.34.

Methyl 4-(2-bromoacetyl)benzoate (5). Methyl 4-acetylbenzoate (2 g, 0.011 mol) and *p*-toluenesulfonic acid (0.2 g, 1.2 mmol) were suspended in anhydrous acetonitrile (10 ml). *N*-Bromosuccinimide (2.2 g, 11.2 mmol) was added under inert conditions. The mixture was stirred for 15 min at room temperature and subsequently heated to 60 °C for 2 h. After cooling, the

mixture was stirred in a mixture of water (50 ml) and toluene (50 ml) for 15 min. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to dryness resulting in a light yellow solid, which was again washed with toluene to remove remaining bromine (67–85%). Mp: 78–80 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.02 (s, 4H, ph-2,3,5,6*H*), 3.82 (s, 3H, OCH_3), 2.60 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 195.8 (*C*=O ketone), 165.6 (*C*=O ester), 137.2 (ph-4*C*), 133.1 (ph-1*C*), 129.6 (ph-2,6*C*), 129.0 (ph-3,5*C*), 70.9 (ph-*C*H_2), 57.7 (O*C*H_3). HRMS (m/z): $[M+H]^+$ calcd. for $C_{10}H_9BrO_3$, 256.9813; found, 256.9818.

Methyl 4-(2-(4-(hydroxydiphenylmethyl)piperidin-1-yl)acetyl)benzoate (6). Diphenyl-piperidin-4-yl-methanol hydrochloride (1.3 g, 4.3 mmol), compound **5** (1.0 g, 3.9 mmol) and potassium carbonate (1.2 g, 8.7 mmol) were stirred in acetone (30 ml) at room temperature. After 12 h the inorganic salts were filtered off and the filtrate concentrated under reduced pressure. The product was purified by column chromatography (TEA 0.5%; DCM: methanol = 100: 0 → 98: 2). Re-crystallization from acetonitrile gave a yellow solid (0.5 g, 30%). Mp: 140 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.08-8.03 (m, 4H, ph-2,3,5,6*H*), 7.48 (d, J = 8.1 Hz, 4H, ph-2,6*H*), 7.24 (t, J = 7.8 Hz, 4H, ph'-3,5*H*), 7.10 (t, J = 7.3 Hz, 2H, ph'-4*H*), 5.23 (s, 1H, O*H*), 3.87 (s, 3H, OCH_3), 3.77 (s, 2H, NCH_2), 2.84 (d, J = 10.7 Hz, 2H, pip-2,6H^{eq}), 2.49 (overlay with DMSO peak, pip-4*H*), 2.10 (t, J = 11.1 Hz, 2H, pip-2,6H^{ax}), 1.50-1.43 (m, 2H, pip-3,5H^{ax}), 1.21 (d, J = 12.3 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 197.2 (*C*=O ketone), 165.6 (*C*=O ester), 147.3 (ph'-1*C*), 139.4 (ph-4*C*), 133.1 (ph-1*C*), 129.3 (ph-2,6*C*), 128.5 (ph-3,5*C*), 127.8 (ph'-3,5*C*), 125.7 (ph'-2,4,6*C*), 78.5 (*C*OH), 64.6 (N*C*H_2), 53.7 (pip-2,6*C*), 52.5 (O*C*H_3), 43.0 (pip-4*C*), 25.9 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{28}H_{29}NO_4$, 444.2175; found, 444.2172.

4-(2-(4-(Hydroxydiphenylmethyl)piperidin-1-yl)acetyl)benzoic acid (7). Compound **6** (0.3 g, 0.7 mmol) was dissolved in methanol (1 ml) and THF (1 ml). Aqueous potassium hydroxide (2 M, 2 ml) was added and the mixture was heated in the microwave oven for 15 min at 70 °C. After cooling the organic solvents were removed under reduced pressure and the aqueous solution neutralized with HCl. The product precipitated as an off-white solid that was recrystallized from acetonitrile under addition of a drop of water (0.1 g, 35%). Mp: 187 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.04-8.00 (m, 4H, ph-2,3,5,6*H*), 7.48 (d, J = 8.0 Hz, 4H, ph'-2,6*H*), 7.24 (t, J = 7.7 Hz, 4H, ph'-3,5*H*), 7.10 (t, J = 7.2 Hz, 2H, ph'-4*H*), 5.24 (s, 1H, O*H*), 3.80 (s, 2H, NCH_2), 2.86 (d, J = 9.2 Hz, 2H, pip-2,6H^{eq}), 2.49 (overlay with DMSO peak, pip-4*H*), 2.14-2.10 (m, 2H, pip-2,6H^{ax}), 1.52-1.47 (m, 2H, pip-3,5H^{ax}), 1.22 (d, J = 11.6, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 197.0 (*C*=O ketone), 166.7 (*C*=O ester), 147.2 (ph'-1*C*), 138.8 (ph-4*C*), 135.1 (ph-1*C*), 129.4 (ph-3,5*C*), 128.3 (ph-2,6*C*), 127.8 (ph'-3,5*C*), 125.8 (ph'-4*C*), 125.7 (ph'-2,6*C*), 78.5 (*C*OH), 64.4 (N*C*H$_2$), 53.6 (pip-2,6*C*), 43.9 (pip-4*C*), 25.8 (pip-3,5*C*). HRMS (m/z): $[M-H]^+$ calcd. for $C_{27}H_{27}NO_4$, 428.1862; found, 428.1843. Elemental analysis: calcd. for $C_{27}H_{27}NO_4 \cdot 0.5\ H_2O$, C 73.95, H 6.44, N 3.19; found, C 74.10, H 6.23, N 3.29.

Methyl 2-(4-(bromomethyl)phenyl)-2-methylpropanoate (8). Under inert atmosphere, *p*-tolylacetic acid (5 g, 0.033 mol) was dissolved in methanol (60 ml) and under cooling (ice bath) chlorotrimethyl silane (6.5 ml) was added dropwise. The solution was stirred overnight at room temperature and subsequently concentrated to dryness. The resulting colorless oil was dissolved in THF (25 ml) and added to a solution of sodium hydride (4 g, 60% dispersion in mineral oil, 0.1 mol) in THF (100 ml). Methyl iodide (5 ml, 0.08 mol) was added dropwise and under cooling. The resulting suspension was stirred overnight at room temperature. After concentration

to dryness the crude was taken up in water (50 ml) and extracted with ethyl acetate (3 x 50 ml). The organic layer was dried ($MgSO_4$) and concentrated to give methyl 2-methyl-2-(*p*-tolyl)propanoate (**8a**) as a yellow oil (4.6 g; 73%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32 (d, J = 8.3 Hz, 2H, ph-2,6*H*), 7.17 (d, J = 8.1 Hz, 2H, ph-3,5*H*), 3.66 (s, 3H, OCH_3), 2.34 (s, 3H, ph-CH_3), 1.58 (s, 6H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.4 (*C*=O), 141.8 (ph-4*C*), 136.3 (ph-1*C*), 129.1 (ph-3,5*C*), 125.5 (ph-2,6*C*), 52.2 (O*C*H$_3$), 46.2 (*C*(CH$_3$)$_2$), 26.6 (C(*C*H$_3$)$_2$), 21.0 (ph-*C*H$_3$). HRMS (m/z): $[M+H]^+$ calcd. for $C_{12}H_{16}O_2$, 193.1229; found, 193.1236. A solution of 2,2′-azobis(2-methylpropionitrile) in toluene (5 ml, 0.2 M) was concentrated to dryness without heating and under exclusion of light. 2,2′-Azobis(2-methylpropionitrile), *N*-bromosuccinimide (1.0 g, 5 mmol) and compound **8a** (1.0 g, 5 mmol) were dissolved in bromobenzene or chlorobenzene (5 ml). The solution was heated in an oil bath kept at 110 °C. When the reaction mixture reached 80-90 °C, an exothermic reaction started with the development of bromine (orange color). Subsequently, the mixture turned dark brown and was quenched by cooling with an ice bath and adding ice water. The product was extracted with petrol. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to dryness. The product was purified by column chromatography (petrol: ethyl acetate = 100: 0 → 98: 2) giving a yellow oil (50 – 61%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.38 (d, J = 8.3 Hz, 2H, ph-2,6*H*), 7.29 (d, J = 8.4 Hz, 2H, ph-3,5*H*), 4.67 (s, 2H, CH_2Br), 3.57 (s, 3H, OCH_3), 1.48 (s, 6H, C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 176.4 (*C*=O), 142.9 (ph-4*C*), 140.9 (ph-1*C*), 126.6 (ph-2,6*C*), 125.1 (ph-3,5*C*), 62.5 (*C*H$_2$Br), 52.0 (O*C*H$_3$), 46.0 (*C*(CH$_3$)$_2$), 26.4 (C(*C*H$_3$)$_2$). HRMS (m/z): $[M-H]^+$ calcd. for $C_{12}H_{15}BrO_2$, 269.0172; found, 269.0163.

Methyl 2-(4-((4-(hydroxydiphenylmethyl)piperidin-1-yl)methyl)phenyl)-2-methyl-propanoate (9). Diphenyl(piperidin-4-yl)methanol hydrochloride (1.0 g, 3.3 mmol), compound

8 (0.7 g, 2.6 mmol), potassium carbonate (1.46 g, 10.6 mmol) and potassium iodide (0.05 g, 0.33 mmol) were suspended in acetonitrile (25 ml). Under inert atmosphere the mixture was refluxed overnight. After cooling the inorganic components were removed by filtration. The filtrate was concentrated to dryness and the crude purified by column chromatography (TEA 0.5%; DCM: methanol = 100: 0 → 98: 2). The product was isolated as colorless oil. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.48 (d, J = 7.6 Hz, 4H, ph'-2,6*H*), 7.29-7.18 (m, 8H, ph-2,3,5,6*H*, ph'-3,5*H*), 7.10 (t, J = 7.1 Hz, 2H, ph'-4*H*), 5.22 (s, 1H, O*H*), 3.56 (s, 3H, OCH_3), 3.37 (s, 2H, NCH_2), 2.75 (d, J = 10.8 Hz, 2H, pip-2,6H^{eq}), 2.49-2.44 (m, 1H, pip-4*H*), 1.91 (t, J = 11.3 Hz, 2H, pip-2,6H^{ax}), 1.50-1.43 (m, 8H, pip-3,5H^{ax}, C(CH_3)$_2$), 1.23 (d, J = 12.2 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.4 (*C*=O), 147.3 (ph'-1*C*), 143.0 (ph-1*C*), 137.0 (ph-4*C*), 128.7 (ph-3,5*C*), 127.7 (ph'-3,5*C*), 125.8 (ph'-2,6*C*, ph-2,6*C*), 125.2 (ph'-4*C*), 78.5 (*C*OH), 61.9 (N*C*H$_2$), 53.5 (pip-2,6*C*), 52.0 (*C*(CH$_3$)$_2$), 45.8 (O*C*H$_3$), 43.4 (pip-4*C*), 26.4 (C(*C*H$_3$)$_2$), 25.5 (pip-3,5*C*). HRMS (m/z): [M+H]$^+$ calcd. for $C_{30}H_{35}NO_3$, 458.2695; found, 458.2696.

2-(4-((4-(Hydroxydiphenylmethyl)piperidin-1-yl)methyl)phenyl)-2-methylpropanoic acid (10). Compound **9** (0.5 g, 1.0 mmol) was dissolved in methanol (1 ml) and THF (1 ml). Aqueous potassium hydroxide (2 M, 2 ml) was added and the mixture was heated in the microwave oven for 15 min at 70 °C. After cooling the organic solvents were evaporated and the aqueous solution neutralized with HCl. The product precipitated as white solid that was recrystallized from acetonitrile under addition of a drop of water (0.23 g, 48%). Mp: 246 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.48 (d, J = 7.7 Hz, 4H, ph'-2,6*H*), 7.30-7.19 (m, 8H, ph-2,3,5,6*H*, ph'-3,5*H*), 7.09 (t, J = 7.3 Hz, 2H, ph'-4*H*), 5.22 (s, 1H, O*H*), 3.39 (s, 2H, NCH_2), 2.77 (d, J = 10.6 Hz, 2H, pip-2,6H^{eq}), 2.49-2.44 (m, 1H, pip-4*H*), 1.94 (t, J = 11.2 Hz, 2H, pip-2,6H^{ax}), 1.50-1.43 (m, 8H, pip-3,5H^{ax}, C(CH_3)$_2$), 1.20 (d, J = 12.4 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ

177.6 (*C*=O), 147.3 (ph'-1*C*), 143.6 (ph-4*C*), 136.2 (ph-1*C*), 128.6 (ph-2,6*C*), 127.7 (ph'-3,5*C*), 125.8 (ph'-2,6*C*, ph-3,5*C*), 125.3 (ph'-4*C*), 78.5 (*C*OH), 61.9 (N*C*H_2), 53.5 (pip-2,6*C*), 45.5 (*C*(CH_3)$_2$), 43.3 (pip-4*C*), 26.4 (C(*C*H_3)$_2$), 25.9 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{29}H_{33}NO_3$, 444.2539; found, 444.2520. Elemental analysis: calcd. for $C_{29}H_{33}NO_3 \cdot 0.5\ H_2O$, C 76.96, H 7.57, N 3.09; found, C 77.19, H 7.38, N 3.09.

Methyl 4-(bromomethyl)-3-fluorobenzoate (11). 3-Fluoro-4-methylbenzoic acid (2.0 g, 13.0 mmol) was dissolved in methanol (20 ml). Chlorotrimethyl silane (2.6 ml) was added dropwise and under cooling and the mixture was stirred overnight at room temperature. The resulting crude was concentrated on silica and the product was purified by column chromatography (DCM). Methyl 3-fluoro-4-methylbenzoate (**11a**) was isolated as a colorless oil (1.4 g, 64%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.70 (d, J = 7.9 Hz, 1H, ph-6*H*), 7.64 (d, J = 10.2 Hz, 1H, ph-2*H*), 7.23 (t, J = 7.7 Hz, 1H, ph-5*H*), 3.89 (s, 3H, OC*H*$_3$), 2.29 (s, 3H, ph-C*H*$_3$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.2 (*C*=O), 162.2 (d, $^1J_{C,F}$ = 118.8 Hz, ph-3*C*), 131.4 (d, $^3J_{C,F}$ = 5.0, ph-5*C*), 130.6 (d, $^2J_{C,F}$ = 17.3 Hz, ph-4*C*), 129.7 (d, $^3J_{C,F}$ = 7.5 Hz, ph-1*C*), 125.1 (d, $^4J_{C,F}$ = 3.6 Hz, ph-6*C*), 116.1 (d, $^2J_{C,F}$ = 23.9 Hz, ph-2*C*), 52.2 (O*C*H_3), 14.9 (d, $^3J_{C,F}$ = 3.5 Hz, ph-*C*H_3). ^{19}F NMR ($CDCl_3$, 282 MHz): δ –117.16 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_9H_9FO_2$, 169.0665; found, 169.0662. A solution of 2,2'-azobis(2-methylpropionitrile) in toluene (10 ml, 0.2 M) was concentrated to dryness without heating and under exclusion of light. 2,2'-Azobis(2-methylpropionitrile), *N*-bromosuccinimide (1.9 g, 10.0 mmol) and compound **11a** (1.4 g, 8.3 mmol) were dissolved in chlorobenzene and treated as described for compound **8**, giving the desired product as yellow oil (1.5 g, quantitative conversion). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.80 (d, J = 8.0 Hz, 1H, ph-6*H*), 7.71 (d, J = 10.1 Hz, 1H, ph-2*H*), 7.46 (t, J = 7.7 Hz, 1H, ph-5*H*), 4.51 (s, 2H, C*H*$_2$Br), 3.92 (s, 3H, C*H*$_3$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.6 (*C*=O), 160.3

(d, $^{1}J_{C,F}$ = 249.3, ph-3*C*), 132.5 (d, $^{3}J_{C,F}$ = 7.9, ph-5*C*), 131.4 (d, $^{2}J_{C,F}$ = 22.0, ph-4*C*), 130.1 (ph-1*C*), 125.7 (ph-6*C*), 117.0 (d, $^{2}J_{C,F}$ = 23.1, ph-2*C*), 52.6 (O*C*H$_3$), 24.6 (*C*H$_2$Br).

Methyl 3-fluoro-4-((4-(hydroxydiphenylmethyl)piperidin-1-yl)methyl)benzoate hydrochloride (12). Under inert atmosphere, diphenyl(piperidin-4-yl)methanol hydrochloride (2.0 g, 6.6 mmol), compound **11** (1.5 g, 6.0 mmol) and potassium carbonate (2.7 g, 19.6 mmol) were suspended in acetonitrile (50 ml) and refluxed overnight. After cooling, the inorganic components were filtered off and the filtrate concentrated under reduced pressure. The product was purified by column chromatography (TEA 0.5%; DCM: methanol = 100: 0 → 98: 2). The oily product was crystallized with HCl in ethanol to give a white solid (1.7 g, 61%). Mp: 151–154 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.72 (br s, 1H, N*H*$^{+}$), 7.91-7.84 (m, 2H, ph-5,6*H*), 7.78 (d, *J* = 10.0, 1H, ph-2*H*), 7.45 (d, *J* = 7.5 Hz, 4H, ph'-2,6*H*), 7.26 (t, *J* = 7.7 Hz, 4H, ph'-3,5*H*), 7.13 (t, *J* = 7.3 Hz, 2H, ph'-4*H*), 5.54 (s, 1H, O*H*), 4.32 (s, 2H, NC*H*$_2$), 3.88 (s, 3H, OC*H*$_3$), 3.36 (d, *J* = 11.1 Hz, 2H, pip-2,6*H*^{eq}), 3.02-2.95 (m, 2H, pip-2,6*H*^{ax}), 2.81 (t, *J* = 11.7 Hz, 1H, pip-4*H*), 1.83-1.76 (m, 2H, pip-3,5*H*^{ax}), 1.42 (d, *J* = 13.7 Hz, 2H, pip-3,5*H*^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 164.8 (*C*=O), 161.0 (d, $^{1}J_{C,F}$ = 219.8 Hz, ph-3*C*), 164.5 (ph'-1*C*), 134.9 (ph-5*C*), 133.1 (ph-4*C*), 127.9 (ph'-3,5*C*), 126.1 (ph'-4*C*), 125.7 (ph'-2,6*C*), 125.2 (ph-6*C*), 121.6 (ph-1*C*), 116.2 (d, $^{2}J_{C,F}$ = 23.8 Hz, ph-2*C*), 78.1 (*C*OH), 56.0 (NCH$_2$), 52.7 (OCH$_3$), 51.4 (pip-2,6*C*), 40.4 (pip-4*C*), 23.8 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –113.23 (ph-*F*). HRMS (m/z): [M+H]$^{+}$ calcd. for $C_{27}H_{28}FNO_3$, 434.2131; found, 434.2131.

3-Fluoro-4-((4-(hydroxydiphenylmethyl)piperidin-1-yl)methyl)benzoic acid (13). Compound **12** (0.5 g, 1.0 mmol) was dissolved in methanol (1 ml) and THF (1 ml) and treated with aqueous potassium hydroxide (2 M, 2 ml). The mixture was heated in the microwave oven for 15 min at 70 °C. After cooling the organic solvents were removed under reduced pressure

and the aqueous solution neutralized with HCl. The product precipitated as white solid that was recrystallized from acetonitrile under addition of a drop of water (0.15 g, 33%). Mp: 247 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.72 (d, J = 7.9 Hz, 1H, ph-6*H*), 7.58 (d, J = 10.4 Hz, 1H, ph-2*H*), 7.50-7.48 (m, 5H, ph-5*H*, ph'-2,6*H*), 7.23 (t, J = 7.8 Hz, 4H, ph'-3,5*H*), 7.10 (t, J = 7.3 Hz, 2H, ph'-4*H*), 5.24 (s, 1H, O*H*), 3.55 (s, 2H, NCH_2), 2.79 (d, J = 11.0 Hz, 2H, pip-2,6H^{eq}), 2.50-2.45 (m, 1H, pip-4*H*), 2.02 (t, J = 11.1 Hz, 2H, pip-2,6H^{ax}), 1.52-1.44 (m, 2H, pip-3,5H^{ax}), 1.23 (d, J = 12.6 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 166.3 (d, $^{4}J_{C,F}$ = 2.3 Hz, *C*=O), 160.4 (d, $^{1}J_{C,F}$ = 243.9 Hz, ph-3*C*), 147.3 (ph'-1*C*), 132.2 (d, $^{3}J_{C,F}$ = 7.2 Hz, ph-5*C*), 131.6 (d, $^{3}J_{C,F}$ = 4.4 Hz, ph-1*C*), 129.8 (d, $^{2}J_{C,F}$ = 13.1 Hz, ph-4*C*), 127.8 (ph'-3,5*C*), 125.8 (ph'-4*C*), 125.7 (ph'-2,6*C*), 125.1 (d, $^{4}J_{C,F}$ = 2.6 Hz, ph-6*C*), 115.7 (d, $^{2}J_{C,F}$ = 23.6 Hz, ph-2*C*), 78.5 (*C*OH), 54.5 (N*C*H$_2$), 53.3 (pip-2,6*C*), 43.1 (pip-4*C*), 25.9 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –117.833 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{26}FNO_3$, 420.1975; found, 420.1961. Elemental analysis: calcd. for $C_{26}H_{26}FNO_3 \cdot 0.5\ H_2O$, C 79.16, H 7.15, N 3.55; found, C 78.95, H 6.90, N 3.48.

Methyl 4-((4-((4-fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)methyl)benzoate (14). Under inert atmosphere, compound **34** (0.4 g, 1.24 mmol), methyl 4-(bromomethyl)benzoate (0.43 g, 1.86 mmol) and potassium carbonate (0.43 g, 3.1 mmol) were suspended in acetone (15 ml) and stirred for 2 h at room temperature. The inorganic components were subsequently filtered off and the filtrate was concentrated under reduced pressure. Purification by column chromatography (TEA 0.5%; DCM: methanol = 100: 0 → 99: 1) gave the product as a colorless oil (0.19 g, 35%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.87 (d, J = 8.2 Hz, 2H, ph-2,6*H*), 7.53-7.48 (m, 4H, ph'-2,6*H*, ph''-2,6*H*), 7.42 (d, J = 8.2 Hz, 2H, ph-3,5*H*), 7.25 (t, J = 7.7 Hz, 2H, ph'-3,5*H*), 7.12 (t, J = 7.3 Hz, 1H, ph'-4*H*), 7.06 (t, J = 8.9 Hz, 2H, ph''-3,5*H*),

5.33 (s, 1H, CO*H*), 3.83 (s, 3H, OCH_3), 3.49 (s, 2H, NCH_2), 2.77 (d, J = 8.1 Hz, 2H, pip-2,6H^{eq}), 2.48 (t, J = 11.8 Hz, 1H, pip-4*H*), 1.95 (t, J = 11.8 Hz, 2H, pip-2,6H^{ax}), 1.52-1.44 (m, 2H, pip-3,5H^{ax}), 1.21 (t, J = 11.5 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 166.2 (*C*=O), 160. 5 (d, $^1J_{C,F}$ = 240.6 Hz, ph''-4*C*), 147.1 (ph'-1*C*), 144.7 (ph-4*C*), 143.5 (d, $^4J_{C,F}$ = 2.7 Hz, ph''-1*C*), 129.1 (ph-2,6*C*), 128.8 (ph'-3,5*C*), 128.2 (ph-1*C*), 127.9 (ph-3,5*C*), 127.7 (d, $^3J_{C,F}$ = 7.7 Hz, ph''-2,6*C*), 125.9 (ph'-4*C*), 125.7 (ph'-2,6*C*), 114.4 (d, $^2J_{C,F}$ = 20.7 Hz, ph''-3,5*C*), 78.3 (*C*OH), 61.9 (N*C*H$_2$), 53.5 (pip-2,6*C*), 52.1 (O*C*H$_3$), 43.3 (pip-4*C*), 26.0 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –118.08 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{27}H_{28}FNO_3$, 434.2131; found, 434.2122.

4-((4-((4-Fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)methyl)benzoic acid (15). Compound **14** (0.18 g, 0.4 mmol) was dissolved in methanol (0.5 ml) and THF (0.5 ml). Aqueous potassium hydroxide (2 M, 1 ml) was added and the mixture was stirred overnight at room temperature. The organic solvents were evaporated and the aqueous solution neutralized with hydrogen chloride solution. The product precipitated as a white solid that was recrystallized from acetonitrile under addition of a drop of water (0.06 g, 35%). Mp: > 250 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.86 (d, J = 8.1 Hz, 2H, ph-2,6*H*), 7.53-7.48 (m, 4H, ph'-2,6*H*, ph''-2,6*H*), 7.39 (d, J = 8.1 Hz, 2H, ph-3,5*H*), 7.25 (t, J = 7.7 Hz, 2H, ph'-3,5*H*), 7.12 (t, J = 7.3 Hz, 1H, ph'-4*H*), 7.06 (t, J = 8.9 Hz, 2H, ph''-3,5*H*), 5.34 (s, 1H, O*H*), 3.49 (s, 2H, NCH_2), 2.78 (d, J = 8.0 Hz, 2H, pip-2,6H^{eq}), 2.48 (t, J = 11.9 Hz, 1H, pip-4*H*), 1.96 (t, J = 11.7 Hz, 2H, pip-2,6H^{ax}), 1.51-1.45 (m, 2H, pip-3,5H^{ax}), 1.21 (d, J = 11.1 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 167.3 (*C*=O), 160.5 (d, $^1J_{C,F}$ = 240.8 Hz, ph''-4*C*), 143.9 (ph-4*C*), 143.5 (d, $^4J_{C,F}$ = 2.6 Hz, ph''-1*C*), 129.5 (ph-1*C*), 129.2 (ph-2,6*C*), 128.7 (ph'-3,5*C*), 127.9 (ph-3,5*C*), 127.7 (d, $^3J_{C,F}$ = 7.8 Hz, ph''-2,6*C*), 125.9 (ph'-4*C*), 125.7 (ph'-2,6*C*), 114.4 (d, $^2J_{C,F}$ = 20.7 Hz, ph''-

3,5*C*), 78.3 (*C*OH), 61.9 (N*C*H$_2$), 53.5 (pip-2,6*C*), 43.3 (pip-4*C*), 25.9 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –118.09 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{26}FNO_3$, 420.1975; found, 420.1964. Elemental analysis: calcd. for $C_{26}H_{26}FNO_3 \cdot 0.25\ H_2O$, C 73.65, H 6.30, N 3.30; found, C 73.39, H 6.13, N 3.27.

Methyl 4-((4-(bis(4-fluorophenyl)(hydroxy)methyl)piperidin-1-yl)methyl)benzoate (16). Compound **35** (0.22 g, 0.65 mmol), methyl 4-(bromomethyl)benzoate (0.3 g, 1.3 mmol) and potassium carbonate (0.18 g, 1.3 mmol) were suspended in acetone (15 ml), refluxed overnight and stirred at room temperature for 24 h. The inorganic components were filtered off and the filtrate concentrated under reduced pressure. Purification by column chromatography (TEA 0.5%; DCM: methanol = 98: 2) gave the product as a colorless oil (0.12 g, 41%). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.87 (d, J = 8.3 Hz, 2H, ph-2,6*H*), 7.50-7.47 (m, 4H, ph'-2,6*H*), 7.40 (d, J = 8.2 Hz, 2H, ph-3,5*H*), 7.10 (t, J = 8.9 Hz, 4H, ph'-3,5*H*), 5.38 (s, 1H, CO*H*), 3.82 (s, 3H, OCH_3), 3.47 (s, 2H, NCH_2), 2.75 (d, J = 10.9 Hz, 2H, pip-2,6H^{eq}), 2.49-2.42 (m, 1H, pip-4*H*), 1.94 (t, J = 11.2 Hz, 2H, pip-2,6H^{ax}), 1.50-1.42 (m, 2H, pip-3,5H^{ax}), 1.21 (d, J = 12.6 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.2 (*C*=O), 160.5 (d, $^1J_{C,F}$ = 241.3 Hz, ph'-4*C*), 144.6 (ph-4*C*), 143.3 (ph'-1*C*), 129.1 (ph-2,6*C*), 128.8 (ph-3,5*C*), 127.7 (d, $^3J_{C,F}$ = 7.8 Hz, ph'-2,6*C*), 114.4 (d, $^2J_{C,F}$ = 20.9 Hz, ph'-3,5*C*), 78.1 (*C*OH), 61.8 (N*C*H$_2$), 53.4 (pip-2,6*C*), 52.0 (O*C*H$_3$), 43.3 (pip-4*C*), 25.9 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –117.89 (ph-*F*). HRMS (m/z): $[M]^+$ calcd. for $C_{27}H_{27}F_2NO_3$, 452.2037; found, 452.2024.

4-((4-(Bis(4-fluorophenyl)(hydroxy)methyl)piperidin-1-yl)methyl)benzoic acid (17). Compound **16** (0.5 g, 1.1 mmol) was dissolved in methanol (1 ml) and THF (1 ml). Aqueous potassium hydroxide (2 M, 2 ml) was added and the mixture was heated in the microwave oven for 15 min at 70 °C. After cooling the organic solvents were evaporated and the aqueous solution

neutralized with HCl. The product precipitated as a white solid that was recrystallized from acetonitrile under addition of a drop of water (0.15 g, 31%). Mp: > 250 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.85 (d, J = 8.2 Hz, 2H, ph-2,6*H*), 7.50-7.48 (m, 4H, ph'-2,6*H*), 7.38 (d, J = 8.2 Hz, 2H, ph-3,5*H*), 7.09-7.04 (m, 4H, ph'-3,5*H*), 5.38 (s, 1H, O*H*), 3.48 (s, 2H, NCH_2), 2.76 (d, J = 11.1 Hz, 2H, pip-2,6H^{eq}), 2.45 (t, J = 11.8 Hz, 1H, pip-4*H*), 1.96 (t, J = 11.0 Hz, 2H, pip-2,6H^{ax}), 1.50-1.42 (m, 2H, pip-3,5H^{ax}), 1.21 (d, J = 12.5 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.3 (*C*=O), 160.5 (d, $^{1}J_{C,F}$ = 241.0 Hz, ph'-4*C*), 143.8 (ph-4*C*), 143.2 (ph'-1*C*), 129.5 (ph-1*C*), 129.2 (ph-2,6*C*), 128.7 (ph-3,5*C*), 127.7 (d, $^{3}J_{C,F}$ = 7.9 Hz, ph'-2,6*C*), 114.4 (d, $^{2}J_{C,F}$ = 20.8 Hz, ph'-3,5*C*), 78.1 (*C*OH), 61.8 (N*C*H$_2$), 53.4 (pip-2,6*C*), 43.3 (pip-4*C*), 25.9 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –117.89 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{26}H_{25}F_2NO_3$, 438.1881; found, 438.1884. Elemental analysis: calcd. for $C_{26}H_{25}F_2NO_3 \cdot 0.25\ H_2O$, C 70.65, H 5.82, N 3.17; found, C 70.67, H 5.67, N 3.25.

3-Fluorodihydrofuran-2(3*H*)-one (18). Under inert atmosphere, 3-hydroxy-dihydro-furan-2-one (2.0 g, 19.6 mmol) was dissolved in DCM (30 ml). DAST (3.9 ml, 29.4 mmol) was added dropwise at 0 °C. The solution was allowed to come to room temperature and stirred for 3 h at this temperature. The mixture was diluted with DCM and quenched with sodium bicarbonate solution at 0 °C. The separated organic layer was washed with brine and water, dried ($MgSO_4$), filtered and concentrated to dryness. The product was purified by column chromatography (DCM) and was collected as colorless oil (1.15 g, 58%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.23/5.13 (t/t, J = 7.7/7.7 Hz, 0.5H/0.5H, C*H*F), 4.50-4.46/4.32-4.27 (m/m, 1H/1H, CH_2O), 2.72-2.65/2.54-2.45 (m/m, 1H/1H, CHFCH_2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.8 (d, $^{2}J_{C,F}$ = 20.9 Hz, *C*=O), 85.3 (d, $^{1}J_{C,F}$ = 188.8 Hz, *C*HF), 64.9 (d, $^{3}J_{C,F}$ = 5.9 Hz, *C*H$_2$O), 29.5 (d, $^{2}J_{C,F}$ = 20.0

Hz, CHF*C*H$_2$). ^{19}F NMR ($CDCl_3$, 282 MHz): δ –196.16 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_4H_5FO_2$, 105.0352; found, 105.0348.

Methyl 2-fluoro-4-hydroxybutanoate (19). Compound **18** (1.73 g, 16.6 mmol) was dissolved in methanol (75 ml). Sodium methanolate (2.2 g, 41.5 mmol) was added in one portion. After stirring the solution for 1 h at room temperature it was quenched with HCl (2 N). Methanol was evaporated under reduced pressure and the product was subsequently extracted into ethyl acetate. Purification was performed by column chromatography (DCM: ethyl acetate = 10: 0 → 9: 1) giving the product as colorless oil (0.58 g, 26%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.20/5.04 (dd/dd, J = 7.3/7.0 Hz, 0.5H/0.5H, C*H*F), 3.90-3.70 (m, 2H, C*H*$_2$OH), 3.80 (s, 3H, OC*H*$_3$), 2.26-2.08 (m, 2H, CHFC*H*$_2$). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 170.8 (d, $^2J_{C,F}$ = 23.4 Hz, *C*=O), 86.5 (d, $^1J_{C,F}$ = 168.5 Hz, *C*HF), 57.8 (d, $^3J_{C,F}$ = 4.1 Hz, *C*H$_2$OH), 52.8 (O*C*H$_3$), 35.1 (d, $^2J_{C,F}$ = 20.6 Hz, CHF*C*H$_2$). ^{19}F NMR ($CDCl_3$, 282 MHz): δ –195.16 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_5H_9FO_3$, 137.0614; found, 137.0610.

Methyl 2-fluoro-4-(tosyloxy)butanoate (20). Compound **19** (0.78 g, 5.9 mmol) was dissolved in DCM (30 ml) and the solution was cooled to 0 °C. Pyridine (0.51 ml, 6.2 mmol) and tosyl chloride (1.2 g, 6.2 mmol) were subsequently added. The mixture was stirred overnight at room temperature. It was diluted with DCM and the reaction was quenched with water. The separated organic layer was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The product was purified by column chromatography (DCM) giving a colorless oil (0.43 g, 25%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.78 (d, J = 8.3 Hz, 2H, ph-3,5*H*), 7.37 (d, J = 8.0 Hz, 2H, ph-2,6*H*), 5.03/4.95 (dd/dd, J = 8.4/8.5 Hz, 0.5H/0.5H, C*H*F), 4.19-4.15 (m, 2H, C*H*$_2$O), 3.78 (s, 3H, OC*H*$_3$), 2.45 (s, 3H, ph-C*H*$_3$), 2.36-2.15 (m, 2H, CHFC*H*$_2$). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 169.5 (d, $^2J_{C,F}$ = 23.3 Hz, *C*=O), 145.3 (ph-4*C*), 132.6 (ph-1*C*), 130.1 (ph-2,6*C*), 128.1 (ph-

3,5*C*), 85.0 (d, $^{1}J_{C,F}$ = 184.4 Hz, *C*HF), 64.9 (d, $^{3}J_{C,F}$ = 3.6 Hz, *C*H_2O), 52.8 (O*C*H_3), 32.1 (d, $^{2}J_{C,F}$ = 20.9 Hz, CHF*C*H_2), 21.8 (ph-*C*H_3). ^{19}F NMR ($CDCl_3$, 282 MHz): δ –195.79 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{12}H_{15}FO_5S$, 291.0703; found, 291.0692.

Methyl 4-(4-benzhydrylpiperazin-1-yl)-2-fluorobutanoate (21). TEA (0.2 ml, 0.5 mmol) was dissolved in dry acetonitrile. Under inert atmosphere, 1-benzhydryl-piperazine (0.37 g, 1.49 mmol) and compound **20** (0.43 g, 1.49 mmol) were subsequently added. The solution was stirred for 6 h at 70 °C. After cooling it was concentrated to dryness and the product was purified by column chromatography (TEA; DCM: methanol = 99: 1) giving a light yellow oil (0.22 g, 40%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.40 (d, *J* = 7.4 Hz, 4H, ph-2,6*H*), 7.28 (t, *J* = 7.6 Hz, 4H, ph-3,5*H*), 7.17 (t, *J* = 7.4 Hz, 2H, ph-4*H*), 5.17/5.09 (t/t, *J* = 5.4/5.4 Hz, 0.5H/0.5H, C*H*F), 4.25 (s, 1H, benzh.-C*H*), 3.67 (s, 3H, OCH_3), 2.50-2.15 (m, 10H, prz-2,3,5,6H_2, NCH_2), 1.98-1.93 (m, 2H, CHFCH_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 169.9 (d, $^{2}J_{C,F}$ = 23.7 Hz, *C*=O), 142.9 (ph-1*C*), 128.5 (ph-3,5*C*), 127.6 (ph-2,6*C*), 126.9 (ph-4*C*), 87.0 (d, $^{1}J_{C,F}$ = 179.7 Hz, *C*HF), 75.1 (benzh.-*C*H), 55.0 (N*C*H_2), 52.8 (prz-2,6*C*), 52.0 (O*C*H_3), 51.5 (prz-3,5*C*), 29.1 (d, $^{2}J_{C,F}$ = 20.6 Hz, CHF*C*H_2). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –193.32 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{27}FN_2O_2$, 371.2135; found, 371.2140.

4-(4-Benzhydrylpiperazin-1-yl)-2-fluorobutan-1-ol dihydrochloride (22). Lithium aluminium hydride (0.031 g, 0.81 mmol) was dissolved in THF (5 ml). Compound **21** (0.1 g, 0.27 mmol), dissolved in THF (5 ml), was added dropwise under inert atmosphere. The mixture was stirred 1 h at room temperature and subsequently quenched with brine. THF was evaporated and the aqueous solution was adjusted to pH = 10 using aqueous NaOH (2 N). The product was extracted into DCM. Drying ($MgSO_4$) and concentration afforded a colorless oil (0.08 g, 87%). The product was crystallised with HCl in ethanol and precipitated as white solid (0.046 g, 42%).

Mp: 209 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C): δ 7.68 (d, J = 7.2 Hz, 4H, ph-2,6*H*), 7.38 (t, J = 7.5 Hz, 4H, ph-3,5*H*), 7.29 (t, J = 7.3 Hz, 2H, ph-4*H*), 5.09 (br s, 1H, benzh.-C*H*), 4.71-4.66/4.59-4.54 (m/m, 0.5H/0.5H, C*H*F), 3.64-3.53 (m, 2H, CH_2OH), 3.48 (s, 4H, prz-2,6H_2), 3.23 (t, J = 8.0 Hz, 2H, NCH_2), 3.05 (s, 4H, prz-3,5H_2), 2.18-2.07 (m, 2HCHFCH_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 135.3 (ph-1*C*), 129.3 (ph-3-5*C*), 128.2 (ph-2,6*C*), 92.1 (d, $^{1}J_{C,F}$ = 169.1 Hz, *C*HF), 74.1 (benzh.-*C*H), 62.5 (d, $^{2}J_{C,F}$ = 19.7 Hz, *C*H_2OH), 52.1 (N*C*H_2), 48.5 (prz-2,6*C*), 44.2 (prz-3,5*C*), 25.2 (CHF*C*H_2). ^{19}F NMR ($CDCl_3$, 282 MHz): δ –188.20 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{27}FN_2O$, 343.2186; found, 343.2184. Elemental analysis: calcd. for $C_{21}H_{27}FN_2O \cdot 2\ HCl \cdot 0.25\ H_2O$, C 60.07, H 7.08, N 6.69; found, C 60.72, H 7.04, N 6.74.

4-(4-Benzhydrylpiperazin-1-yl)-2-fluorobutyl acetate (23). Compound **22** (liberated base; 0.1 g, 0.29 mmol) was dissolved in DCM (5 ml). TEA (43 µl, 0.3 mmol) and acetic anhydride (35 µl, 0.3 mmol) were added under cooling (ice bath). The mixture was stirred 3 h at room temperature and subsequently quenched with aqueous NaOH (2 M). The organic layer was dried ($MgSO_4$), filtered and purified by column chromatography (TEA; DCM: methanol = 99.5: 0.5). The product was isolated as colorless oil (0.09 g, 80%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.41 (d, J = 7.3 Hz, 4H, ph-2,6*H*), 7.27 (t, J = 7.5 Hz, 4H, ph-3,5*H*), 7.16 (t, J = 7.2 Hz, 2H, ph-4*H*), 4.83-4.63 (m, 1H, C*H*F), 4.23 (s, 1H, benzh.-C*H*), 4.17-4.03 (m, 2H, CH_2OCOCH$_3$), 2.52-2.26 (m, 10H, prz-2,3,5,6H_2, NCH_2), 2.02 (s, 3H, COCH_3), 1.81-1.65 (m, 2H, CH_2CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.2 (*C*=O), 143.0 (ph-1*C*), 128.6 (ph-3,5*C*), 127.6 (ph-2,6*C*), 126.9 (ph-4*C*), 90.1 (d, $^{1}J_{C,F}$ = 170.0 Hz, but-2*C*), 75. 2 (benzh.-*C*H), 65.4 (d, $^{2}J_{C,F}$ = 20.4 Hz, but-1*C*), 53.0 (d, $^{3}J_{C,F}$ = 5.2 Hz, but-4*C*), 52.8/52.7 (prz-2,6*C*), 51.6 (prz-3,5*C*), 28.0 (d, $^{2}J_{C,F}$ = 20.4 Hz, but-3*C*), 20.6 (CO*C*H$_3$). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –186.59 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{23}H_{29}FN_2O_2$, 384.2213; found, 384.2210.

4-(4-Benzhydrylpiperazin-1-yl)-2-fluorobutanoic acid (24). Compound **21** (0.1 g, 0.27 mmol) was dissolved in methanol (0.5 ml) and THF (0.5 ml). Aqueous potassium hydroxide (2 M, 1 ml) was added and the mixture was stirred overnight at room temperature. The organic solvents were evaporated and the aqueous solution neutralized with hydrogen chloride solution. The product precipitated as a white solid that was recrystallized from acetonitrile under addition of a drop of water (0.05 g, 49%). Mp: 227–230 °C (carbonization). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.38 (d, J = 7.6 Hz, 4H, ph-2,6*H*), 7.25 (t, J = 7.5 Hz, 4H, ph-3,5*H*), 7.15 (t, J = 7.2 Hz, 2H, ph-4*H*), 4.95/4.87 (t/t, J = 5.8/5.8 Hz, 0.5H/0.5H, C*H*F), 4.39 (s, 1H, benzh.-C*H*), 3.50 (br s, 4H, prz-2,6H_2), 3.36-3.31/3.23-3.17 (m/m, 1H/1H, NCH_2), 2.99 (br s, 4H, prz-3,5H_2), 2.39-2.22 (m, 2H, CHFCH_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 171.0 (d, $^{2}J_{C,F}$ = 22.1 Hz, *C*=O), 141.6 (ph-1*C*), 128.6 (ph-3,5*C*), 127.4 (ph-2,6*C*), 127.1 (ph-4*C*), 86. 9 (d, $^{1}J_{C,F}$ = 181.5 Hz, *C*HF), 73.9 (benzh.-*C*H), 51.8 (N*C*H$_2$), 51.2 (prz-2,6*C*), 48.2 (prz-3,5*C*), 26.6 (d, $^{2}J_{C,F}$ = 22.1 Hz, CHF*C*H$_2$). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –186.28 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{25}FN_2O_2$, 357.1978; found, 357.1980. HPLC (analytical Chromolith column (RP-18e, 100-4.6 mm), 3 ml/min, methanol (0.1% trifluoroacetic acid) 20–60% in 8 min) RT: 5.2 min; purity: 99.62%.

Methyl 2-fluoro-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butanoate (25). Diphenyl(piperidin-4-yl)methanol hydrochloride (1.1 g, 3.6 mmol) was dissolved in DMSO (25 ml). TEA (1 ml, 7.2 mmol) and compound **20** (1.4 g, 4.8 mmol) were subsequently added. The mixture was stirred overnight at 60 °C. After cooling the product was extracted into DCM and purified by column chromatography (TEA; DCM: methanol = 99: 1) giving a light yellow oil (0.35 g, 25%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.49 (d, J = 8.2 Hz, 4H, ph-2,6*H*), 7.25 (t, J = 7.7 Hz, 4H, ph-3,5*H*), 7.12 (t, J = 7.3 Hz, 2H, ph-4*H*), 5.27 (s, 1H, O*H*), 5.16/5.08 (t/t, J =

5.4/5.4 Hz, 0.5H/0.5H, C*H*F), 3.67 (s, 3H, CH_3), 2.82-2.76 (m, 2H, pip-2,6H^{eq}), 2.45 (t, J = 11.9 Hz, 1H, pip-4*H*), 2.36-2.30 (m, 2H, NCH_2), 1.98-1.85 (m, 4H, CH_2CHF, pip-2,6H^{ax}), 1.44-1.39 (pip-3,5H^{eq}), 1.25 (d, J = 12.0 Hz, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.0 (d, $^2J_{C,F}$ = 23.6 Hz, *C*=O), 147.3 (ph-1*C*), 127.8 (ph-3-5*C*), 125.8 (ph-2,6*C*), 87.1 (d, $^1J_{C,F}$ = 179.7 Hz, *C*HF), 78.5 (*C*OH), 55.0 (N*C*H$_2$), 53.5 (pip-2,6*C*), 52.1 (*C*H$_3$), 43.4 (pip-4*C*), 29.4 (d, $^2J_{C,F}$ = 20.6 Hz, CHF*C*H$_2$), 26.0 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –193.29 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{23}H_{28}FNO_3$, 386.2131; found, 386.2121.

2-Fluoro-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butan-1-ol (26). Compound **25** (0.2 g, 0.52 mmol) in THF (5 ml) was added dropwise to a suspension of lithium aluminium hydride (75 mg, 1.95 mmol) in THF (5 ml). After stirring for 1 h at room temperature the solution was quenched with brine and concentrated to dryness. The crude mixture was taken up in aqueous NaOH (2 N) and the product was extracted into DCM. Concentration gave the product as a white solid, which was purified by column chromatography (TEA; DCM: methanol = 9: 1); (0.15 g, 83%). Mp: 144 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.50 (d, J = 8.2 Hz, 4H, ph-2,6*H*), 7.25 (t, J = 7.5 Hz, 4H, ph-3,5*H*), 7.12 (t, J = 7.3 Hz, 2H, ph-4*H*), 5.26 (s, 1H, O*H*), 5.00 (br s, 1H, CH$_2$O*H*), 4.56-4.52/4.48-4.44 (m/m, 0.5H/0.5H, C*H*F), 3.54-3.41 (m, 2H, CH_2OH), 2.86 (br s, 2H, pip-2,6H^{eq}), 2.46 (t, J = 11.6 Hz, 1H, pip-4*H*), 2.33 (br s, 2H, NCH_2), 1.93-1.87 (m, 2H, pip-2,6H^{ax}), 1.74-1.64 (CH_2CHF), 1.50-1.42 (pip-3,5H^{eq}), 1.25 (br s, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 147.3 (ph-1*C*), 127.8 (ph-3-5*C*), 125.8 (ph-2,6*C*), 93.3 (d, $^1J_{C,F}$ = 167.6 Hz, *C*HF), 78.5 (*C*OH), 64.0 (d, $^2J_{C,F}$ = 21.8 Hz, *C*H$_2$OH), 53.8 (pip-2,6*C*), 53.3 (N*C*H$_2$), 43.4 (pip-4*C*), 28.5 (d, $^2J_{C,F}$ = 22.8 Hz, *C*H$_2$CHF), 25.9 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –186.46 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{28}FNO_2$, 358.2182; found, 358.2170.

2-Fluoro-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butyl acetate (27). Compound **26** (0.1 g, 0.28 mmol) was dissolved in DCM (5 ml). TEA (43 µl, 0.3 mmol) and acetic anhydride (35 µl, 0.3 mmol) were added under cooling (ice bath). The mixture was stirred 3 h at room temperature and subsequently quenched with aqueous NaOH (2 N). The organic layer was dried ($MgSO_4$), filtered and purified by column chromatography (TEA; DCM: methanol = 95: 5). The product was isolated as colorless oil (0.11 g, quantitative conversion). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.49 (d, J = 7.9 Hz, 4H, ph-2,6*H*), 7.25 (t, J = 7.3 Hz, 4H, ph-3,5*H*), 7.11 (t, J = 7.6 Hz, 2H, ph-4*H*), 5.26 (s, 1H, O*H*), 4.80-4.77/4.72-4.68 (m/m, 0.5H/0.5H, C*H*F), 4.23-4.08 (m, 2H, CH_2OAc), 2.86-2.81 (m, 2H, pip-2,6H^{eq}), 2.46 (t, J = 11.8 Hz, 1H, pip-4*H*), 2.37-2.30 (m, 2H, NCH_2), 2.04 (s, 3H, COCH_3), 1.91-1.69 (m, 4H, CH_2CHF, pip-2,6H^{ax}), 1.49-1.44 (pip-3,5H^{eq}), 1.21 (br s, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.2 (*C*=O), 147.3 (ph-1*C*), 127.8 (ph-3-5*C*), 125.8 (ph-2,6*C*), 90.6 (d, $^1J_{C,F}$ = 168.9 Hz, *C*HF), 78.5 (*C*OH), 65.4 (d, $^2J_{C,F}$ = 20.4 Hz, *C*H$_2$OAc), 54.0 (N*C*H$_2$), 53.3 (pip-2,6*C*), 43.5 (pip-4*C*), 28. 2 (d, $^2J_{C,F}$ = 20.3 Hz, *C*H$_2$CHF), 26.0 (pip-3,5*C*), 20.6 (CO*C*H$_3$). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –186.72 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{30}FNO_3$, 400.2288; found, 400.2286.

2-Fluoro-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butanoic acid (28). Compound **25** (0.13 g, 0.34 mmol) was dissolved in methanol (0.5 ml) and THF (0.5 ml). Aqueous potassium hydroxide (2 M, 1 ml) was added and the mixture was stirred overnight at room temperature. The organic solvents were subsequently evaporated and the aqueous solution neutralized with hydrogen chloride solution. The product precipitated as a white solid that was recrystallized from acetonitrile under addition of a drop of water (0.03 g, 24%). Mp: 242–244 °C (carbonisation). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.43 (d, J = 7.7 Hz, 4H, ph-2,6*H*), 7.21 (t, J = 7.7 Hz, 4H, ph-3,5*H*), 7.07 (t, J = 7.3 Hz, 2H, ph-4*H*), 5.23 (s, 1H, O*H*), 4.92/4.84 (s/s, 0.5H/0.5H, C*H*F), 3.43

(br s, 2H, pip-2,6H^{eq}), 3.13-3.04 (m, 2H, NCH_2), 2.91 (m, 2H, pip-2,6H^{ax}), 2.74 (s, 1H, pip-4*H*), 2.31-2.10 (CH_2CHF), 1.49/1.25 (overlay with CD_3COOD peak: pip-3,5H_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 171.0 (*C*=O), 146.0 (ph-1*C*), 127.8 (ph-3,5*C*), 126.0 (ph-4*C*), 125.5 (ph-2,6*C*), 86.8 (d, $^1J_{C,F}$ = 181.8 Hz, *C*HF), 77.9 (*C*OH), 51.8 (pip-2,6*C*, NCH_2), 40.5 (pip-4*C*), 26.6 (d, $^2J_{C,F}$ = 21.6 Hz, *C*H_2CHF), 23.8 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –187.15 (CH*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{26}FNO_3$, 372.1975; found, 372.1984.

***tert*-Butyl 4-(bis(4-fluorophenyl)(hydroxy)methyl)piperidine-1-carboxylate (33).** To a cooled (ice bath) solution of 4-fluorophenylmagnesium bromide in THF (1 M, 8.75 ml) was added di-*tert*-butyl piperidine-1,4-dicarboxylate (0.5 g, 2.0 mmol), dissolved in anhydrous THF (10 ml). The mixture was heated to 60 °C for 3 h. After quenching with brine at 0 °C (ice bath) the mixture was concentrated to dryness. The crude product was taken up in water and extracted into ethyl acetate. The organic layer was dried ($MgSO_4$), filtered and concentrated to give a light yellow solid. Recrystallization from ethanol gave a white solid (0.38 g, 47%). Mp: 152–152 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42-7.37 (m, 2H, ph-2,6*H*), 7.01-6.96 (m, 2H, ph-3,5*H*), 4.15 (br s, 2H, pip-2,6H^{eq}), 2.69 (t, *J* = 10.2 Hz, 2H, pip-2,6H^{ax}), 2.51-2.42 (m, 1H, pip-4*H*), 1.50 (br s, 2H, pip-3,5H^{eq}), 1.42 (s, 9H, C(CH_3)$_3$), 1.34-1.21 (m, 2H, pip-3,5H^{ax}). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.7 (d, $^1J_{C,F}$ = 244.5 Hz, ph-4*C*), 154.8 (*C*=O), 141.3 (ph-1*C*), 127.6 (d, $^3J_{C,F}$ = 7.8 Hz, ph-2,6*C*), 115.2 (d, $^2J_{C,F}$ = 21.0 Hz, ph-3,5*C*), 79.6 (*C*OH), 79.2 (*C*$(CH_3)_3$), 44.6 (pip-4*C*), 43.9 (pip-2,6*C*), 28.5 (C(CH$_3$)$_3$), 26.5 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –117.77 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{23}H_{27}F_2NO_3$, 402.1881; found, 402.1898.

(4-Fluorophenyl)(phenyl)(piperidin-4-yl)methanol hydrochloride (34). To a solution of compound **32** (0.56 g, 1.46 mmol) in THF (1.0 ml) was added HCl in dioxane (4 M, 0.8 ml). The solution was stirred overnight at room temperature, while the product slowly crystallized as

hydrochloric salt. The precipitate was filtered off and washed with THF and diethyl ether to give the product as a white solid (0.32 g, 68%). Mp: 240–242 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.20/8.54 (br s/br s, 1H/1H, NH_2^+), 7.53-7.48 (m, 4H, ph-2,6*H*, ph'-2,6*H*), 7.28 (t, J = 7.7 Hz, 2H, ph-3,5*H*), 7.14 (t, J = 7.3 Hz, 2H, ph-4*H*), 7.09 (t, J = 8.8 Hz, 2H, ph'-3,5*H*), 5.66 (CO*H*), 3.19 (d, J = 12.0 Hz, 2H, pip-2,6H^{eq}), 2.84 (br s, 3H, pip-2,6H^{ax}, pip-4*H*), 1.64-1.56 (m, 2H, pip-3,5H^{ax}), 1.37 (t, J = 10.5, 2H, pip-3,5H^{eq}). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.6 (d, $^1J_{C,F}$ = 241.4 Hz, ph'-4*C*), 146.4 (ph-1*C*), 142.8 (ph'-1*C*), 128.0 (ph-3,5*C*), 127.8 (d, $^3J_{C,F}$ = 7.9 Hz, ph'-2,6*C*), 126.2 (ph-4*C*), 125.7 (ph-2,6*C*), 114.5 (d, $^2J_{C,F}$ = 20.9 Hz, ph'-3,5*C*), 78.2 (*C*OH), 43.2 (pip-2,6*C*), 40.9 (pip-4*C*), 23.3 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –117.66 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{20}FNO$, 286.1607; found, 286.1594.

Bis(4-fluorophenyl)(piperidin-4-yl)methanol hydrochloride (35). Compound **33** (0.5 g, 1.2 mmol) was dissolved in THF (2 ml). HCl in dioxane (4 M, 0.6 ml) was added dropwise and the solution was stirred overnight at room temperature. During this time a white precipitate formed, which was filtered off, washed with DCM and diethyl ether and dried (0.24 g, 60%). Mp: 244 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.16/8.52 (d/d, J = 9.7/9.7 Hz, 1H/1H, NH_2^+), 7.51-7.49 (m, 4H, ph-2,6*H*), 7.10 (t, J = 8.8 Hz, 4H, ph-3,5*H*), 5.73 (s, 1H, CO*H*), 3.19 (d, J = 12.0 Hz, 2H, pip-2,6H^{eq}), 2.84-2.82 (m, 3H, pip-2,6H^{ax}, pip-4*H*), 1.61-1.54 (m, 2H, pip-3,5H^{ax}), 1.38 (d, J = 13.5 Hz, 2H, pip-3,5H^{eq}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 160.6 (d, $^1J_{C,F}$ = 241.4 Hz, ph-4*C*), 142.6 (ph-1*C*), 127.8 (d, $^3J_{C,F}$ = 7.8 Hz, ph-2,6*C*), 114.6 (d, $^2J_{C,F}$ = 20.9 Hz, ph-3,5*C*), 78.0 (*C*OH), 43.2 (pip-2,6*C*), 40.9 (pip-4*C*), 23.3 (pip-3,5*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ: –117.47 (ph-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{19}F_2NO$, 304.1513; found, 304.1516.

4-(4-((4-Fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)butyl acetate (38). Under inert atmosphere, compound **34** (0.1 g, 0.31 mmol), 4-bromobutyl acetate (0.09 ml, 0.62 mmol)

and potassium carbonate (0.13 g, 0.93 mmol) were dissolved in anhydrous acetone (5 ml). After heating at 60 °C for 3 h the mixture was stirred overnight at room temperature. The inorganic compounds were filtered off and the crude purified by column chromatography (DCM: methanol = 100: 0 → 95: 5). The product was isolated as colorless oil (117 mg, 94%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.53-7.48 (m, 4H, ph-2,6*C*, ph'-2,6*C*), 7.27 (t, J = 7.8 Hz, 2H, ph-3,5*H*), 7.13 (t, J = 7.3 Hz, 1H, ph-4*H*), 7.07 (d, J = 8.9 Hz, 2H, ph'-3,5*H*), 5.37 (s, 1H, CO*H*), 3.98 (t, J = 6.5 Hz, 2H, but-1H_2), 2.90 (br s, 2H, pip-2,6H^{eq}), 2.50 (br s, 1H, pip-4*H*), 2.31 (br s, 2H, but-4H_2), 1.98 (m, 5H, CH_3, pip-2,6H^{ax}), 1.56-1.46 (m, 6H, but-2,3H_2, pip-3,5H^{eq}), 1.24 (br s, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.5 (*C*=O), 160.5 (d, $^{1}J_{C,F}$ = 240.8 Hz, ph'-4*C*), 147.0 (ph-1*C*), 143.4 (ph'-1*C*), 127.9 (ph-3,5*C*), 127.8 (d, $^{3}J_{C,F}$ = 7.7 Hz, ph'-2,6*C*), 126.0 (ph-4*C*), 125.7 (ph-2,6*C*), 114.1 (d, $^{2}J_{C,F}$ = 20.9 Hz, ph'-3,5*C*), 78.2 (*C*OH), 63.8 (but-1*C*), 57.3 (but-4*C*), 53.4 (pip-2,6*C*), 43.3 (pip-4*C*), 26.0 (but-2*C*), 25.7 (pip-3,5*C*), 22.6 (but-3*C*), 20.8 (*C*H$_3$). ^{19}F NMR (DMSO-d_6, 282 MHz): δ: –118.01 (ph'-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{30}FNO_3$, 400.2288; found, 400.2296.

4-(4-((4-Fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)butan-1-ol (39). Under inert atmosphere, compound **34** (0.1 g, 0.31 mmol), 4-bromo-1-butanol (0.1 g, 0.62 mmol) and potassium carbonate (0.13 g, 0.93 mmol) were dissolved in anhydrous acetone (5 ml). After heating at 60 °C for 3 h the mixture was stirred overnight at room temperature. The inorganic compounds were filtered off and the crude purified by column chromatography (DCM: methanol = 98: 2 → 95: 5). The product was isolated as colorless oil (87 mg, 78%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.53-7.48 (m, 4H, ph-2,6*C*, ph'-2,6*C*), 7.29 (t, J = 7.7 Hz, 2H, ph-3,5*H*), 7.15 (t, J = 7.3 Hz, 1H, ph-4*H*), 7.10 (d, J = 8.8 Hz, 2H, ph'-3,5*H*), 5.56 (s, 1H, CO*H*), 3.56 (s, 1H, CH_2O*H*), 3.39 (t, J = 6.3 Hz, 2H, but-1H_2), 3.33 (pip-2,6H^{eq}, overlay with water), 3.19 (br s, 2H,

pip-2,6H^{ax}), 2.68 (br s, 3H, but-4H_2, pip-4H), 1.59-1.55 (m, 4H, but-3H_2, pip-3,5H^{eq}), 1.43-1.34 (m, 4H, but-2H_2, pip-3,5H^{ax}),. ^{13}C NMR (DMSO-d_6, 150 MHz): δ 160.6 (d, $^1J_{C,F}$ = 241.1 Hz, ph'-4C), 146.6 (ph-1C), 143.0 (ph'-1C), 128.0 (ph-3,5C), 127.8 (d, $^3J_{C,F}$ = 7.8 Hz, ph'-2,6C), 126.2 (ph-4C), 125.7 (ph-2,6C), 114.5 (d, $^2J_{C,F}$ = 20.9 Hz, ph'-3,5C), 78.1 (COH), 60.3 (but-1C), 56.8 (but-4C), 53.5 (pip-2,6C), 41.9 (pip-4C), 29.9 (but-2C), 24.7 (pip-3,5C), 21.6 (but-3C). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –117.74 (ph'-F). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{28}FNO_2$, 358.2182; found, 358.2185.

1-(3-(1,3-Dioxolan-2-yl)propyl)-4-((4-fluorophenyl)(phenyl)methylene)piperidine (41). Under inert atmosphere, compound **36** (0.14 g, 0.53 mmol), 2-(3-chloropropyl)-1,3-dioxolane (0.15 ml, 1.06 mmol), potassium carbonate (0.3 g, 2.1 mmol) and potassium iodide (0.02 g, 0.1 mmol) were dissolved in anhydrous acetonitrile (10 ml). The mixture was heated overnight at 80 °C. After cooling, the inorganic compounds were filtered off and the crude purified by column chromatography (DCM: methanol = 100: 0 → 95: 5). The product was isolated as colorless oil (50 mg, 25%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.28 (t, J = 7.6 Hz, 2H, ph-3,5H), 7.20 (t, J = 7.3 Hz, 1H, ph-4H), 7.10-7.06 (m, 4H, ph-2,6H, ph'-2,6H), 6.96 (t, J = 8.6 Hz, 2H, ph'-3,5H), 4.88 (t, J = 4.1 Hz, 1H, diox-CH), 3.96/3.84 (t/t, J = 6.9/6.9 Hz, 2H/2H, diox-4,5H_2), 2.51 (s, 4H, pip-2,6H_2), 2.40-2.39 (m, 6H, prop-1H_2, pip-3,5H_2), 1.67 (m, 4H, prop-2,3H_2). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 161.6 (d, $^1J_{C,F}$ = 243.0 Hz, ph'-4C), 142.4 (ph-1C), 138.4 (d, $^4J_{C,F}$ = 3.0 Hz, ph'-1C), 135.9 (C=C), 134.9 (C=$C^{pip\text{-}4C}$), 131.5 (d, $^3J_{C,F}$ = 7.5 Hz, ph'-2,6C), 129.9 (ph-2,6C), 128.2 (ph-3,5C), 126.6 (ph-4C), 115.0 (d, $^2J_{C,F}$ = 21.0 Hz, ph'-3,5C), 104.5 (diox-CH), 65.0 (diox-4,5C), 58.3 (prop-1C), 55.2 (pip-2,6C), 31.9 (pip-3,5C), 31.6 (prop-3C), 21.4 (prop-2C). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –116.74 (ph'-F). HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{28}FNO_2$, 382.2182; found, 382.2175.

4-(4-((4-Fluorophenyl)(phenyl)methylene)piperidin-1-yl)butanal (42). Compound **41** (30 mg, 0.08 mmol) was dissolved in THF (0.5 ml) and HCl (1 M; 1.5 ml), and the solution was stirred overnight at room temperature. The mixture was subsequently diluted with DCM and extracted with aqueous NaOH (2 M). The separated organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield the product as colorless oil (20 mg, 74%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.71 (C*H*O), 7.28 (t, J = 7.6 Hz, 2H, ph-3,5*H*), 7.20 (t, J = 7.4 Hz, 1H, ph-4*H*), 7.10-7.06 (m, 4H, ph-2,6*H*, ph'-2,6*H*), 6.96 (t, J = 8.6 Hz, 2H, ph'-3,5*H*), 2.50 (br s, 4H, pip-2,6H_2), 2.40 (br s, 6H, but-4H_2, pip-3,5H_2), 1.60 (m, 4H, but-2,3H_2). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 201.4 (*C*HO), 161.6 (d, $^1J_{C,F}$ = 245.3 Hz, ph'-4*C*), 142.4 (ph-1*C*), 138.4 (ph'-1*C*), 136.1 (*C*=C), 134.8 (C=*C*$^{pip\text{-}4C}$), 131.4 (d, $^3J_{C,F}$ = 7.8 Hz, ph'-2,6*C*), 129.9 (ph-2,6*C*), 128.2 (ph-3,5*C*), 126.6 (ph-4*C*), 115.0 (d, $^2J_{C,F}$ = 21.1 Hz, ph'-3,5*C*), 55.3 (but-4*C*), 52.9 (pip-2,6*C*), 31.6 (but-2*C*), 30.6 (pip-3,5*C*), 22.1 (but-3*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –116.53 (ph'-*F*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{24}FNO$, 338.1920; found, 338.1912.

4-(4-((4-Fluorophenyl)(phenyl)methylene)piperidin-1-yl)butanoic acid (44). Compound **43** (0.3 g, 0.8 mmol) was dissolved in methanol (1 ml) and THF (1 ml). Aqueous potassium hydroxide (2 M, 2 ml) was added and the mixture was stirred for 3 h at room temperature. The organic solvents were subsequently removed under reduced pressure and the aqueous solution neutralized with hydrogen chloride solution. An oily product separating from the mixture was extracted into DCM. The dried and concentrated product was purified by column chromatography (DCM: methanol = 97: 3 → 7: 3) and isolated as white solid (0.1 g, 36%). Mp: 115–117 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.32 (t, J = 7.6 Hz, 2H, ph-3,5*H*), 7.23 (t, J = 7.4 Hz, 1H, ph-4*H*), 7.16-7.09 (m, 6H, ph-2,6*H*, ph'-2,3,5,6*H*), 2.60 (br s, 4H, pip-2,6H_2), 2.27 (t, J = 7.0 Hz, 2H, but-4H_2), 2.30 (t, J = 5.5 Hz, 4H, pip-3,5H_2), 2.26 (t, J = 7.1 Hz, 2H, but-2H_2),

1.72-1.67 (m, 2H, but-3H_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 174.4 (C=O), 160.9 (d, $^1J_{C,F}$ = 242.0, ph'-4C), 141.8 (ph-1C), 138.2 (d, $^4J_{C,F}$ = 3.2 Hz, ph'-1C), 134.7 (C=C), 134.5 (C=$C^{pip\text{-}4C}$), 131.3 (d, $^3J_{C,F}$ = 8.0 Hz, ph'-2,6C), 129.4 (ph-3,5C), 128.3 (ph-2,6C), 126.7 (ph-4C), 115.1 (d, $^2J_{C,F}$ = 21.2 Hz, ph'-3,5C), 56.5 (but-4C), 54.0 (pip-2,6C), 32.1/21.3 (but-2,3C), 30.4 (pip-3,5C). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –116.35 (ph'-F). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{24}FNO_2$, 354.1864; found, 354.1863.

Ethyl 4-(4-(4-fluorobenzoyl)piperidin-1-yl)butanoate (47). Under inert atmosphere, compound **37** (as HCl salt; 0.25 g, 1.0 mmol), 4-bromo-butyric acid ethyl ester (0.28 ml, 2.0 mmol) and potassium carbonate (0.57 g, 4.0 mmol) were dissolved in anhydrous acetone (10 ml). After heating at 60 °C for 3 h the mixture was stirred overnight at room temperature. The inorganic compounds were filtered off and the crude purified by column chromatography (DCM: methanol = 100: 0 → 96: 4). The product was isolated as colorless oil (0.28 g, 88%). An analytical sample of the product was crystallized as HCl salt. Mp: 138–139 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.67 (br s, 1H, NH^+), 8.11-8.09 (dd, J = 8.7 Hz, 2H, ph-2,6H), 7.40 (dd, J = 8.8 Hz, 2H, ph-3,5H), 4.08 (q, 2H, OCH_2CH$_3$), 3.72 (m, 1H, pip-4H), 3.52 (d, J = 11.8 Hz, 2H, pip-2,6H^{eq}), 3.08-3.02 (m, 4H, pip-2,6H^{ax}, but-4H_2), 2.42 (t, J = 7.4 Hz, 2H, but-2H_2), 2.00-1.92 (m, 6H, pip-3,5H_2, but-3H_2), 1.20 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 199.6 (C=O^{ketone}), 172.0 (C=O^{ester}), 165.2 (d, $^1J_{C,F}$ = 250.7 Hz, ph-4C), 131.9 (d, $^4J_{C,F}$ = 2.7, ph-1C), 131.4 (d, $^3J_{C,F}$ = 9.3, ph-2,6C), 116.01 (d, $^2J_{C,F}$ = 21.8, ph-3,5C), 60.1 (OCH$_2$CH$_3$), 55.2 (but-4C), 52.4 (pip-2,6C), 30.6 (but-2C), 25.6 (pip-3,5C), 18.7 (but-3C), 14.2 (OCH$_2$$CH_3$). ^{19}F NMR (DMSO-$d_6$, 282 MHz): δ –106.04 (ph'-F). HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{24}FNO_2$, 322.1818; found, 322.1813.

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl 4-methylbenzenesulfonate (48). 2-(2,2-Dimethyl-[1,3]dioxolan-4-yl)-ethanol (5 g, 34 mmol) was dissolved in DCM (100 ml). The solution was cooled (ice bath) and pyridine (3 ml, 37.4 mmol) and tosyl chloride (7.1 g, 37.4 mmol) were subsequently added. The mixture was stirred overnight at room temperature. After dilution with DCM the reaction was quenched with water. The separated organic layer was washed with brine, dried ($MgSO_4$) and concentrated to dryness. The product was purified by column chromatography (DCM) giving a colorless oil (2.8 g, 27.5%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.78 (d, J = 8.3 Hz, 2H, ph-2,6*H*), 7.35 (d, J = 7.5 Hz, 2H, ph-3,5*H*), 4.19-4.09 (m, 3H, diox-CH_2, diox-4*H*), 4.01/3.51 (t/t, J = 4.7/5.0 Hz, 1H/1H, diox-5H_2), 2.45 (s, 3H, ph-CH_3), 1.94-1.86 (m, 2H, OCH_2), 1.33/1.28 (s/s, 3H/3H, C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 145.0 (ph-4*C*), 133.0 (ph-1*C*), 130.1 (ph-3,5*C*), 128.1 (ph-2,6*C*), 109.2 (*C*(CH$_3$)$_2$), 72.4 (diox-4*C*), 69.2 (diox-5*C*), 67.5 (O-*C*H$_2$), 33.2 (diox-*C*H$_2$), 27.0/25.7 (C(*C*H$_3$)$_2$), 21.8 (ph-*C*H$_3$). HRMS (m/z): [M+H]$^+$ calcd. for $C_{14}H_{20}O_5S$, 301.1110; found, 301.1113.

1-Benzhydryl-4-(2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)piperazine (49). TEA (1.24 ml, 9.3 mmol) was dissolved in dry acetonitrile (100 ml). Under inert atmosphere, 1-benzhydryl-piperazine (2.3 g, 9.3 mmol) and compound **48** (2.8 g, 9.3 mmol) were subsequently added. The solution was stirred for 6 h at 70 °C. After cooling it was concentrated to dryness and the product was purified by column chromatography (TEA; DCM: methanol = 100: 0 → 95: 5) giving a yellow oil (1.36 g, 39%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.40 (d, J = 7.7 Hz, 4H, ph-2,6*H*), 7.27 (t, J = 7.7 Hz, 4H, ph-3,5*H*), 7.17 (t, J = 7.4 Hz, 2H, ph-4*H*), 4.23 (s, 1H, benzh.-C*H*), 4.02-3.98 (m, 1H, diox-4*H*), 3.97/3.42 (t/t, J = 4.6/7.4 Hz, 1H/1H, diox-5H_2), 2.49-2.24 (m, 10H, prz-2,3,5,6H_2, NCH_2), 1.63-1.59 (m, 2H, OCH_2), 1.28/1.23 (s/s, 3H/3H, C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 143.0 (ph-1*C*), 128.5 (ph-3,5*C*), 127.6 (ph-2,6*C*), 126.8 (ph-4*C*), 107.7

($C(CH_3)_2$), 75.3 (benzh.-*C*H), 74.2 (diox-4*C*), 68.8 (diox-5*C*), 55.0 (NCH_2), 53.0 (prz-2,6*C*), 51.6 (prz-3,5*C*), 30.6 (diox-CH_2), 26.9/25.7 ($C(CH_3)_2$). HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{32}N_2O_2$, 381.2542; found, 381.2550.

4-(4-Benzhydrylpiperazin-1-yl)butane-1,2-diol (50). Compound **49** (1.36 g, 3.6 mmol) was dissolved in methanol (13 ml). PTSA (1.5 g, 7.9 mmol) was added and the solution was stirred for 4 h at 40 °C. After cooling it was quenched with aqueous NaOH (2 N). The organic solvent was removed under reduced pressure and the crude product extracted in DCM. Final purification was performed by column chromatography (TEA; DCM: methanol = 95: 5 → 90: 10) giving the product as colorless oil (1.1 g, 90%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.40 (d, J = 7.6 Hz, 4H, ph-2,6*H*), 7.28 (t, J = 7.7 Hz, 4H, ph-3,5*H*), 7.17 (t, J = 7.4 Hz, 2H, ph-4*H*), 4.64/4.60 (br s/br s, 2H, O*H*/O*H*), 4.24 (s, 1H, benzh.-C*H*), 3.46-3.42 (m, 1H, C*H*OH), 3.28-3.25/3.21-3.16 (m/m, 1H/1H, CH_2OH), 2.43-2.30 (m, 10H, prz-2,3,5,6H_2, NCH_2), 1.61-1.56/1.40-1.42 (m/m, 2H, CH_2CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 143.0 (ph-1*C*), 128.55 (ph-3,5*C*), 127.57 (ph-2,6*C*), 126.85 (ph-4*C*), 75.24 (benzh.-*C*H), 70.46 (*C*HOH), 65.9 (CH_2OH), 55.0 (NCH_2), 53.0 (prz-2,6*C*), 51.6 (prz-3,5*C*), 30.7 (CH_2CH). HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{28}N_2O_2$, 341.2229; found, 341.2226.

4-(4-Benzhydrylpiperazin-1-yl)-2-hydroxybutyl acetate (51). Compound **50** (1.5 g, 1.5 mmol) was dissolved in DCM (20 ml). TEA (0.23 ml, 1.65 mmol) and acetic anhydride (0.14 ml, 1.5 mmol) were added under cooling (ice bath). The mixture was stirred 3 h at room temperature and subsequently quenched by adding aqueous NaOH (2 N). The separated and washed (brine, water) organic layer was concentrated and purified by column chromatography (TEA; DCM: methanol = 97: 3) giving the product as colorless oil (0.26 g, 46%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.41 (d, J = 7.5 Hz, 4H, ph-2,6*H*), 7.28 (t, J = 7.7 Hz, 4H, ph-3,5*H*), 7.17 (t, J = 7.3 Hz,

2H, ph-4*H*), 5.02 (br s, 1H, O*H*), 4.24 (s, 1H, benzh.-C*H*), 3.87 (d, *J* = 5.6 Hz, 2H, CH_2OCOCH$_3$), 3.70-3.66 (m, 1H, C*H*OH), 2.52-2.09 (m, 10H, prz-2,3,5,6H_2, NCH_2), 1.99 (s, 3H, COCH_3), 1.58-1.51/1.46-1.38 (m/m, 2H, CH_2CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.4 (*C*=O), 143.0 (ph-1*C*), 128.5 (ph-3,5*C*), 127.6 (ph-2,6*C*), 126.9 (ph-4*C*), 75.2 (benzh.-*C*H), 68.0 (*C*H$_2$OCOCH$_3$), 66.9 (*C*HOH), 54.5 (NCH$_2$), 53.0 (prz-2,6*C*), 51.6 (prz-3,5*C*), 30.2 (*C*H$_2$CH), 21.1 (CO*C*H$_3$). HRMS (m/z): [M+H]$^+$ calcd. for $C_{23}H_{30}N_2O_3$, 383.2335; found, 383.2324.

4-(4-Benzhydrylpiperazin-1-yl)-2-((methylsulfonyl)oxy)butyl acetate (52). Compound **51** (1.3 g, 3.4 mmol) was dissolved in DCM (20 ml). TEA (0.5 ml, 3.7 mmol) and methanesulfonyl chloride (0.3 ml, 3.7 mmol) were added dropwise and under cooling (ice bath). The mixture was stirred 1 h at room temperature and subsequently quenched with aqueous NaOH (2 N). The separated, dried ($MgSO_4$) and filtered organic layer was purified by column chromatography (TEA; DCM: methanol = 100: 0 → 97: 3). The product was isolated as colorless oil (1.1 g, 70%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.41 (d, *J* = 8.0 Hz, 4H, ph-2,6*H*), 7.28 (t, *J* = 7.4, 4H, ph-3,5*H*), 7.17 (t, *J* = 7.2 Hz, 2H, ph-4*H*), 4.84-4.80 (m, 1H, C*H*OMs), 4.25/4.14 (dd/dd, *J* = 12.1/12.4 Hz, 1H/1H, CHCH_2O), 4.25 (s, 1H, benzh.-C*H*), 3.18 (s, 3H, SCH_3), 2.50-2.10 (m, 10H, prz-2,3,5,6H_2, NCH_2), 2.03 (s, 3H, COCH_3), 1.85-1.80 (m, 2H, CH_2CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.1 (*C*=O), 143.0 (ph-1*C*), 128.6 (ph-3,5*C*), 127.6 (ph-2,6*C*), 126.9 (ph-4*C*), 78.5 (*C*HOMs), 75.3 (benzh.-*C*H), 64.7 (*C*H$_2$O), 55.0 (NCH$_2$), 52.6 (prz-2,6*C*), 51.6 (prz-3,5*C*), 37.7 (SCH$_3$), 27.9 (*C*H$_2$CH), 20.6 (CO*C*H$_3$). HRMS (m/z): [M+H]$^+$ calcd. for $C_{24}H_{32}N_2O_5S$, 461.2110; found, 461.2086.

(1-(2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl)piperidin-4-yl)diphenylmethanol (53). TEA (4 ml, 29.3 mmol) was dissolved in dry acetonitrile (50 ml). Under inert atmosphere, diphenyl(piperidin-4-yl)methanol hydrochloride (8.64 g, 24.1 mmol) and compound **48** (7.2 g g,

24.1 mmol) were subsequently added. The solution was stirred for 6 h at 70 °C. After cooling it was concentrated to dryness and the product was purified by column chromatography (TEA; DCM: methanol = 100: 0 → 95: 5) giving a light yellow oil (1.5 g, 16%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.50 (d, J = 8.2 Hz, 4H, ph-2,6H), 7.25 (t, J = 7.7 Hz, 4H, ph-3,5H), 7.11 (t, J = 7.3 Hz, 2H, ph-4H), 5.23 (s, 1H, OH), 4.01-3.95 (m, 2H, diox-4H, diox-5H), 3.42 (t, J = 7.2 Hz, 1H, diox-5H_2), 2.82 (br s, 2H, pip-2,6H^{eq}), 2.45 (t, J = 11.5 Hz, 1H, pip-4H), 2.28/2.24 (br s/br s, 2H, NCH_2), 1.86-1.77 (m, 2H, pip-2,6H^{ax}), 1.62-1.60 (m, 2H, CH_2CH), 1.47-1.43 (m, 2H, pip-3,5H^{eq}), 1.28/1.23 (s/s, 3H/3H, C(CH_3)$_2$), 1.22 (br s, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 147.4 (ph-1C), 127.8 (ph-3-5C), 125.6 (ph-2,6C), 107.7 (C(CH$_3$)$_2$), 78.4 (COH), 75.0 (diox-4C), 68.3 (diox-5C), 66.1 (NCH$_2$), 53.6 (pip-2,6C), 43.6 (pip-4C), 35.2 (diox-CH$_2$), 26.9/25.8 (C(CH$_3$)$_2$), 25.9 (pip-3,5C). HRMS (m/z): [M+H]$^+$ calcd. for $C_{25}H_{33}NO_3$, 396.2539; found, 396.2533.

4-(4-(Hydroxydiphenylmethyl)piperidin-1-yl)butane-1,2-diol (54). Compound **53** (1.5 g, 3.9 mmol) was dissolved in methanol (15 ml). PTSA (1.1 g, 5.8 mmol) was added and the solution was stirred for 4 h at 40 °C. After cooling it was quenched with aqueous NaOH (2 N). The organic solvent was removed under reduced pressure and the crude product extracted in DCM. Final purification was performed by column chromatography (TEA; DCM: methanol = 95: 5 → 90: 10) giving the product as colorless oil (1.35 g, quantitative conversion). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.48 (d, J = 7.6 Hz, 4H, ph-2,6H), 7.24 (t, J = 7.6 Hz, 4H, ph-3,5H), 7.10 (t, J = 7.2 Hz, 2H, ph-4H), 5.26 (s, 1H, OH), 4.78 (br s, 2H, 2x OH), 3.46-3.42 (m, 1H, CHOH), 3.29-3.15 (m, 2H, CH_2OH), 2.88 (br s, 2H, pip-2,6H^{eq}), 2.49-2.34 (m, 3H, pip-4H, NCH_2), 1.90 (br s, 2H, pip-2,6H^{ax}), 1.56-1.35 (m, 4H, CH_2OH, pip-3,5H^{eq}), 1.25 (d, J = 12.2 Hz, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 147.3 (ph-1C), 127.8 (ph-3-5C), 125.8 (ph-

2,6*C*), 78.5 (*C*OH), 70.7 (*C*HOH), 65.9 (*C*H_2OH), 55.3 (N*C*H_2), 53.6 (pip-2,6*C*), 43.4 (pip-4*C*), 30.4 (*C*H_2CH(OH)CH_2OH), 25.9 (pip-3,5*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{29}NO_3$, 356.2226; found, 356.2216.

2-Hydroxy-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butyl acetate (55). Compound **54** (1.35 g, 3.9 mmol) was dissolved in DCM (50 ml). TEA (0.6 ml, 4.3 mmol) and acetic anhydride (0.4 ml, 3.9 mmol) were subsequently added and the solution was stirred for 3 h at room temperature. The reaction was quenched with aqueous NaOH (2 N) and the organic layer was separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Final purification was performed by column chromatography (TEA; DCM: methanol = 99: 1 → 97: 3) giving the product as colorless oil (0.6 g, 41%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.50 (d, J = 8.2 Hz, 4H, ph-2,6*H*), 7.25 (t, J = 7.7 Hz, 4H, ph-3,5*H*), 7.11 (t, J = 7.2 Hz, 2H, ph-4*H*), 5.26 (s, 1H, CO*H*), 4.76 (br s, 1H, CHO*H*), 3.86 (d, J = 5.5 Hz, 2H, CH_2OCOCH_3), 3.71-3.67 (m, 1H, C*H*OH), 2.86 (t, J = 13.9 Hz, 2H, pip-2,6H^{eq}), 2.47 (t, J = 8.7 Hz, 1H, pip-4*H*), 2.39-2.31 (m, 2H, NCH_2), 2.00 (s, 3H, COCH_3), 1.86 (t, J = 13.1 Hz, 2H, pip-2,6H^{ax}), 1.53-1.40 (m, 4H, CH_2CH(OH), pip-3,5H^{eq}), 1.22 (d, J = 12.0 Hz, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.4 (*C*=O), 147.3 (ph-1*C*), 127.8 (ph-3-5*C*), 125.8 (ph-2,6*C*), 78.5 (*C*OH), 68.0 (*C*H_2OCOCH_3), 67.2 (*C*HOH), 55.0 (N*C*H_2), 53.7 (pip-2,6*C*), 43.5 (pip-4*C*), 30.1 (*C*H_2CH(OH)CH_2), 26.0 (pip-3,5*C*), 20.8 (CO*C*H_3). HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{31}NO_4$, 398.2331; found, 398.2331.

4-(4-(Hydroxydiphenylmethyl)piperidin-1-yl)-2-((methylsulfonyl)oxy)butyl acetate (56). To a cooled (ice bath) solution of compound **55** (0.32 g, 0.8 mmol) and TEA (0.12 ml, 0.88 mmol) in DCM (10 ml) was added methanesulfonyl chloride (0.07 ml, 0.88 mmol) dropwise. The mixture was stirred 1 h at room temperature and subsequently quenched with brine. The separated, dried ($MgSO_4$) and filtered organic layer was purified by column chromatography

(TEA; DCM: methanol = 99: 1 → 98: 2). The product was isolated as colorless solid (0.1 g, 26%). Mp: 78 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.50 (d, J = 8.0 Hz, 4H, ph-2,6*H*), 7.25 (t, J = 7.3 Hz, 4H, ph-3,5*H*), 7.11 (t, J = 7.3 Hz, 2H, ph-4*H*), 5.26 (s, 1H, O*H*), 4.83-4.79 (m, 1H, C*H*OMs), 4.27-4.12 (m, 2H, CH_2OAc), 3.19 (s, 3H, SO_2CH_3), 2.84 (t, J = 13.3 Hz, 2H, pip-2,6H^{eq}), 2.46 (t, J = 11.9 Hz, 1H, pip-4*H*), 2.38-2.27 (m, 2H, NCH_2), 2.03 (s, 3H, COCH_3), 1.90-1.73 (m, 4H, CH_2CHF, pip-2,6H^{ax}), 1.47-1.44 (m, 2H, pip-3,5H^{eq}), 1.21 (d, J = 12.0 Hz, 2H, pip-3,5H^{ax}). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 170.1 (*C*=O), 147.3 (ph-1*C*), 127.8 (ph-3-5*C*), 125.7 (ph-2,6*C*), 78.5 (*C*OH), 64.7 (*C*H_2OAc), 53.8 (N*C*H_2), 53.1 (pip-2,6*C*), 43.5 (pip-4*C*), 37.7 (SO_2CH_3), 28.1 (*C*H_2CH(OMs)), 26.0 (pip-3,5*C*), 20.6 (CO*C*H_3). HRMS (m/z): $[M+H]^+$ calcd. for $C_{25}H_{33}NO_6S$, 476.2107; found, 476.2088.

Ethyl 4-(4-(4-(phenylthio)benzoyl)piperidin-1-yl)butanoate (63). Under inert atmosphere, compound **60** (0.26 g, 0.8 mmol), 4-bromo-butyric acid ethyl ester (0.22 ml, 1.6 mmol) and potassium carbonate (0.44 g, 3.2 mmol) were dissolved in anhydrous acetone (10 ml). After heating at 60 °C for 3 h the mixture was stirred overnight at room temperature. The inorganic compounds were filtered off and the crude purified by column chromatography (DCM: methanol = 100: 0 → 97: 3). The product was isolated as colorless oil (0.26 g, 79%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.79 (d, J = 8.5 Hz, 2H, ph-2,6*H*), 7.50-7.49 (m, 2H, ph'-2,6*H*), 7.40-7.39 (m, 3H, ph'-3-5*H*), 7.20 (d, J = 8.5 Hz, 2H, ph-3,5*H*), 4.12 (q, 2H, OCH_2CH$_3$), 3.18-3.15 (m, 1H, pip-4*H*), 2.98 (d, J = 11.6 Hz, 2H, pip-2,6H^{eq}), 2.40 (t, J = 7.2 Hz, 2H, but-4H_2), 2.33 (t, J = 7.3 Hz, 2H, but-2H_2), 2.11 (br s, 2H, pip-2,6H^{ax}), 1.85-1.80 (m, 6H, pip-3,5H_2, but-3H_2), 1.25 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 201.7 (*C*=O^{ketone}), 173.7 (*C*=O^{ester}), 144.9 (ph-1*C*), 134.1 (ph-2,6*C*), 133.4 (ph-1*C*), 132.1 (ph'-1*C*), 129.91/129.89 (ph-3,5*C*, ph'-2,6*C*), 129.0 (ph'-3,5*C*), 127.6 (ph'-4*C*), 60.5 (O*C*H$_2$CH$_3$), 57.8 (but-4*C*), 53.1 (pip-2,6*C*), 43.4 (pip-4*C*), 32.4

(but-2*C*), 28.6 (pip-3,5*C*), 22.2 (but-3*C*), 14.4 (OCH_2CH_3). HRMS (m/z): $[M+H]^+$ calcd. for $C_{24}H_{30}NO_3S$, 412.1946; found, 412.1936.

(4-(1-(4-Ethoxy-4-oxobutyl)piperidine-4-carbonyl)phenyl)diphenylsulfonium trifluoromethanesulfonate (64). To a solution of compound **63** (0.3 g, 0.73 mmol) in chlorobenzene (3 ml) were added trifluoromethanesulfonic acid (65.0 µl, 0.73 mmol), diphenyliodonium triflate (0.31 g, 0.73 mmol) and copper(II) benzoate hydrate (11.5 mg, 0.036 mmol) and the mixture was heated at 125 °C for 1 h. After cooling, the resulting brown oil was washed with diethyl ether (3 × 20 ml). The product was purified by column chromatography (DCM: methanol = 10: 0 → 9: 1). The isolated product was dissolved in DCM (5 ml) and washed with aqueous NaOH (2 M; 5 ml) and a saturated solution of sodium triflate (5 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated to give the product as a brown oil (0.37 g, 79%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.27 (d, J = 8.5 Hz, 2H, ph-2,6*H*), 7.94 (d, J = 8.2 Hz, 2H, ph-3,5*H*), 7.90-7.86 (m, 6H, ph'-2,4,6*H*), 7.81-7.78 (m, 4H, ph'-3,5*H*), 4.05 (q, 2H, OCH_2CH_3), 3.01/2.34/2.08/1.78 (v br s, 15H, pip-2,3,5,6H_2, pip-4*H*, but-1-3H_2), 1.19 (t, J = 7.1 Hz, 3H, OCH_2CH_3). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 169.2 (C=O^{ester}), 134.6 (ph-1*C*), 131.7 (ph'-3,5*C*), 131.6 (ph-3,5*C*), 131.5 (ph-2,6*C*), 130.1 (ph'-2,6*C*), 124.9 (ph-4*C*), 120.7 (q, $^1J_{C,F}$ = 320.4 Hz, CF_3), 59.9 (OCH_2CH_3), 14.2 (OCH_2CH_3). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –78.21 (CF_3). HRMS (m/z): $[M]^+$ calcd. for $C_{30}H_{34}NO_3S$, 488.2259; found, 488.2242.

1-(3-(1,3-Dioxolan-2-yl)propyl)-4-((4-((4-methoxyphenyl)thio)phenyl)(phenyl)methylene)piperidine (73). Under inert atmosphere, compound **68** (0.42 g, 1.1 mmol), 2-(3-chloropropyl)-1,3-dioxolane (0.3 ml, 2.2 mmol), potassium carbonate (0.6 g, 4.4 mmol) and potassium iodide (37 mg, 0.22 mmol) were dissolved in anhydrous acetonitrile (10 ml). After heating at 80 °C for 5 h the mixture was stirred overnight

at room temperature. The inorganic compounds were filtered off and the crude purified by column chromatography (DCM: methanol = 98: 2 → 8: 2). The product was isolated as colorless oil (0.13 g, 24%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.42 (d, J = 8.6 Hz, 2H, ph''-2,6*H*), 7.30 (t, J = 7.6 Hz, 2H, ph-3,5*H*), 7.21 (t, J = 7.4 Hz, 1H, ph-4*H*), 7.06-7.04 (m, 4H, ph-2,6*H*, ph'-2,6*H*), 7.01-6.99 (m, 4H, ph'-3,5*H*, ph''-3,5*H*), 4.77 (t, J = 4.5 Hz, 2H, diox-C*H*), 3.85/3.74 (t/t, J = 6.9/6.9 Hz, 2H/2H, diox-4,5H_2), 3.75 (s, 3H, OCH_3), 2.39-2.23 (m, 10H, pip-2,3,5,6H_2, prop-1H_2), 1.56-1.50 (m, 4H, but-2,3H_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 159.8 (ph''-4*C*), 141.9 (ph-1*C*), 139.7 (ph'-1*C*), 136.0 (ph'-4*C*, *C*=C^{pip}), 135.6 (ph''-2,6*C*), 134.2 (C=*C*^{pip}), 130.3 (ph'-3,5*C*), 129.4 (ph-2,6*C*), 128.2 (ph-3,5*C*), 127.2 (ph'-2,6*C*), 126.5 (ph-4*C*), 122.7 (ph''-1*C*), 115.4 (ph''-3,5*C*), 103.5 (diox-*C*H), 64.2 (diox-4,5*C*), 57.2 (prop-1*C*), 55.3 (O*C*H$_3$), 54.4 (pip-2,6*C*), 31.2 (pip-3,5*C*), 30.6 (prop-3*C*), 21.1 (prop-2*C*). HRMS (m/z): $[M+H]^+$ calcd. for $C_{31}H_{35}NO_3S$, 502.2416; found, 502.2412.

(4-((1-(3-(1,3-Dioxolan-2-yl)propyl)piperidin-4-ylidene)(phenyl)methyl)phenyl)bis(4-methoxyphenyl)sulfonium trifluoromethanesulfonate (74). To a solution of compound **73** (120 mg, 0.24 mmol) in chlorobenzene (1.0 ml) were added trifluoromethanesulfonic acid (21.5 µl, 0.24 mmol), compound **75** (120 mg, 0.24 mmol) and copper(II) benzoate (4 mg, 0.01 mmol) and the mixture was heated at 125 °C for 1 h. After cooling, the resulting brown oil was washed with diethyl ether (3 × 5 ml). The product was purified by column chromatography (DCM: methanol = 10: 0 → 9: 1). The isolated product was dissolved in DCM (5 ml) and washed with aqueous NaOH (2 M; 5 ml) and a saturated solution of sodium triflate (5 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated to give the product as a light yellow oil (50 mg, 28%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.67 (d, J = 8.9 Hz, 2H, ph-2,6*H*), 7.49 (d, J = 8.3 Hz, 2H, ph'-2,6*H*), 7.39 (d, J = 8.4 Hz, 2H, ph'-3,5*H*), 7.30 (t, J = 7.5 Hz, 2H, ph''-3,5*H*), 7.23 (t, J

= 7.4 Hz, 1H, ph''-4*H*), 7.17 (d, *J* = 8.9 Hz, 4H, ph-3,5*H*), 7.07 (d, *J* = 7.7 Hz, 2H, ph''-2,6*H*), 4.87 (t, *J* = 4.3 Hz, 2H, diox-C*H*), 3.95/3.84 (t/t, *J* = 6.9/6.9 Hz, 2H/2H, diox-4,5H_2), 3.90 (s, 6H, OCH_3), 2.53-2.51 (m, 4H, pip-2,6H_2), 2.43-2.40 (m, 2H, prop-1H_2), 2.37 (s, 4H, pip-3,5H_2), 1.68-1.60 (m, 4H, prop-2,3H_2). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 164.6 (ph-4*C*), 148.7 (ph'-1*C*), 141.0/139.1 (ph'-4*C*, ph''-1*C*), 133.6/123.5 (*C*=*C*^{pip}), 133.2 (ph-2,6*C*), 132.8 (ph'-3,5*C*), 130.0 (ph'-2,6*C*), 129.9 (ph''-2,6*C*), 128.6 (ph''-3,5*C*), 127.2 (ph''-4*C*), 121.0 (q, $^1J_{C,F}$ = 318.0 Hz, CF_3), 117.3 (ph-3,5*C*), 114.4 (ph-1*C*), 104.4 (diox-*C*H), 65.0 (diox-4,5*C*), 58.1 (prop-1*C*), 56.2 (O*C*H$_3$), 55.0 (pip-2,6*C*), 31.9 (pip-3,5*C*), 31.7 (prop-3*C*), 21.4 (prop-2*C*). ^{19}F NMR (DMSO-d_6, 282 MHz): δ –78.05 (CF_3). HRMS (m/z): $[M]^+$ calcd. for $C_{38}H_{42}NO_4S$, 608.2662; found, 608.2683.

Radiochemistry – Equipment. All labeling reactions were performed manually using [^{18}F]fluoride in [^{18}O]H_2O. Radio-HPLC was performed with an Agilent 1200 HPLC system equipped with a 1200 Series Diode Array Detector and a GABI Star NaI(Tl) scintillation detector. The system was used for purification as well as characterization of radiotracers.

General procedure for preparation of compounds [^{18}F]23 and [^{18}F]27. [^{18}F]Fluoride in water was trapped on a Sep-Pak® QMA cartridge and released with 0.5 ml of a solution of Kryptofix 222 (30 mM) and potassium carbonate (7.5 mM) dissolved in acetonitrile: water (85: 15). After removing the solvent by heating at 90 °C under a stream of nitrogen, acetonitrile (0.5 ml) was added, and the distillation was continued at 90 °C. This procedure was repeated, the vial was subsequently capped and the mixture was allowed to come to room temperature. The respective mesylate precursor (4 mg in 0.2 ml anhydrous DMSO) was added and the reaction was stirred at 90 °C for 20 min. After quenching with 0.8 ml water the crude mixture was purified by HPLC using a Chromolith® SemiPrep RP18-e column (100 × 10 mm) at room

temperature. The mobile phase consisted of water and methanol (each containing 0.5% TFA). Gradient elution starting with 30% methanol content that was increased to 70% in 9 min allowed for isolation of the radioactive product. The obtained solution was diluted with 20 ml water, trapped on a Sep-Pak® SPE C-18 light cartridge, and the radiolabeled product was released with 0.5 ml ethanol. After having reduced the volume to 100 µl under a stream of nitrogen, the solution was diluted with saline to give a final ethanol concentration of 5% and sterilized by filtration.

[^{18}F]4-(4-Benzhydrylpiperazin-1-yl)-2-fluorobutyl acetate ([^{18}F]23). Starting with 1.2 GBq fluoride-18 and using precursor **52**, the synthetic procedure afforded 182 MBq of formulated [^{18}F]**23** (13% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 30% methanol content that was increased to 90% in 9 min). The radiochemical purity was 100% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 4.6 min).

[^{18}F]2-Fluoro-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butyl acetate ([^{18}F]27). Starting with 1.0 GBq fluoride-18 and using precursor **56**, the synthetic procedure afforded 105 MBq of formulated [^{18}F]**27** (19% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 30% methanol content that was increased to 90% in 9 min). The radiochemical purity was 100% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 4.5 min).

[^{18}F]4-(4-((4-Fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)butyl acetate ([^{18}F]38). [^{18}F]Fluoride in water (1.2 GBq) was trapped on a Sep-Pak® QMA cartridge, released with 0.5 ml of a solution of Kryptofix 222 (30 mM) and potassium hydrogen carbonate (30 mM) dissolved in acetonitrile: water (85: 15). After removing the solvent by heating at 90 °C under a stream of nitrogen, acetonitrile (0.5 ml) was added, and the distillation was continued at 90 °C. This procedure was repeated and the reaction vial was subsequently capped. Compound **62** (4 mg) dissolved in DMSO (0.5 ml) was subsequently added and the mixture was stirred at 110 °C for 15 min. The reaction was quenched and further diluted with water to a volume of 20 ml. This solution was filtered over a Sep-Pak® SPE C-18 light cartridge. The radiolabeled product was released with acetonitrile (1 ml), which was subsequently evaporated under a stream of nitrogen. The dry crude was taken up in THF (100 µl). Phenylmagnesium bromide (100 µl of a 1 M solution in THF) was added and the solution reacted at 90 °C for 30 min. After cooling, the reaction was quenched with formate buffer (pH 3; 1.8 ml) and purified by HPLC using a Chromolith® SemiPrep RP18-e column (100 × 10 mm) at room temperature. The mobile phase consisted of water and methanol (each containing 0.5% TFA). Gradient elution starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min allowed for isolation of [^{18}F]4-(4-((4-fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)butan-1-ol ([^{18}F]**39**). The obtained solution was diluted with water to a final volume of 20 ml, trapped on a Sep-Pak® SPE C-18 light cartridge, and the radiolabeled product was released with acetonitrile (0.5 ml). TEA (50 µl) and acetyl chloride (25 µl) were added and the solution was stirred for 15 min at ambient temperature. The reaction was quenched with water (1.4 ml) and the product was purified by HPLC using the same gradient that was used for purification of [^{18}F]**39**. The obtained solution was diluted with water to a final volume of 20 ml, trapped on a Sep-Pak® SPE C-18

light cartridge, and the radiolabeled product was released with 0.5 ml ethanol. After having reduced the volume to 100 µl under a stream of nitrogen, the solution was diluted with saline to give a final ethanol concentration of 5% and sterilized by filtration. Starting with 3.0 GBq fluoride-18, the three-step synthetic procedure afforded 13.4 MBq of formulated [^{18}F]**38** (6% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min). The radiochemical purity was 98% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 9.5 min).

[^{18}F]4-(4-((4-fluorophenyl)(hydroxy)(phenyl)methyl)piperidin-1-yl)butan-1-ol ([^{18}F]39).

The compound was synthesized as described in the recipe for [^{18}F]**38**. After HPLC purification, the obtained solution was diluted with water to a final volume of 20 ml, trapped on a Sep-Pak® SPE C-18 light cartridge, and the radiolabeled product was released with 0.5 ml ethanol. After having reduced the volume to 100 µl under a stream of nitrogen, the solution was diluted with saline to give a final ethanol concentration of 5% and sterilized by filtration. The synthetic procedure afforded 28.4 MBq of formulated **[^{18}F]39** (6% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min). The radiochemical purity was 100% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 7.8 min).

General procedure for preparation of alcohols [^{18}F]40 and [^{18}F]46. [^{18}F]Fluoride in water was trapped on a Sep-Pak® QMA cartridge, released with 0.5 ml of a solution of Kryptofix 222 (30 mM) and potassium hydrogen carbonate (30 mM) dissolved in acetonitrile: water (85: 15). After removing the solvent by heating at 90 °C under a stream of nitrogen, acetonitrile (0.5 ml) was added, and the distillation was continued at 90 °C. This procedure was repeated and the reaction vial was subsequently capped. The respective sulfonium triflate precursor dissolved in DMSO (0.5 ml) was added and the mixture was stirred at 110 °C for 15 min. The reaction was quenched and further diluted with water to a volume of 20 ml. This solution was filtered over a Sep-Pak® SPE C-18 light cartridge. The radiolabeled product was released with HCl in ethanol (1.25 M, 0.5 ml). The filtrate was heated at 90 °C for 15 min. After cooling, the reaction was quenched with water (1.5 ml) and purified by HPLC using a Chromolith® SemiPrep RP18-e column (100 × 10 mm) at room temperature. The mobile phase consisted of water and methanol (each containing 0.5% TFA). Gradient elution starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min allowed for isolation of the radioactive product. The obtained solution was diluted with water to a final volume of 20 ml, trapped on a Sep-Pak® SPE C-18 light cartridge, and the radiolabeled product was released with 0.5 ml ethanol. After having reduced the volume to 100 µl under a stream of nitrogen, the solution was diluted with saline to give a final ethanol concentration of 5% and sterilized by filtration.

[^{18}F]4-(4-((4-Fluorophenyl)(phenyl)methylene)piperidin-1-yl)butan-1-ol ([^{18}F]40). Starting with 1.5–2.5 GBq fluoride-18, labeling of compound **70** followed by hydrolysis gave [^{18}F]**40** in 5–10% overall RCY. Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 10% methanol content that was increased to 55%

in 12 min and further to 90% in 3 min). The radiochemical purity was in the range of 98–100% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 12.7 min).

[^{18}F](4-Fluorophenyl)(1-(4-hydroxybutyl)piperidin-4-yl)methanone ([^{18}F]46). Starting with 1.75 GBq fluoride-18, labeling of compound **62** followed by hydrolysis gave 36.4 MBq of formulated [^{18}F]**46** (7% overall RCY). Quality control was performed by radio-HPLC using an analytical Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 1% methanol content that was increased to 40% in 15). The radiochemical purity was 96% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 6.8 min).

[^{18}F]4-(4-((4-fluorophenyl)(phenyl)methylene)piperidin-1-yl)butanal ([^{18}F]42). [^{18}F]Fluoride in water (1.78 GBq) was trapped on a Sep-Pak® QMA cartridge, released with 0.5 ml of a solution of Kryptofix 222 (30 mM) and potassium hydrogen carbonate (30 mM) dissolved in acetonitrile: water (85: 15). After removing the solvent by heating at 90 °C under a stream of nitrogen, acetonitrile (0.5 ml) was added, and the distillation was continued at 90 °C. This procedure was repeated and the reaction vial was subsequently capped. Compound **74** dissolved in DMSO (0.5 ml) was added and the mixture was stirred at 110 °C for 15 min. The reaction was quenched and further diluted with water to a volume of 20 ml. This solution was filtered over a Sep-Pak® SPE C-18 light cartridge. The radiolabeled product was released with THF (0.5 ml), and HCl (1 M; 1.5 ml) was added. This solution was heated at 90 °C for 15 min. After cooling, the crude mixture was purified by HPLC using a Chromolith® SemiPrep RP18-e column (100 × 10 mm) at room temperature. The mobile phase consisted of water and methanol

(each containing 0.5% TFA). Gradient elution starting with 10% methanol content that was increased to 70% in 20 min and further to 90% in 5 min allowed for isolation of the radioactive product. The obtained solution was diluted with water to a final volume of 20 ml, trapped on a Sep-Pak® SPE C-18 light cartridge, and the radiolabeled product was released with 0.5 ml ethanol. After having reduced the volume to 100 µl under a stream of nitrogen, the solution was diluted with saline to give a final ethanol concentration of 5% and sterilized by filtration resulting in 37.2 MBq of formulated [^{18}F]**42** (6% overall RCY). Quality control was performed by radio-HPLC using an analytical Agilent Zorbax® Eclipse XDB column (150 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 30% methanol content that was increased to 90% in 15). The radiochemical purity was 96% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 16.4 min).

General procedure for preparation of esters [^{18}F]43 and [^{18}F]47. [^{18}F]Fluoride in water was trapped on a Sep-Pak® QMA cartridge, released with 0.5 ml of a solution of Kryptofix 222 (30 mM) and potassium hydrogen carbonate (30 mM) dissolved in acetonitrile: water (85: 15). After removing the solvent by heating at 90 °C under a stream of nitrogen, acetonitrile (0.5 ml) was added, and the distillation was continued at 90 °C. This procedure was repeated and the reaction vial was subsequently capped. The respective sulfonium triflate dissolved in DMSO (0.5 ml) was subsequently added and the mixture was stirred at 110 °C for 15 min. The reaction was quenched with water (1.5 ml) and the mixture was purified by HPLC using a Chromolith® SemiPrep RP18-e column (100 × 10 mm) at room temperature. The mobile phase consisted of water and methanol (each containing 0.5% TFA). Gradient elution starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min allowed for isolation of the

radioactive product. The obtained solution was diluted with water to a final volume of 20 ml, trapped on a Sep-Pak® SPE C-18 light cartridge, and the radiolabeled product was released with 0.5 ml ethanol. After having reduced the volume to 100 µl under a stream of nitrogen, the solution was diluted with saline to give a final ethanol concentration of 5% and sterilized by filtration.

[^{18}F]Ethyl 4-(4-((4-fluorophenyl)(phenyl)methylene)piperidin-1-yl)butanoate ([^{18}F]43). Starting with 1.7 GBq fluoride-18, labeling of compound **72** gave 26–30 MBq (n = 2) of formulated [^{18}F]**43** (3% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min). The radiochemical purity was 99% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 11.6 min).

[^{18}F]Ethyl 4-(4-(4-fluorobenzoyl)piperidin-1-yl)butanoate ([^{18}F]47). Starting with 1.4 GBq fluoride-18, labeling of compound **64** gave 136 MBq of formulated [^{18}F]**47** (18% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min). The radiochemical purity was 100% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 10.2 min).

[^{18}F]4-(4-((4-fluorophenyl)(phenyl)methylene)piperidin-1-yl)butanoic acid ([^{18}F]44). [^{18}F]**44** was prepared from [^{18}F]**43** using the synthetic procedure described above. After the labeling reaction, the crude mixture was diluted with water to a volume of 20 ml and filtered

over a Sep-Pak® SPE C-18 light cartridge. The radiolabeled product was released from the cartridge with ethanol (1.0 ml), to which was added aqueous NaOH (1 M; 0.5 ml). The ester was hydrolysed in an open vial for 15 min at 90 °C and the resulting carboxylic acid was subsequently purified by HPLC using a Chromolith® SemiPrep RP18-e column (100 × 10 mm) at room temperature. The mobile phase consisted of water and methanol (each containing 0.5% TFA). Gradient elution starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min allowed for isolation of the radioactive product. The isolated product was re-formulated as described above. Starting with 1.0–1.6 GBq fluoride-18, the two-step synthetic procedure afforded 10–15 MBq of formulated [^{18}F]**44** (2.3–2.6% overall RCY). Quality control was performed on a Chromolith® Performance RP18-e column (100 × 4.6 mm) using water and methanol (each containing 0.5% TFA; gradient elution at a flow rate of 3 ml/min starting with 10% methanol content that was increased to 55% in 12 min and further to 90% in 3 min). The radiochemical purity was 100% and the identity of the radiochemical product was confirmed by co-elution with the non-radioactive analogue (RT = 8.7 min).

P-gp ATPase activity assay. The Pgp-Glo™ assay (Promega) was performed following the manufacturer's instructions.[34] Briefly, a membrane preparation from Sf9 cells expressing the recombinant human P-gp was incubated with the respective test compound (20 μM) and ATP (5 mM) for 40 minutes at 37 °C. Sodium orthovanadate (20 μM) and verapamil (200 μM) were added as negative and positive control, respectively. After this time, the enzyme luciferase and the corresponding substrate luciferin were added, and incubation was continued for 20 minutes. Quantification was carried out by determination of luminescence. Results are given as reduction of luminescence (the more avid a substrate the more pronounced is the reduction of luminescence).

***h*H$_1$R binding assay.** CHO-K1 cells stably expressing the *h*H$_1$R were washed with 10 ml ice-cold PBS buffer (140 mM NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 8 mM $NaHPO_4$, pH 7.4), scraped into ice-cold HEPES binding buffer (20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.4), and homogenized with sonication. Membranes were pelleted at 23,000 x g for 30 minutes at 4 °C, homogenized in HEPES buffer using a hand potter, and stored in liquid nitrogen. Prior to experiments, cell membranes were thawed, homogenized with sonication at 4 °C into ice-cold HEPES binding buffer. Competition binding experiments were carried out incubating membranes, 35 µg/well in a final volume of 0.2 ml containing binding buffer and [^{3}H]pyrilamine (1.0 nM; 27 Ci/mmol). Assays were run in triplicates with at least four appropriate concentrations between 1 nM and 1 µM of the test compound. Incubations were performed for 120 min at 25 °C and shaking at 250 rpm. Non-specific binding was determined in the presence of 10 µM chlorpheniramine hydrogenmaleate. Bound radioligand was separated from free radioligand by filtration through GF/B filters pre-treated with 0.3% (mass/vol) polyethyleneimine using an Inotech cell harvester. Unbound radioligand was removed with four washes of 5 ml of ice-cold HEPES buffer.[35]

Animals. All animal work at UCL was performed in compliance with the United Kingdom Home Office's Animals (Scientific Procedures) Act 1986 and with approval of the University College London (UCL) Animal Ethics Committee. Female wild-type albino mice (FVB or Balb/C, Charles River Laboratories, Margate, UK) were allowed to acclimatize for at least one week at the animal facilities at the UCL Centre for Advanced Biomedical Imaging, and they were given food and water *ad libitum*. When used for experiments, they were eight to eleven weeks old and weighing approximately 20 g. For PET scanning experiments at AIT, wild-type FVB as well as P-gp knock-out mice were obtained from Taconic (Germantown, USA). The

study was approved by the local animal welfare committee (Amt der Niederösterreichischen Landesregierung) and all experimental procedures were performed in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU).

Biodistribution Studies. Biodistribution studies were performed at UCL's Centre for Advanced Biomedical Imaging. The respective radiotracer (0.5–3 MBq formulated in saline solution containing max. 5% ethanol) was administered intravenously into the tail vein of female wild type albino mice (Balb/C or FVB) without anesthesia. At designated time points between five minutes and two hours after tracer injection, animals were anesthetized with isoflurane (5% mixed with medical air at a flow of 2 ml/min) and the blood was taken by cardiac puncture. Mice were subsequently sacrificed by cervical dislocation. The organs of interest were sampled, weighed, and the radioactivity content was measured by automated gamma counting (Perkin Elmer Wizard^2). Results were expressed as % ID/g bodyweight. All experiments were performed in duplicate or triplicate. For blocking studies with tariquidar, 15 mg/kg of the P-gp inhibitor formulated in 300 µl of a 2.5% glucose solution was administered intravenously into the tail vein two hours before tracer injection. The dose was administered slowly over the course of approximately 2 min.

Metabolite Analysis. Metabolite analysis was performed as part of the biodistribution studies. Blood samples were collected in heparin-coated tubes and an aliquot (20 µl) was taken for gamma counting. After centrifugation (3 min, 13,000 rpm), the plasma was separated. Plasma proteins were subsequently precipitated with cold ethanol (500 µl) and samples were centrifuged (3 min, 13,000 rpm). 500 µl of the resulting supernatant were separated from the pellet, diluted with water (500 µl) and analyzed by radio-HPLC, using a Phenomenex Luna® C-8 (5 µm, 150 x 4.6 mm) or an Agilent Zorbax® Eclipse XDB C-18 (5 µm, 100 × 4.6 mm) analytical column at

room temperature. The mobile phase consisted of water and methanol containing 0.02% NH_4OH and was used for gradient elution (from 30% of methanol to 95% in 15 min, followed by isocratic elution with 95% for 5 min). The flow rate was 1 ml/min and the UV absorbance detector was set at 254 nm. Brains were homogenized in a mixture of water (500 µl) and ethanol (500 µl). Samples were centrifuged (3 min, 13,000 rpm), the resulting supernatant was separated and deproteinated by adding ethanol a second time (500 µl). After vortexing and centrifugation (3 min, 13,000 rpm), the clear supernatant (500 µl) was separated from the pellet, diluted with water (500 µl) and analyzed by radio-HPLC, using the method reported above. The recovery of radioactivity in the investigated samples from both plasma and brain was almost quantitative (> 95%). All experiments were performed in triplicate.

PET scans. Dynamic PET imaging was performed using a µPET Focus220 scanner (Siemens Medical Solutions, Knoxville, TN) at AIT and a nanoScan® PET-CT system manufactured by Mediso (Medical Imaging Systems, Budapest, Hungary) at UCL, respectively. Mice were anaesthetized with isoflurane (2% in oxygen) and placed on the preheated bed of the scanner (set at 38 °C). The respective radiotracer (5–10 MBq in 100–250 µl saline solution) was injected into the tail vein via intravenous cannulation. After injection, the catheter was carefully removed. Breathing rate and body temperature of the animals were closely monitored during the dynamic PET scans and, if necessary, the isoflurane dose was adjusted. Scans were recorded over two hours, and the animals were subsequently sacrificed by cervical dislocation. Quantification of tissue uptake was carried out using the image analysis software Amide at AIT and the software package VivoQuant 1.23 (inviCRO, Boston, USA) at UCL, respectively.

Blocking studies. Tariquidar (15 mg/kg bodyweight; formulated in 2.5% glucose solution) or elacridar (10 mg/kg bodyweight; formulated in 20% ethanol in water) were injected slowly (over

the course of 1–2 min) into the tail vein 90 min before tracer administration in order to achieve full inhibition of the respective efflux transporters. Control animals received vehicle or saline. This was carried out without anesthesia, and mice were allowed food and water *ad libitum* in between the injections.

Analysis of tracer kinetics. In the absence of an arterial input function, we used a simple model to describe the uptake of the pro-drug tracer from the blood, metabolism and trapping of the tracer in the brain, and the washout of the metabolically activated tracer. Whole-brain time-activity curves were analyzed by fitting the sum of a mono-exponential function and a constant to the time-activity curve data from 10 min p.i. The amplitude of the exponential term A_1 would correspond to tracer being washed out from the brain with s representing the efflux rate, and the constant term A_2 to tracer that gets trapped in brain tissue.

$$C(t) = A_1 e^{-st} + A_2, \quad t > 10\,min$$

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ACKNOWLEDGMENT

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 201380 ("Euripides").

ABBREVIATIONS

ABC, adenosine triphosphate-binding cassette; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; CNS, central nervous system; *h*H_1R, human histamine H_1 receptor; HPLC, high performance liquid chromatography; ID/g, injected dose per gram tissue; MDR, multiple drug resistance; MEM, metabolite extrusion method; PET, positron emission tomography; P-gp, P-glycoprotein; p.i., post injection; RCY, radiochemical yield.

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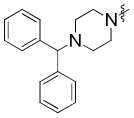
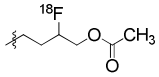
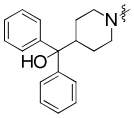
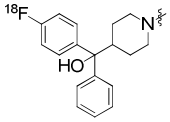
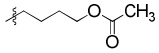
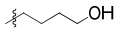
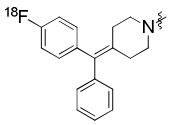
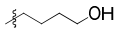
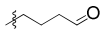
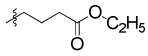
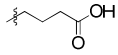
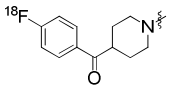
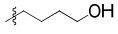
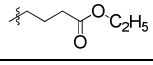
TABLE

Table 1. Blood and Brain Uptake of Tracer Candidates [a)]

Compound	Scaffold	Pro-drug moiety	Tissue uptake (% ID/g)				Brain/blood	
			Blood		Brain			
			5 min p.i.	30 min p.i.	5 min p.i.	30 min p.i.	5 min p.i.	30 min p.i.
[^{18}F]23			1.8	1.4	5.8	1.6	3.2	1.1
[^{18}F]27			2.3	3.5	7.2	4.2	3.1	1.2
[^{18}F]38			0.8	0.8	1.8	1.8	2.3	2.3
[^{18}F]39			0.8	0.7	1.0	1.0	1.3	1.4
[^{18}F]40			0.9	1.0	9.4	7.3	**10.4**	**7.3**
[^{18}F]42			1.4	1.6	3.1	3.4	2.2	2.1
[^{18}F]43			2.5	3.7	2.9	4.9	1.2	1.3
[^{18}F]44			3.0	1.5	1.3	1.7	0.4	1.1
[^{18}F]46			1.3	1.5	6.5	2.7	5.0	1.8
[^{18}F]47			4.0	0.7	3.1	0.9	0.8	1.3

[a)] Biodistribution studies in wild-type mice were performed in duplicate or triplicate: organs of interest were collected at 5 and 30 min p.i., respectively, and analyzed by gamma-counting; average radioactivity uptake given as % injected dose per gram bodyweight (% ID/g).

FIGURE LEGENDS

Figure 1. Illustration of the study design. A) Structures of hydroxyzine and terfenadine, which were used as templates for the development of the pro-drug tracers, and their oxidative metabolism to cetirizine and fexofenadine, respectively; B) Depiction of the structural modifications that were investigated in this study.

Figure 2. Fluorinated non-radioactive analogues of potential pro-drug tracers.

Figure 3. Stimulation of P-gp ATPase activity by selected compounds. Reduction of luminescence in counts per minute; all compounds except verapamil (200 μM) were tested at a concentration of 20 μM; experiments were performed in triplicate; abbreviations: Cet, cetirizine; Con, negative control sodium orthovanadate; Fex, fexofenadine; Ver, positive control verapamil.

Figure 4. Organ distribution and metabolic profile of **[^{18}F]40** and **[^{18}F]44** in wild type FVB mice (*n* = 3). A) Blood and brain uptake of **[^{18}F]40** at 5, 15, 30, 45, 60, 90 and 120 min p.i. in % ID/g; B) Blood and brain uptake of **[^{18}F]44** at 5, 60 and 120 min p.i. in % ID/g; C/D) Metabolic profile of **[^{18}F]40** in brain and blood at 30, 60, 90 and 120 min p.i. as determined by radio-HPLC.

Figure 5. Blocking studies. A) Brain (filled columns) and blood (dotted columns) uptake of **[^{18}F]40** before (black) and after administration of tariquidar (light grey) and elacridar (dark grey) in wild type FVB mice (n = 3; * $p < 0.05$); B) Whole brain time-activity curves from dynamic PET scans in untreated wild type mice (black circles), tariquidar-treated mice (light grey squares) and P-gp knockout mice (open triangles) (experiment performed at AIT); C) Whole brain time-activity curves from dynamic PET scans in untreated wild type mice (black circles) and elacridar-treated mice (dark grey squares) (experiment performed at UCL).

SCHEME LEGENDS

Scheme 1. Synthesis of simplified fexofenadine derivatives

Caption: Reagents and conditions: (i) K_2CO_3, KI, CH_3CN, 80 °C, 12 h; or: K_2CO_3, acetone, 60 °C – rt, 12 h; (ii) oxalyl chloride, DMSO, TEA, DCM, < -50 °C, 30 min; (iii) KOH, H_2O: THF: CH_3OH = 2: 1: 1, MW, 70 °C, 15 min.

Scheme 2. Synthesis of compounds **18**-**28** with an aliphatic fluorinated side chain

Caption: Reagents and conditions: (i) DAST, DCM, 0 °C – rt, 3 h; (ii) $NaOCH_3$, CH_3OH, rt, 1 h; (iii) TsCl, pyridine, DCM, 0 °C – rt, 12 h; (iv) 1-benzhydryl-piperazine (A)/diphenyl-piperidin-4-yl-methanol (B), TEA, CH_3CN, 70 °C, 6 h; (v) $LiAlH_4$, THF, 0 °C – rt, 1 h; (vi) KOH, H_2O: THF: CH_3OH = 2: 1: 1, rt, 1 h; (vii) Ac_2O, TEA, DCM, rt, 3 h.

Scheme 3. Synthesis of the fluorinated scaffolds **C**-**F**

Caption: Reagents and conditions: (i) PCC, celite, DCM, rt, 3 h; (ii) 4F-PhMgBr, THF, 0 – 60 °C, 2 h; (iii) Dess-Martin periodinane, DCM, rt, 2 h; (iv) PhMgBr, THF, 0 – 60 °C, 1 h; (v) HCl, dioxane, THF, rt, 12 h; (vi) TFA, DCM, rt, 3 h.

Scheme 4. Synthesis of precursors for radiolabeling of aliphatic residues

Caption: Reagents and conditions: (i) TsCl, pyridine, DCM, 0 °C – rt, 12 h; (ii) 1-benzhydryl-piperazine (a)/diphenyl-piperidin-4-yl-methanol (b), TEA, CH_3CN, 70 °C, 6 h; (iii) PTSA, CH_3OH, 40 °C, 4 h; (iv) Ac_2O, TEA, DCM, 0 °C – rt, 3 h; (v) MsCl, TEA, DCM, rt, 1 h.

Scheme 5. Synthesis of triarylsulfonium triflates as precursors for labeling with fluoride-18

Caption: Reagents and conditions: (i) 1. *n*-BuLi, THF, -78 °C, 30 min; 2. **58**, THF, -78 °C – 0 °C, 30 min; (ii) HCl, THF, rt, 12 h; (iii) bromoalkane, K_2CO_3, acetone, 60 °C – rt, 12 h; (iv) (Ph)

$_2I^+ \cdot CF_3O_3S^-$, Cu(II) benzoate, CHF_3O_3S, C_6H_5Cl, 125 °C, 1 h; (v) PhMgBr, THF, 0 °C – rt, 3 h; (vi) TFA, DCM, 0 °C – rt, 2 h; (vii) $(4\text{-}OCH_3\text{-}Ph)_2I^+ \cdot CF_3O_3S^-$ (**75**), Cu(II) benzoate, CHF_3O_3S, C_6H_5Cl, 125 °C, 1 h.

Scheme 6. Radiosynthesis of tracers **[^{18}F]23** and **[^{18}F]27**

Caption: Reagents and conditions: (i) [^{18}F]F^-, K_2CO_3, K_{222}, DMSO, 90 °C, 20 min.

Scheme 7. Radiosynthesis of ketanserin- and fexofenadine-like pro-drug tracer candidates

Caption: Reagents and conditions: (i) [^{18}F]F^-, $KHCO_3$, K_{222}, DMSO, 110 °C, 15 min; (ii) HCl, C_2H_5OH, 90 °C, 15 min; (iii) PhMgBr, THF, 90 °C, 30 min; (iv) AcCl, CH_3CN, rt, 15 min.

Scheme 8. Radiosynthesis of ritanserin-like pro-drug tracer candidates

Caption: Reagents and conditions: (i) [^{18}F]F^-, $KHCO_3$, K_{222}, DMSO, 110 °C, 15 min; (ii) HCl, C_2H_5OH, 90 °C, 15 min; (iii) NaOH, C_2H_5OH, 90 °C, 15 min; (iv) HCl, THF, 90 °C, 15 min.

FIGURE 1

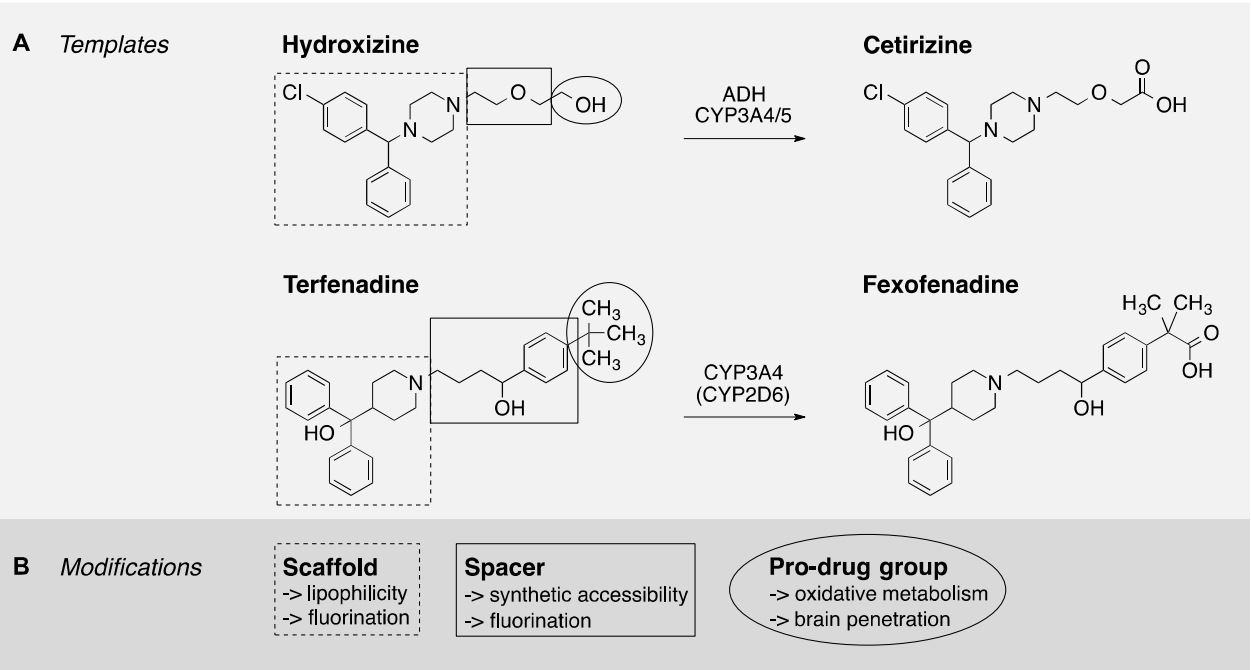

A Templates
Hydroxizine
Cetirizine
ADH
CYP3A4/5
Terfenadine
Fexofenadine
CYP3A4
(CYP2D6)
B Modifications
Scaffold
-> lipophilicity
-> fluorination
Spacer
-> synthetic accessibility
-> fluorination
Pro-drug group
-> oxidative metabolism
-> brain penetration

FIGURE 2

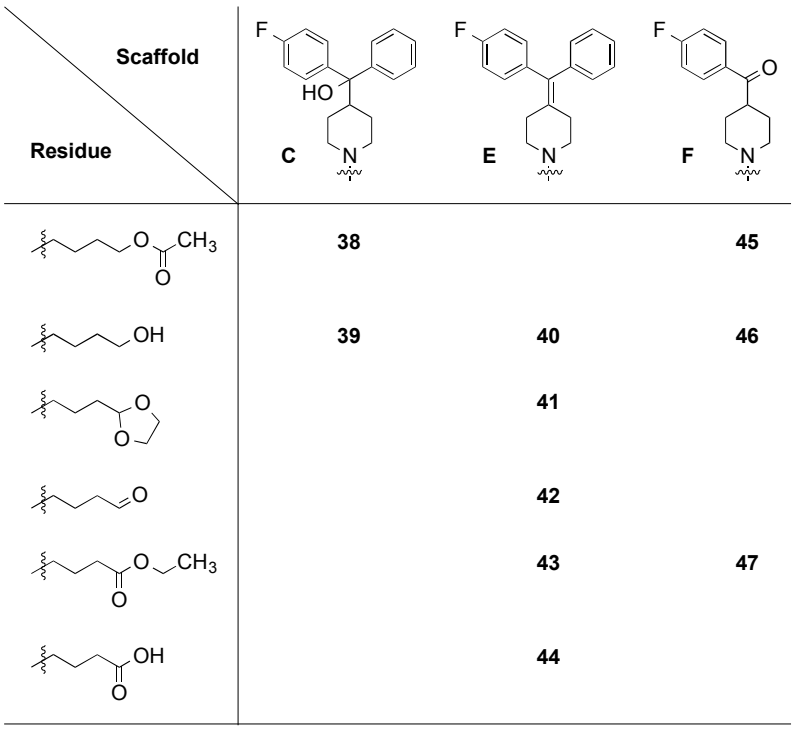


FIGURE 3

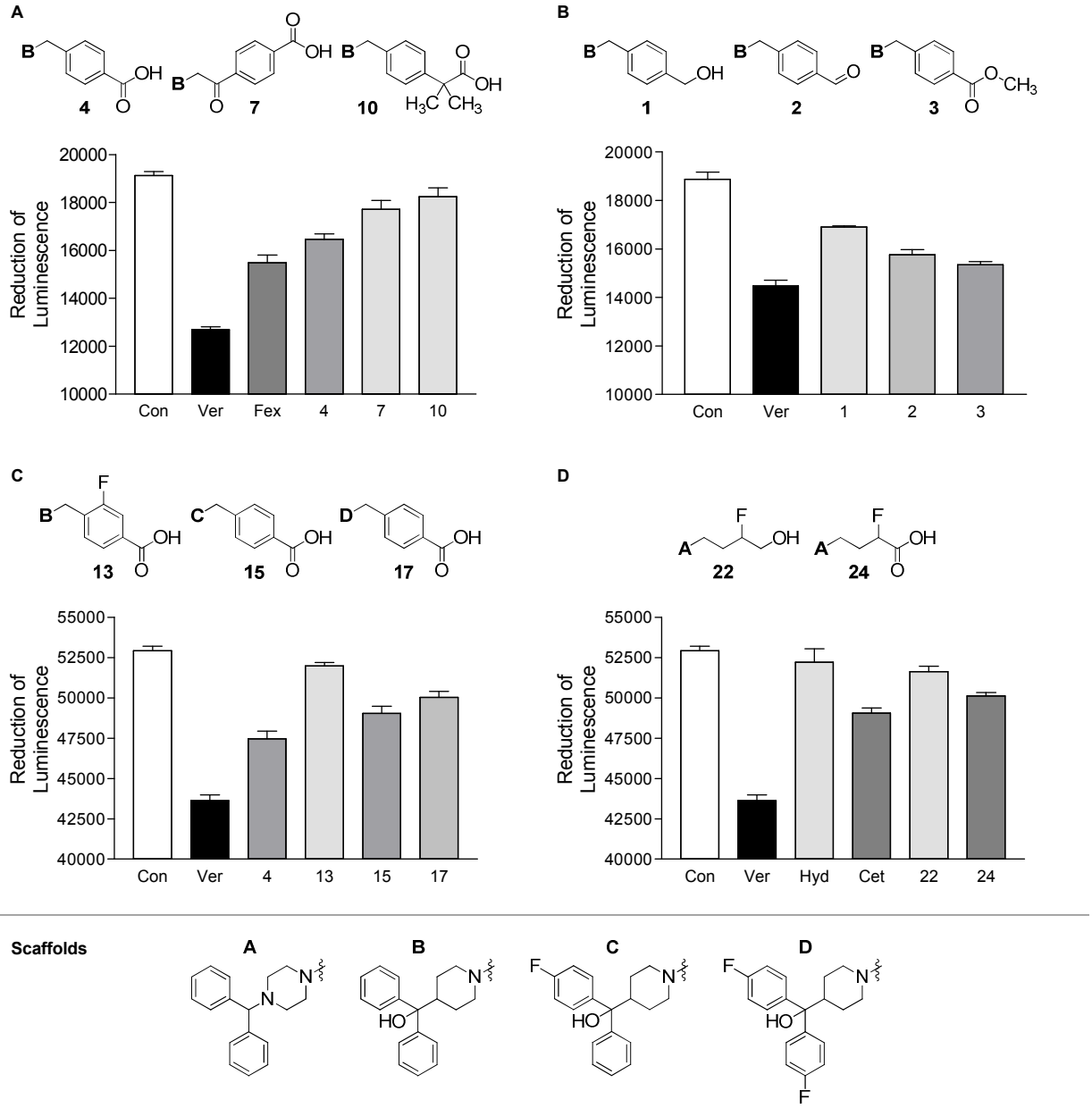

A
B
4
7
10
1
2
3
Reduction of Luminescence
Con
Ver
Fex
Hyd
Cet
C
D
13
15
17
22
24
Scaffolds

FIGURE 4

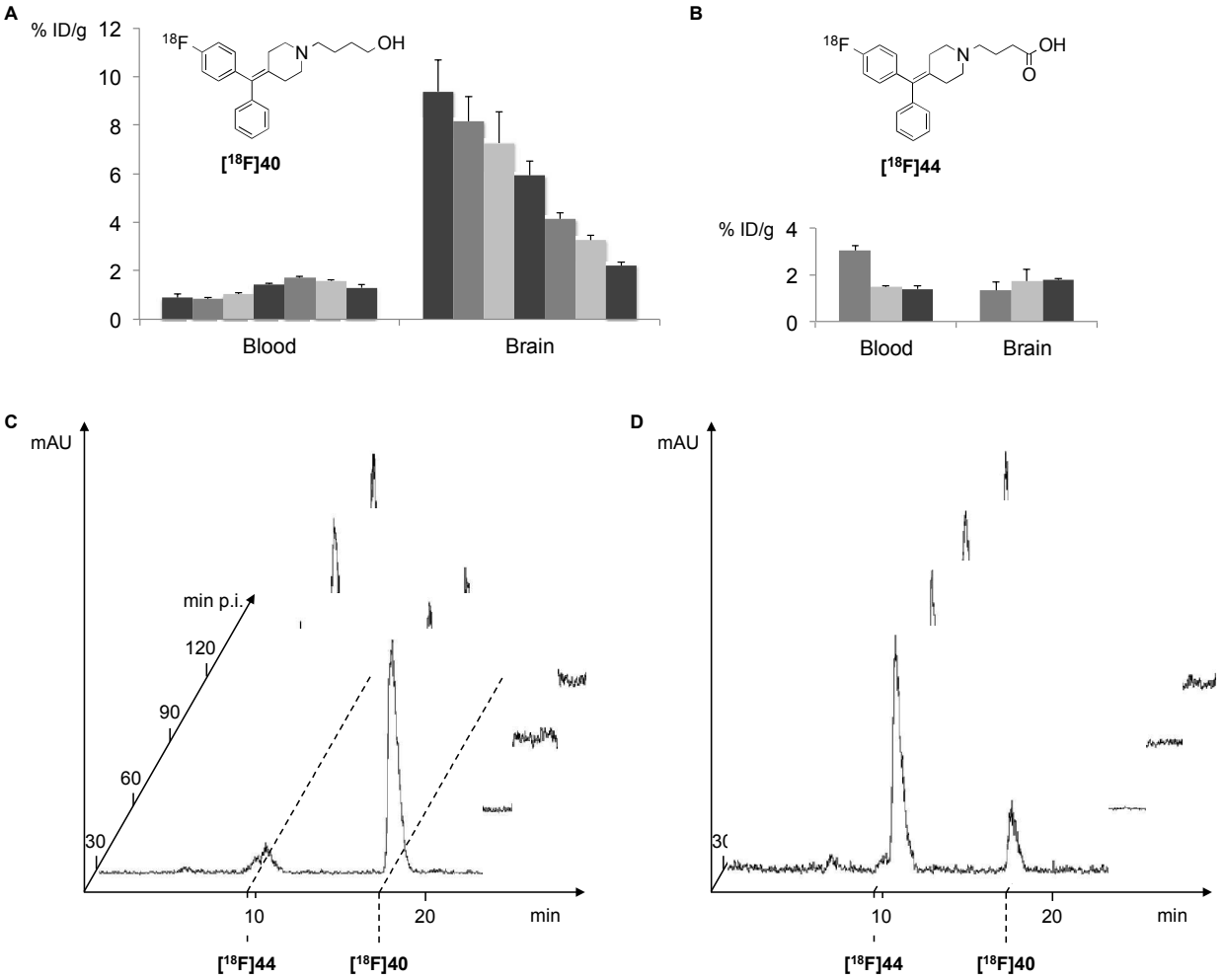
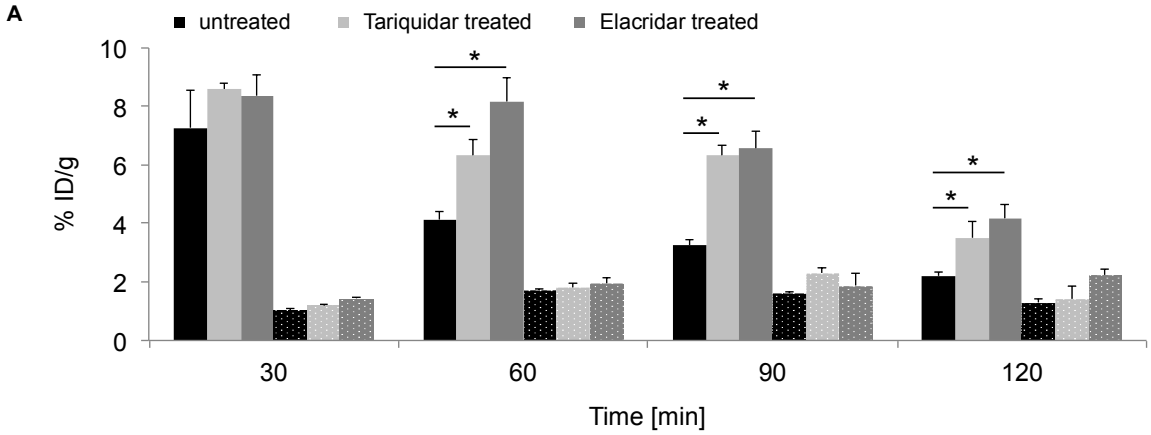
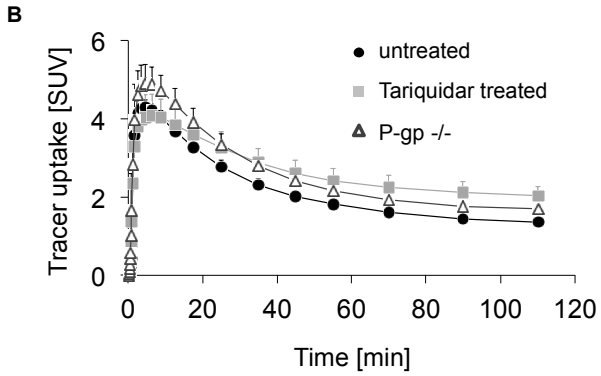
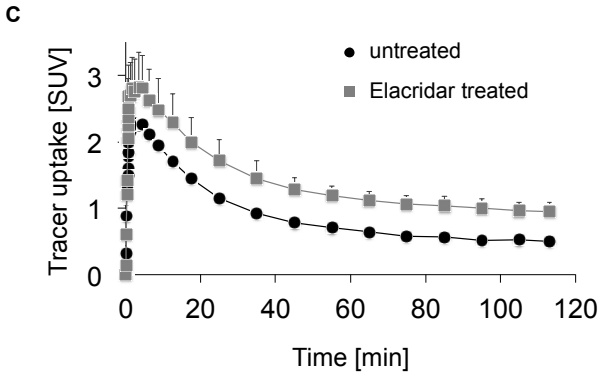

A
% ID/g
12
10
8
6
4
2
0
^{18}F
N
OH
[^{18}F]40
Blood
Brain
B
^{18}F
N
OH
O
[^{18}F]44
% ID/g
4
2
0
Blood
Brain
C
mAU
min p.i.
120
90
60
30
10
20
min
[^{18}F]44
[^{18}F]40
D
mAU
10
20
min
[^{18}F]44
[^{18}F]40

FIGURE 5



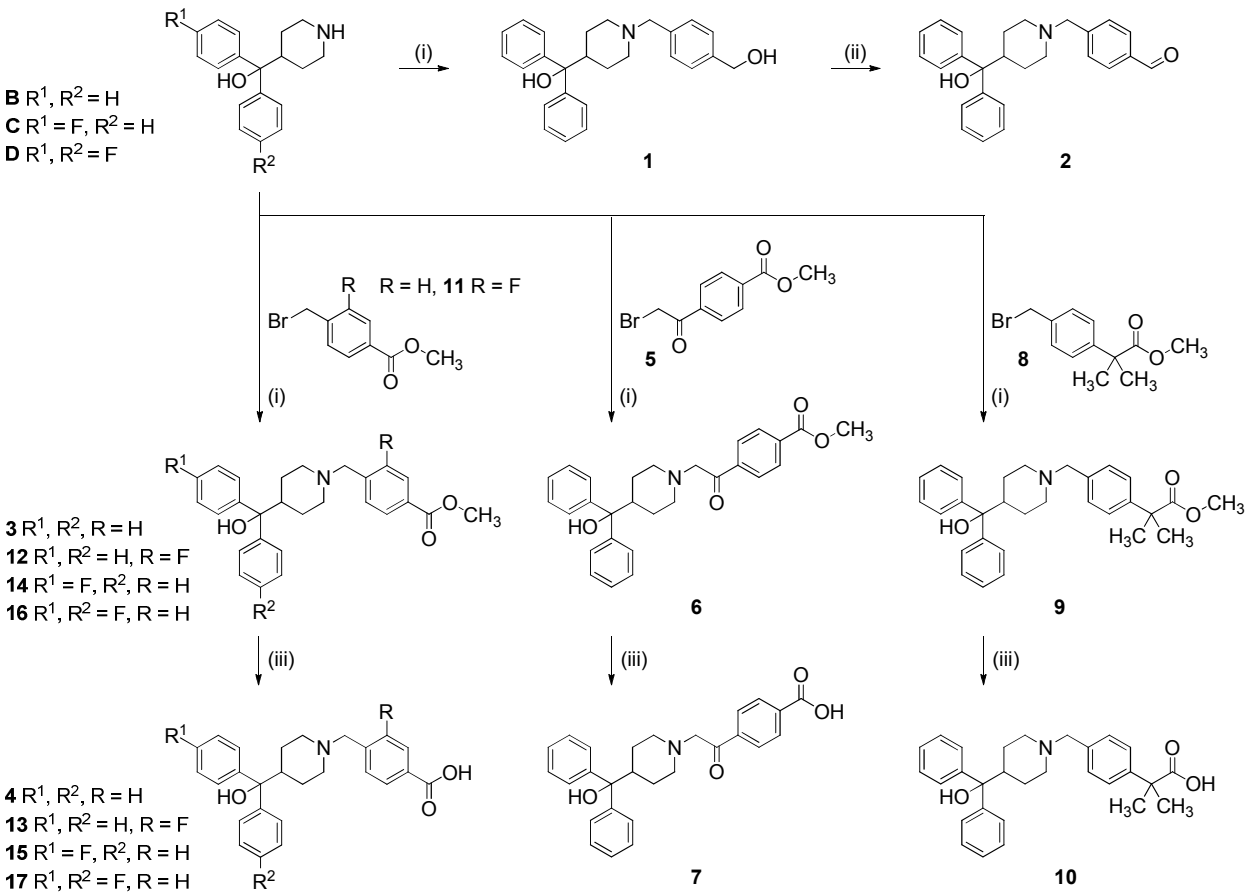




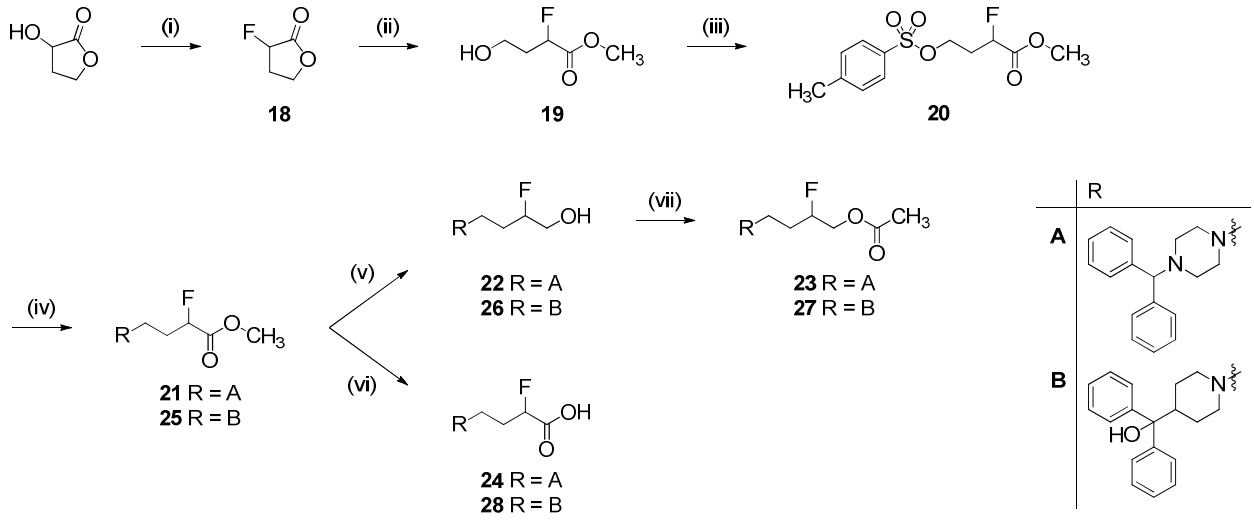
Group	Efflux rate (1/min)	Radioactivity: washout / trapped
Control	0.0378	2.89
Tariquidar	0.0333	1.50
P-gp -/-	0.0387	2.77

Group	Efflux rate (1/min)	Radioactivity: washout / trapped
Control	0.0454	4.14
Elacridar	0.0432	2.33

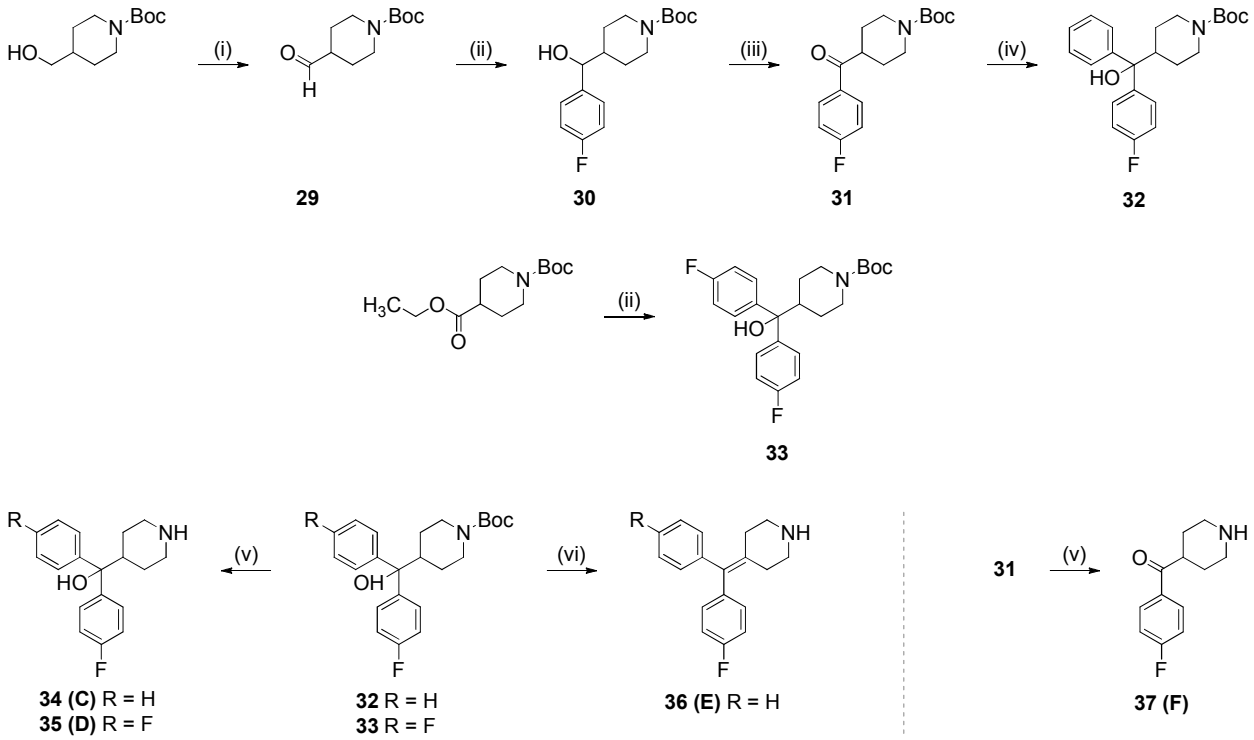
SCHEME 1


B R1, R2 = H
C R1 = F, R2 = H
D R1, R2 = F
(i)
(ii)
1
2
R = H, 11 R = F
5
8
3 R1, R2, R = H
12 R1, R2 = H, R = F
14 R1 = F, R2, R = H
16 R1, R2 = F, R = H
6
9
(iii)
4 R1, R2, R = H
13 R1, R2 = H, R = F
15 R1 = F, R2, R = H
17 R1, R2 = F, R = H
7
10

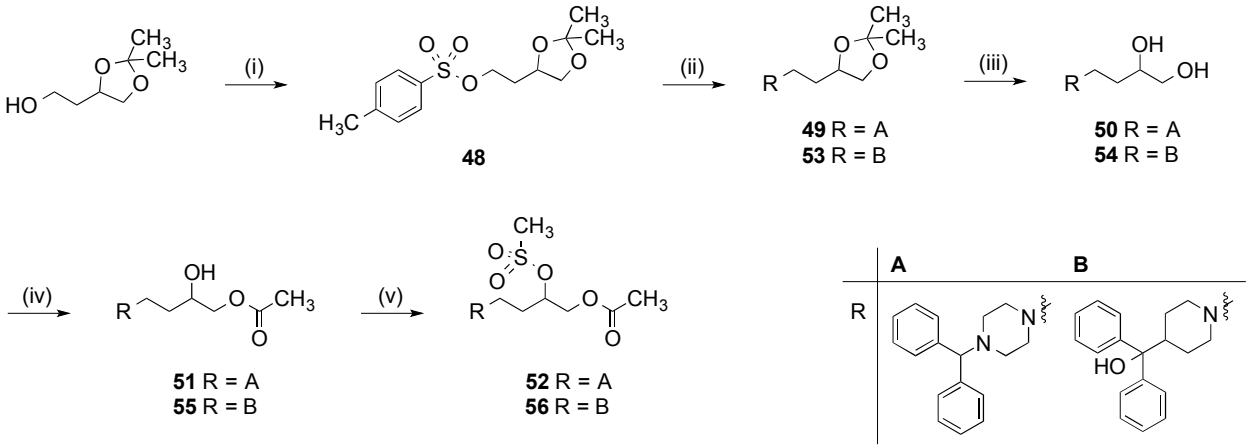
SCHEME 2


(i)
(ii)
(iii)
18
19
20
(iv)
(v)
(vi)
(vii)
21 R = A
25 R = B
22 R = A
26 R = B
24 R = A
28 R = B
23 R = A
27 R = B
R
A
B

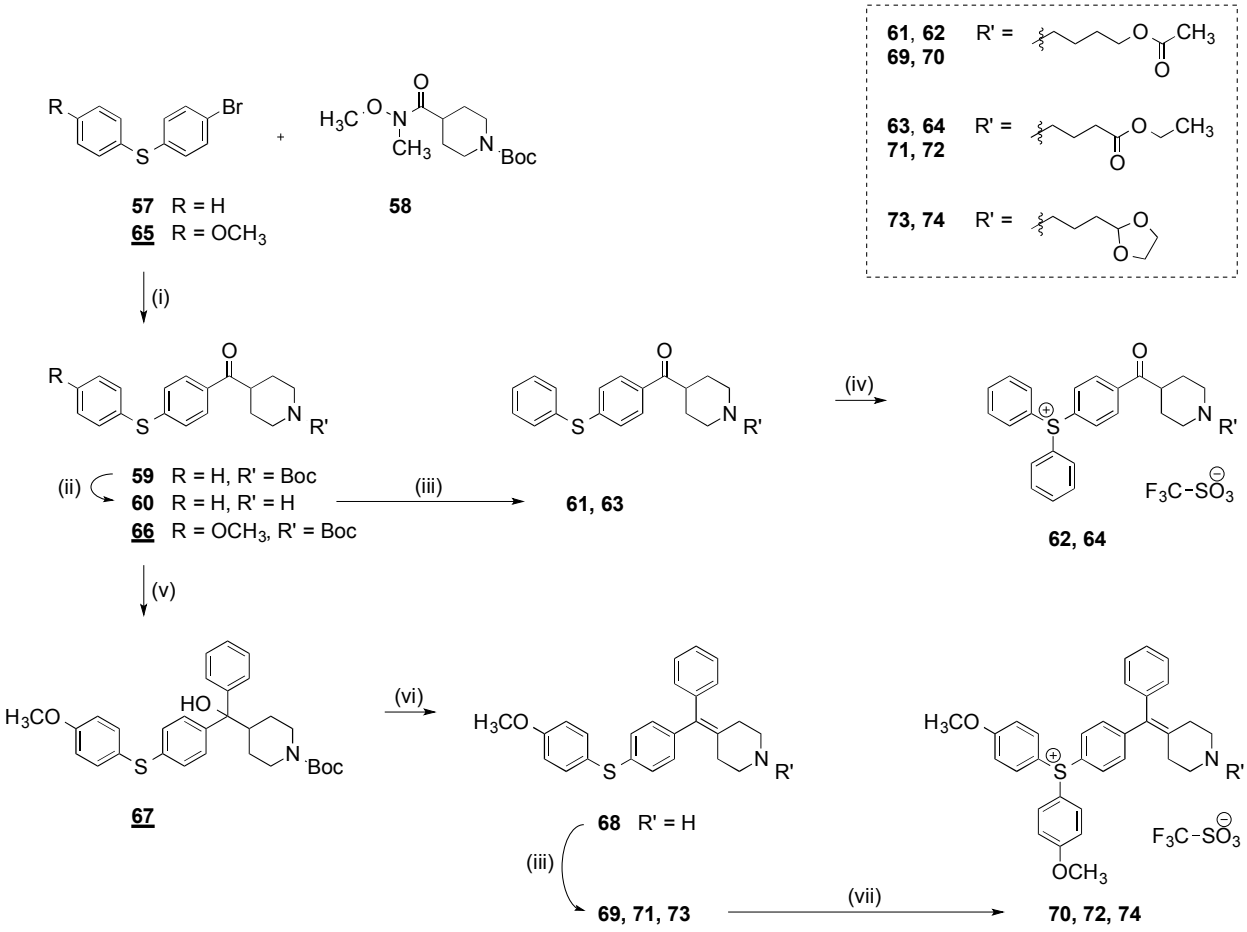
SCHEME 3


HO
N
Boc
(i)
O
H
(ii)
F
(iii)
(iv)
29
30
31
32
H3C
O
(ii)
33
R
NH
(v)
OH
(vi)
34 (C) R = H
35 (D) R = F
32 R = H
33 R = F
36 (E) R = H
31
(v)
37 (F)

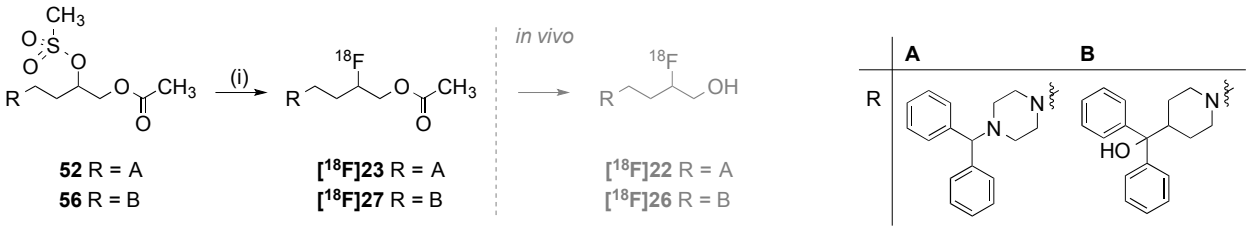
SCHEME 4



SCHEME 5


61, 62 R' =
69, 70
63, 64 R' =
71, 72
73, 74 R' =
57 R = H
65 R = OCH3
58
(i)
59 R = H, R' = Boc
60 R = H, R' = H
66 R = OCH3, R' = Boc
(ii)
(iii)
61, 63
(iv)
62, 64
(v)
67
(vi)
68 R' = H
(iii)
69, 71, 73
(vii)
70, 72, 74

SCHEME 6

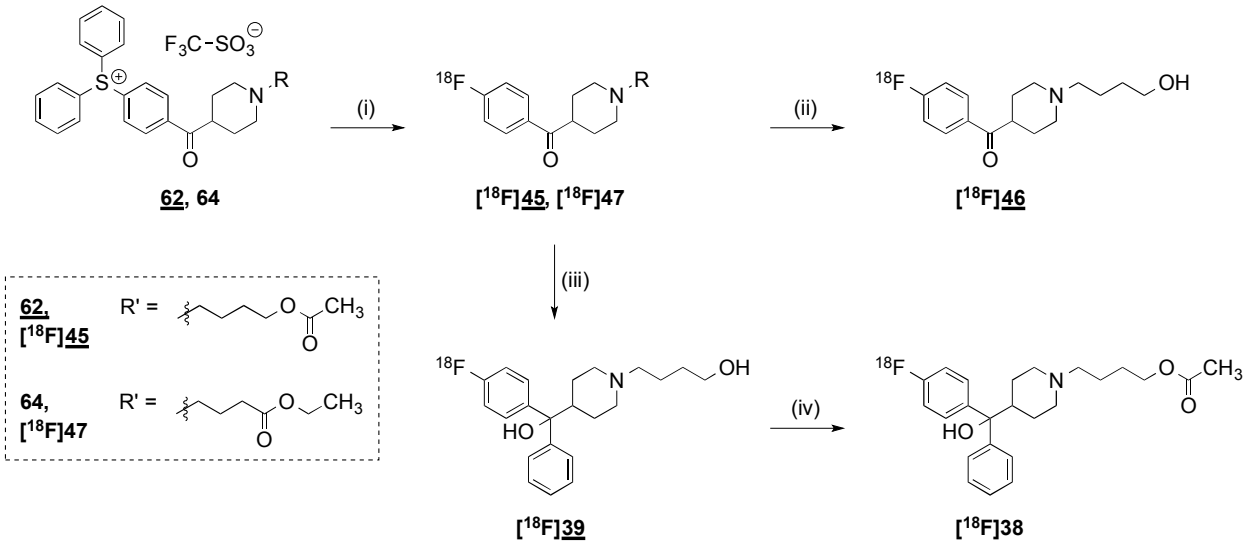


52 R = A
56 R = B

[^{18}F]23 R = A
[^{18}F]27 R = B

[^{18}F]22 R = A
[^{18}F]26 R = B

SCHEME 7


F3C-SO3
S
N
R
O
(i)
18F
(ii)
OH
(iii)
HO
(iv)
62, 64
[18F]45, [18F]47
[18F]46
[18F]39
[18F]38
62, [18F]45
R' =
64, [18F]47
R' =
CH3

SCHEME 8

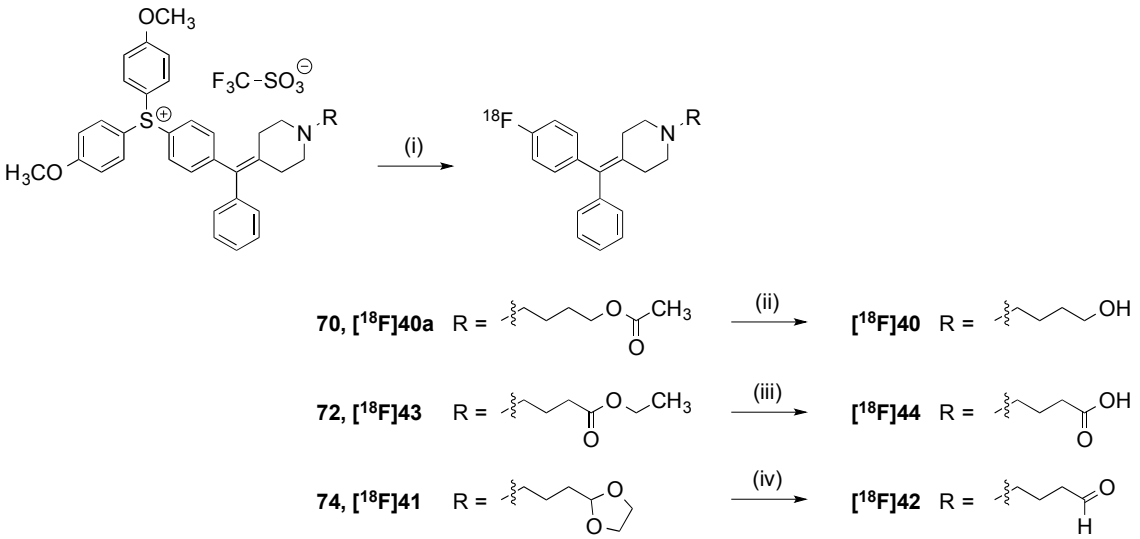
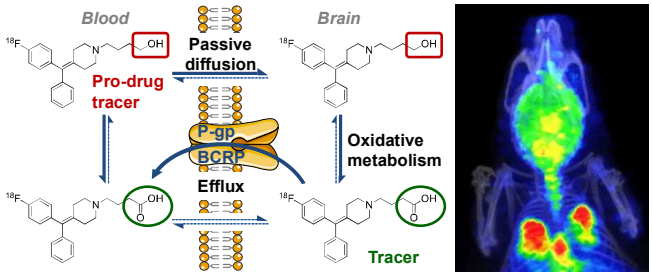
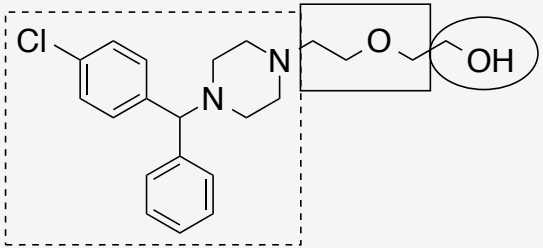

OCH3
F3C-SO3
S
N
R
H3CO
(i)
18F
70, [18F]40a R =
(ii)
[18F]40 R =
OH
72, [18F]43 R =
(iii)
[18F]44 R =
74, [18F]41 R =
(iv)
[18F]42 R =
O
CH3
H

TABLE OF CONTENTS GRAPHIC



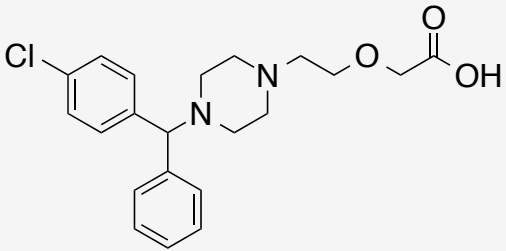
A *Templates*

Hydroxizine

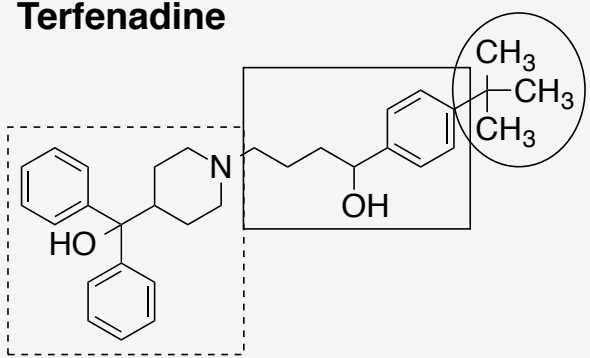


ADH
CYP3A4/5

Cetirizine

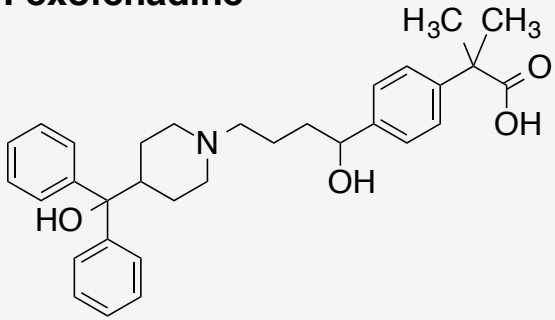


Terfenadine



CYP3A4
(CYP2D6)

Fexofenadine

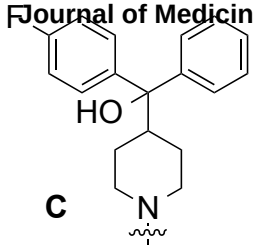
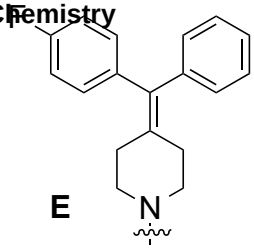
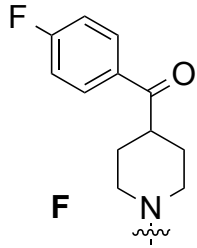
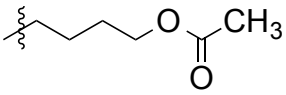
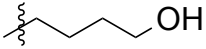
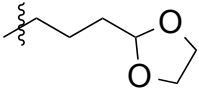
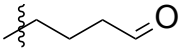
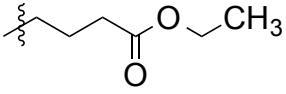
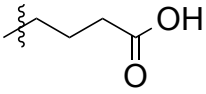


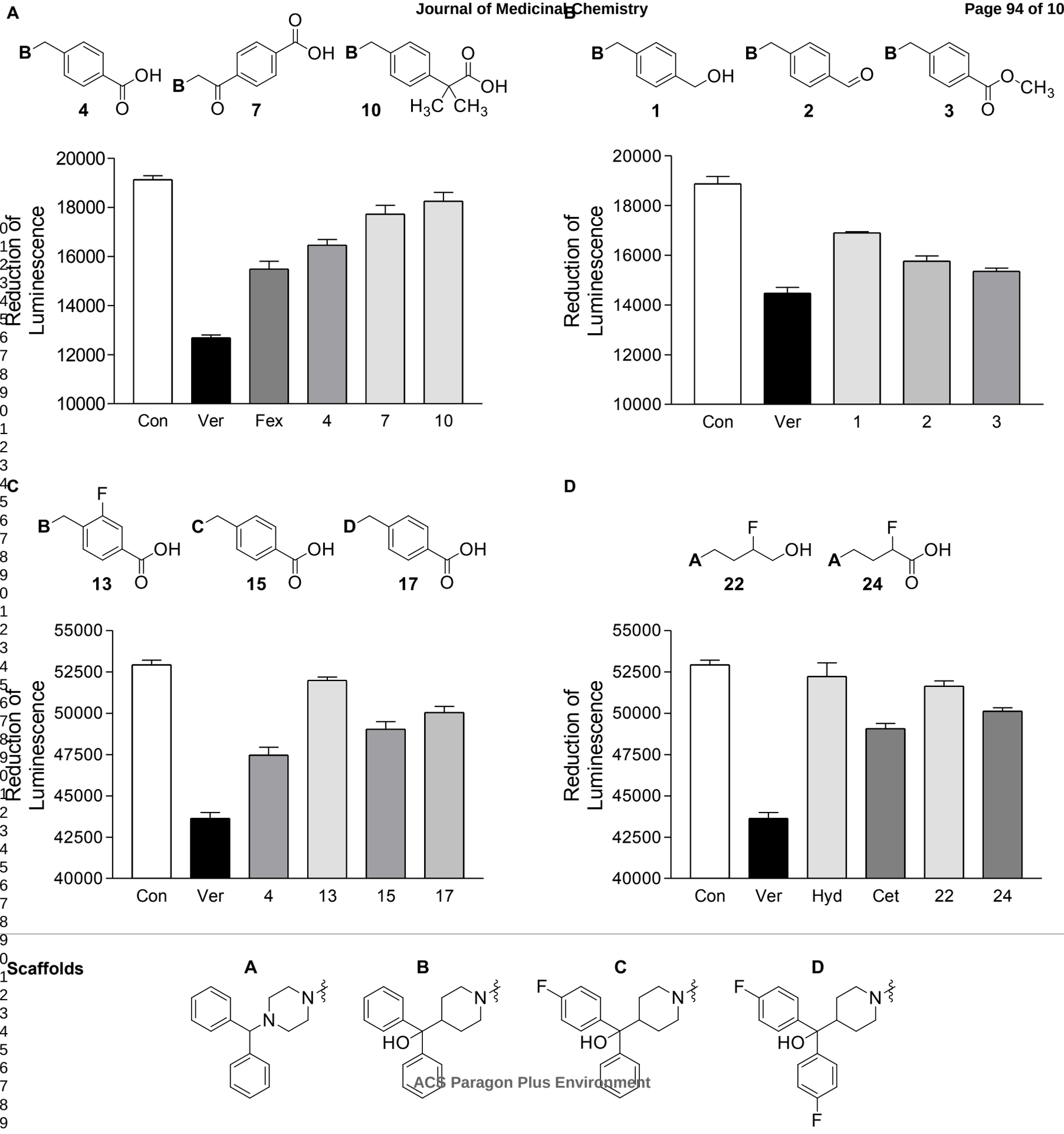
B *Modifications*

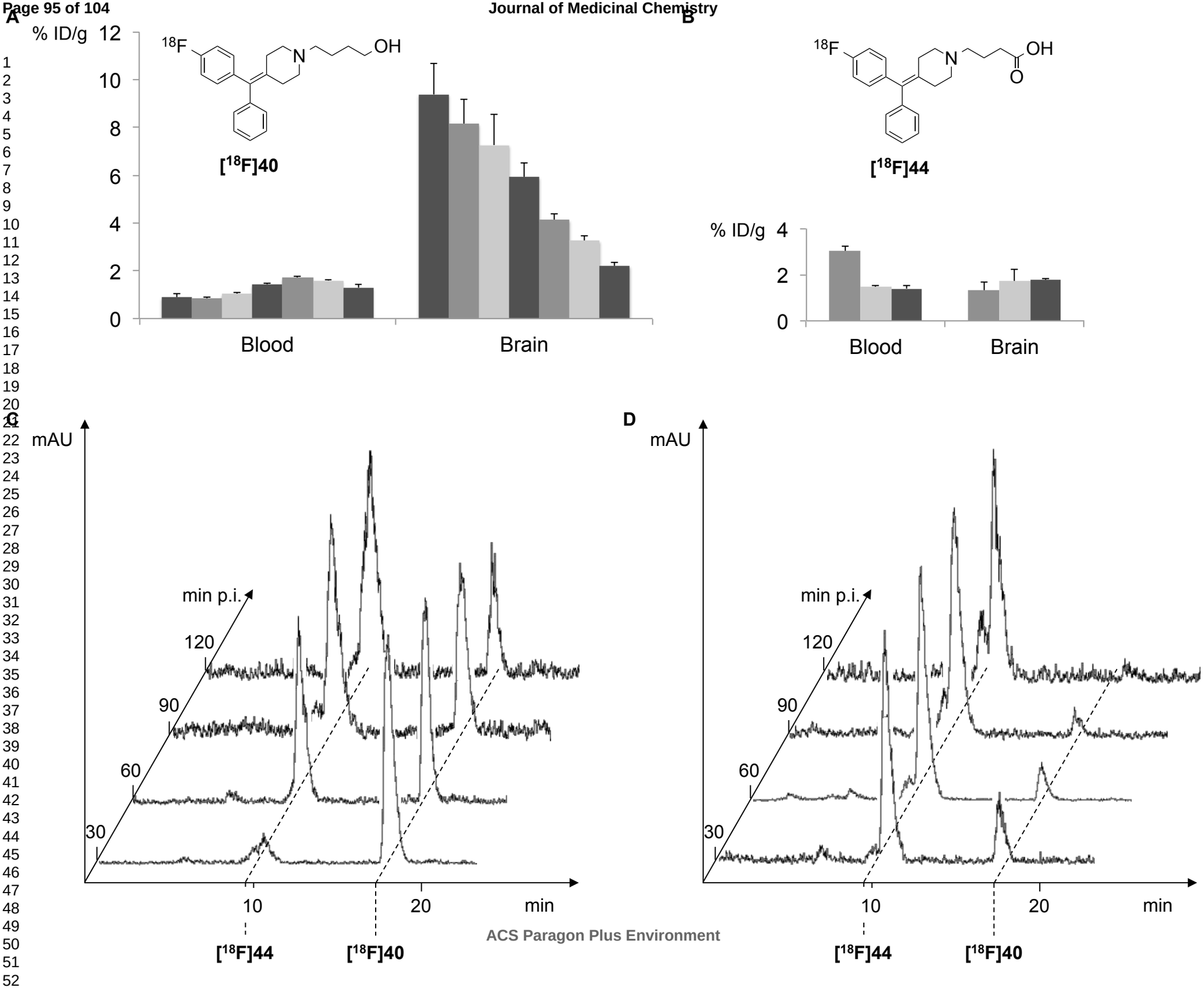
Scaffold
-> lipophilicity
-> fluorination

Spacer
-> synthetic accessibility
-> fluorination

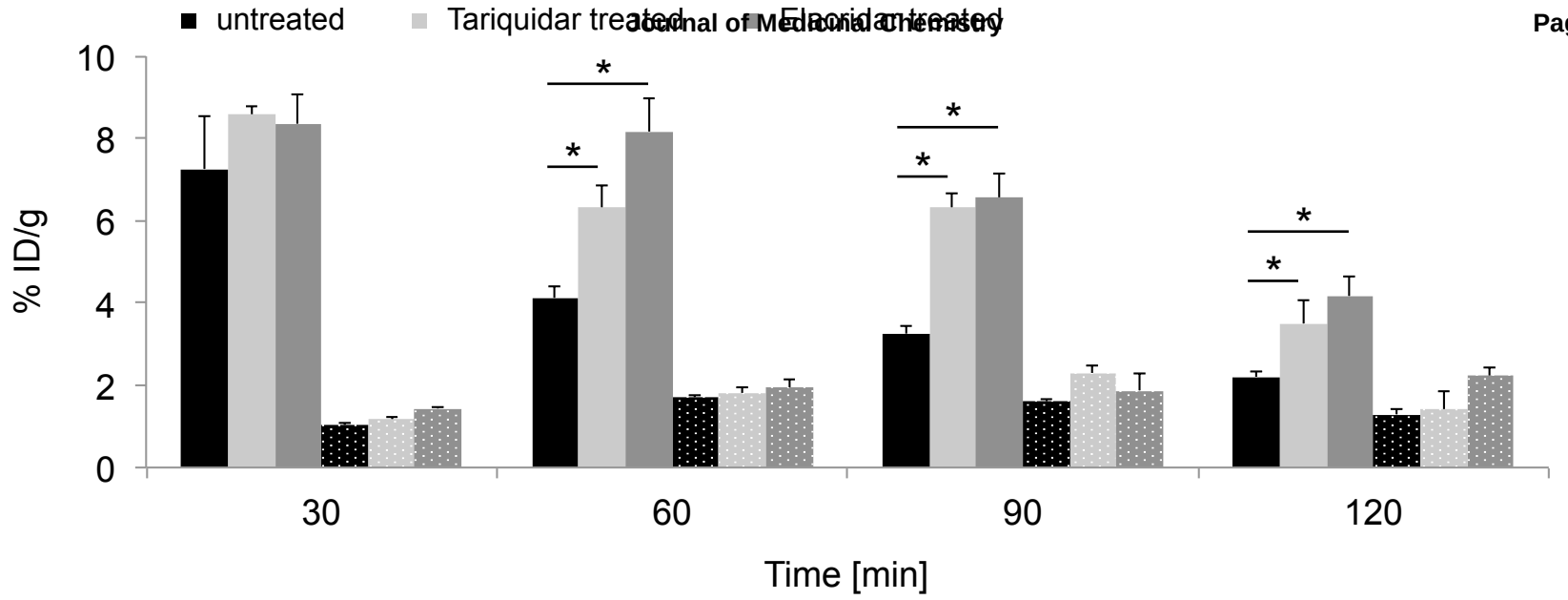
Pro-drug group
-> oxidative metabolism
-> brain penetration

Residue \ Scaffold	 C	 E	 F
	**38**		**45**
	**39**	**40**	**46**
		**41**	
		**42**	
		**43**	**47**
		**44**	

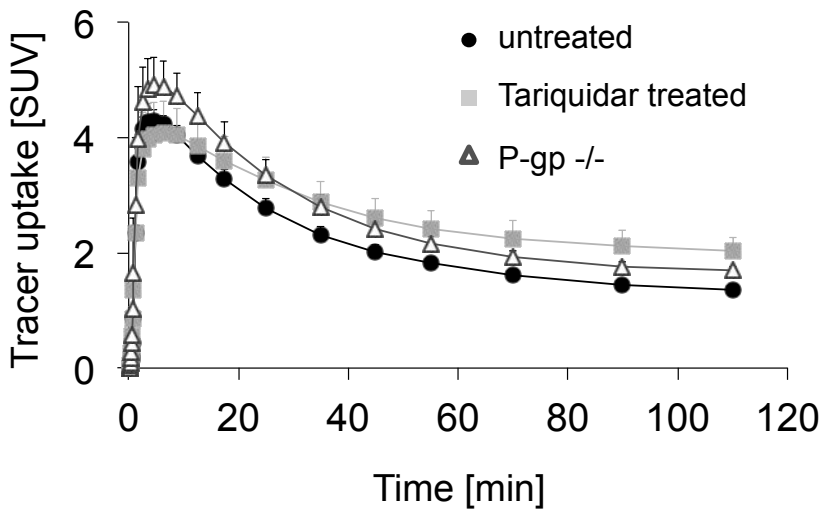



A
% ID/g
12
10
8
6
4
2
0
^{18}F
N
OH
[^{18}F]40
Blood
Brain
B
^{18}F
N
OH
O
[^{18}F]44
% ID/g
4
2
0
Blood
Brain
C
mAU
min p.i.
120
90
60
30
10
20
min
[^{18}F]44
[^{18}F]40
D
mAU
min p.i.
120
90
60
30
10
20
min
[^{18}F]44
[^{18}F]40

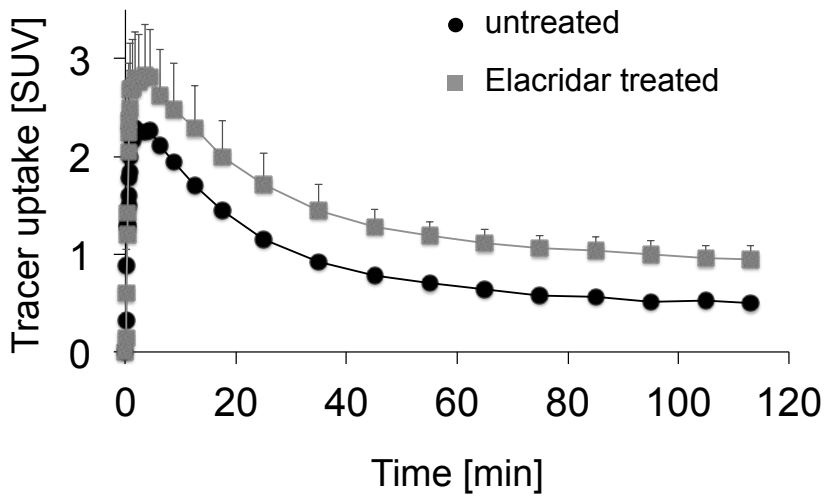
A



B

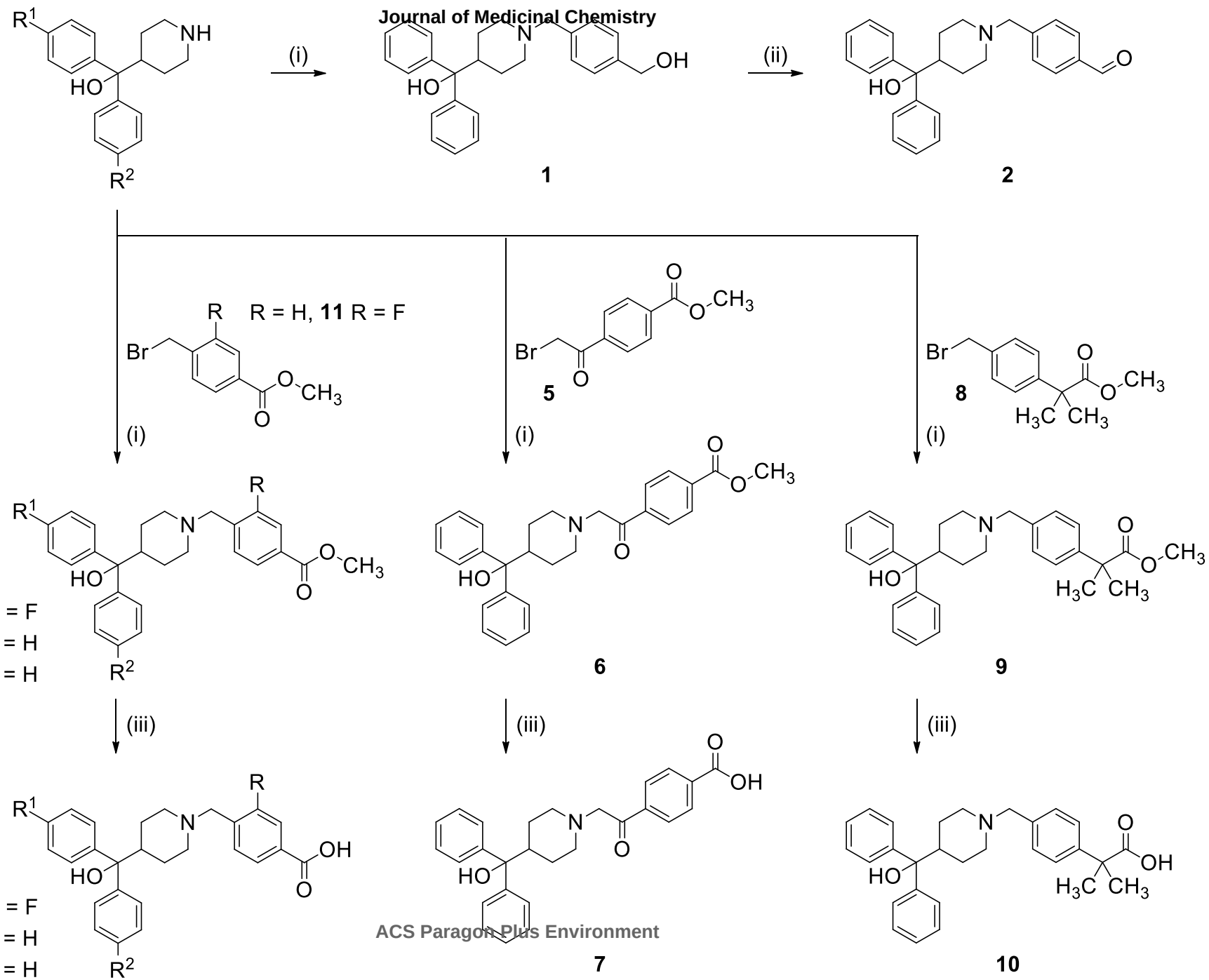


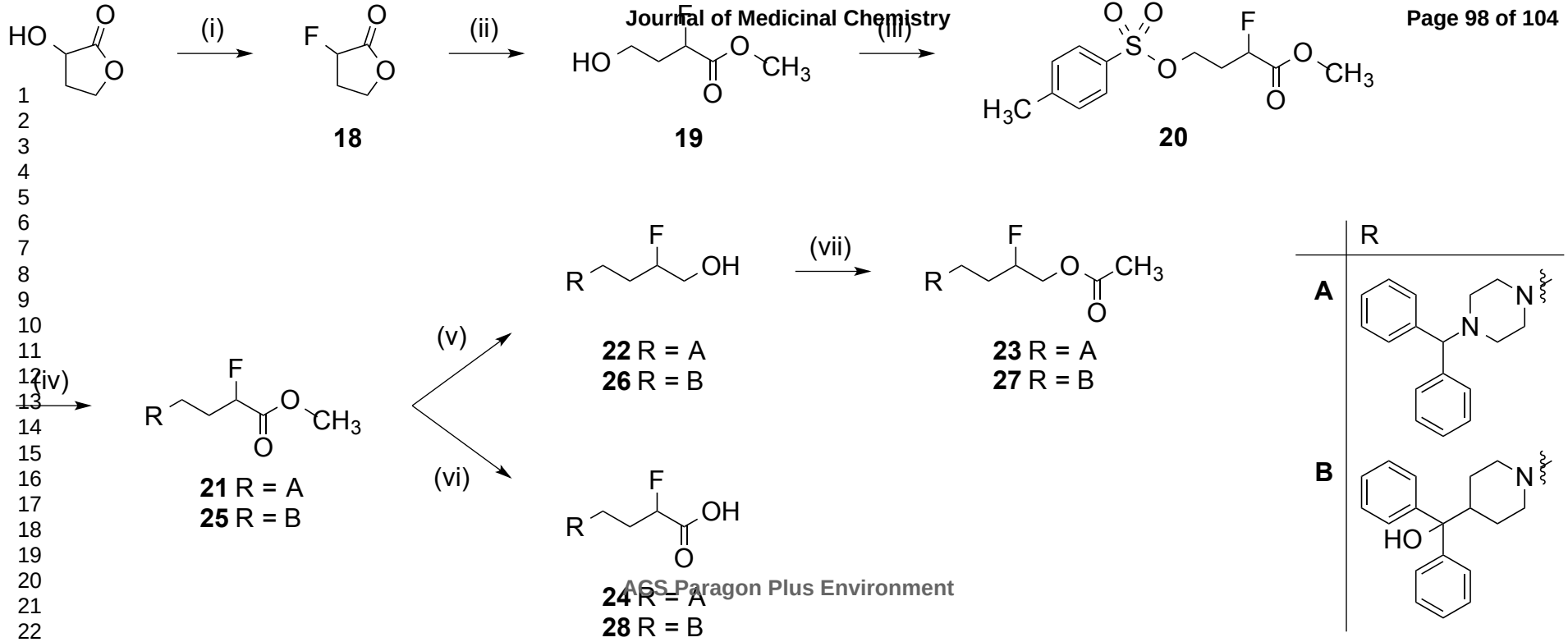
C

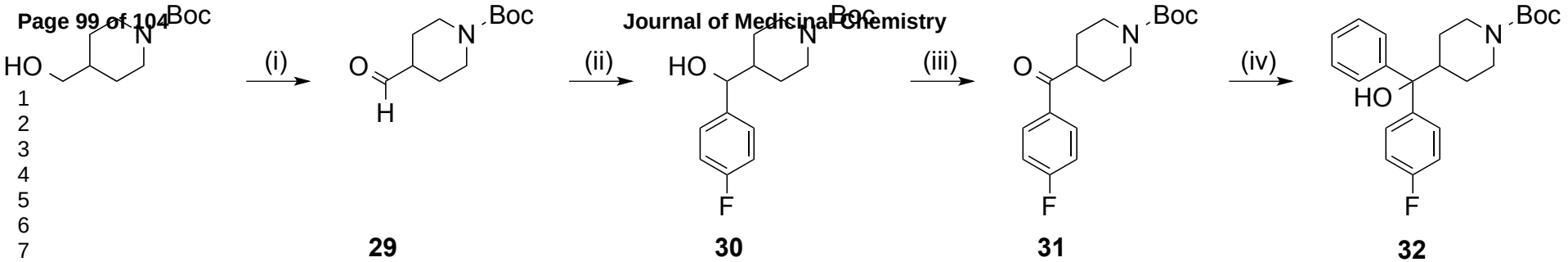


Group	Efflux rate (1/min)	Radioactivity: washout / trapped
Control	0.0378	2.89
Tariquidar	0.0333	1.50
P-gp -/-	0.0387	2.77

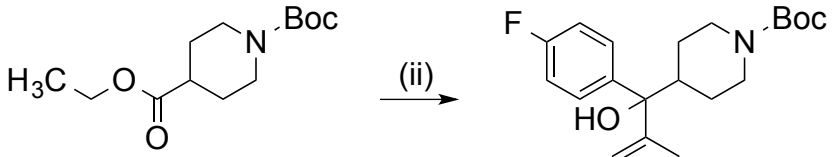
Group	Efflux rate (1/min)	Radioactivity: washout / trapped
Control	0.0454	4.14
Elacridar	0.0432	2.33







29 **30** **31** **32**



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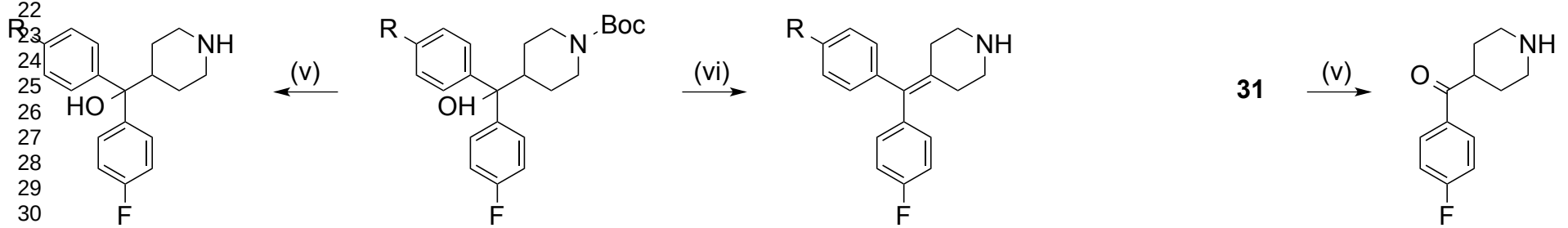
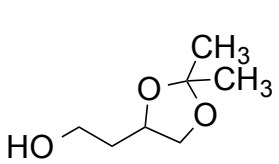


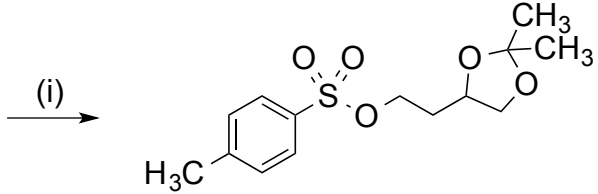
34 (C) R = H
35 (D) R = F

32 R = H
33 R = F

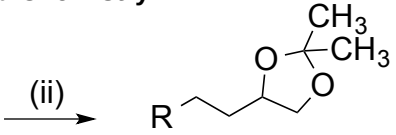
36 (E) R = H

37 (F)

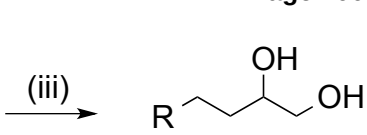




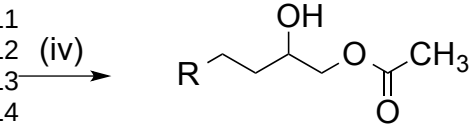
48



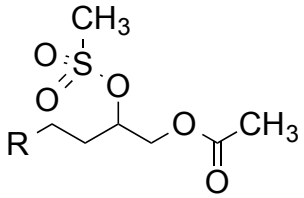
49 R = A
53 R = B



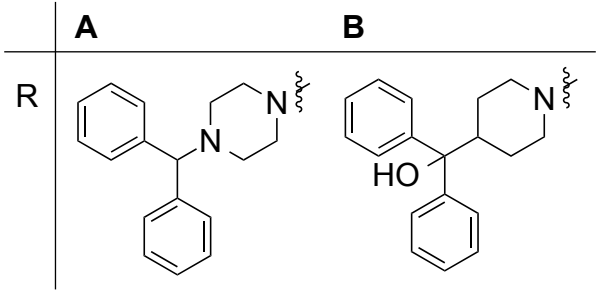
50 R = A
54 R = B

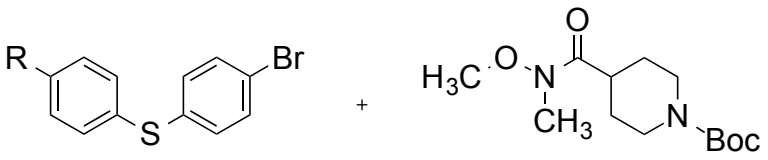


51 R = A
55 R = B



52 R = A
56 R = B

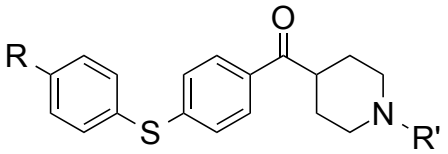




57 R = H
65 R = OCH_3

58

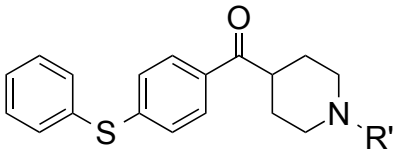
(i)



59 R = H, R' = Boc
60 R = H, R' = H
66 R = OCH_3, R' = Boc

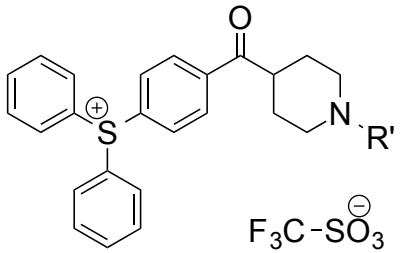
(ii)

(iii)



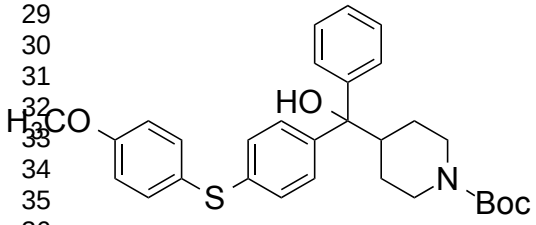
61, 63

(iv)



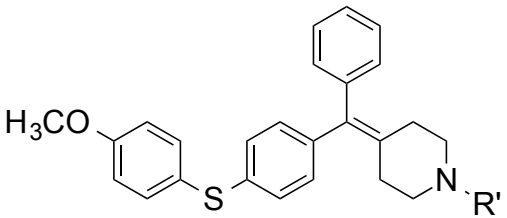
62, 64

(v)



67

(vi)

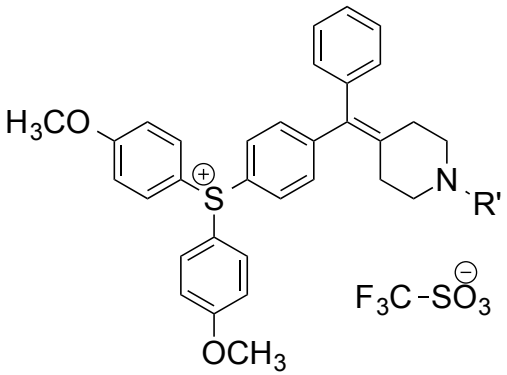


68 R' = H

(iii)

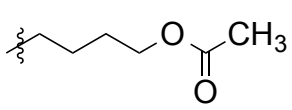
69, 71, 73

(vii)

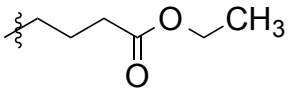


70, 72, 74

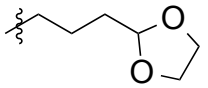
61, **62**
69, **70**
R' =

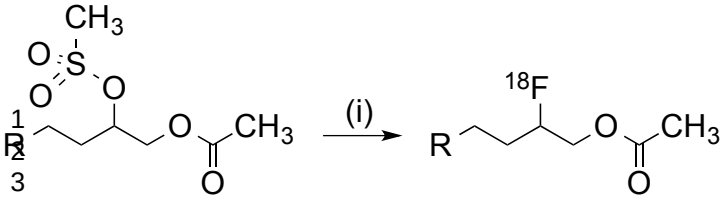


63, **64**
71, **72**
R' =



73, **74**
R' =

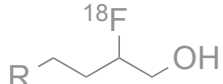




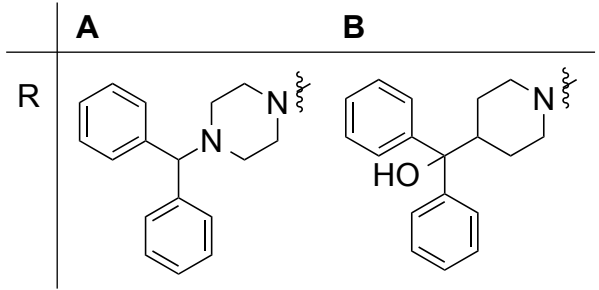
52 R = A
56 R = B

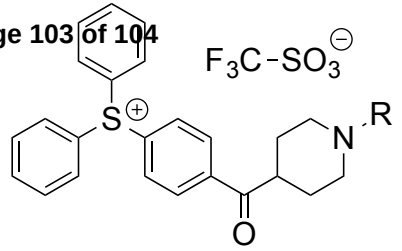
[^{18}F]23 R = A
[^{18}F]27 R = B

in vivo



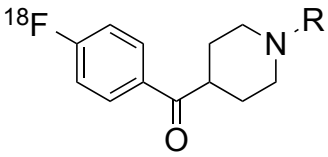
[^{18}F]22 R = A
[^{18}F]26 R = B





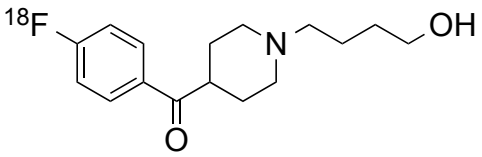
62, 64

(i)



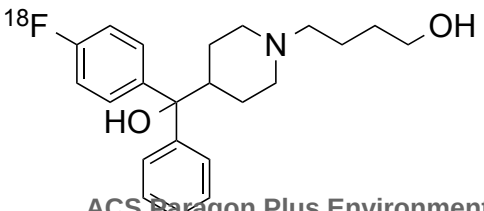
[^{18}F]45, [^{18}F]47

(ii)



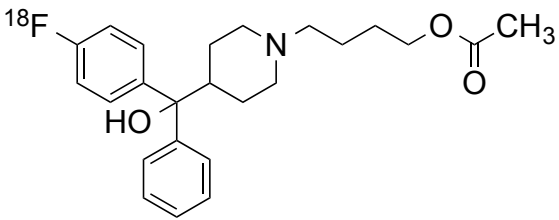
[^{18}F]46

(iii)



[^{18}F]39

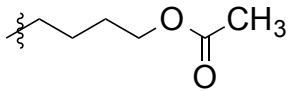
(iv)



[^{18}F]38

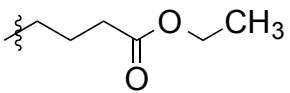
62, [^{18}F]45

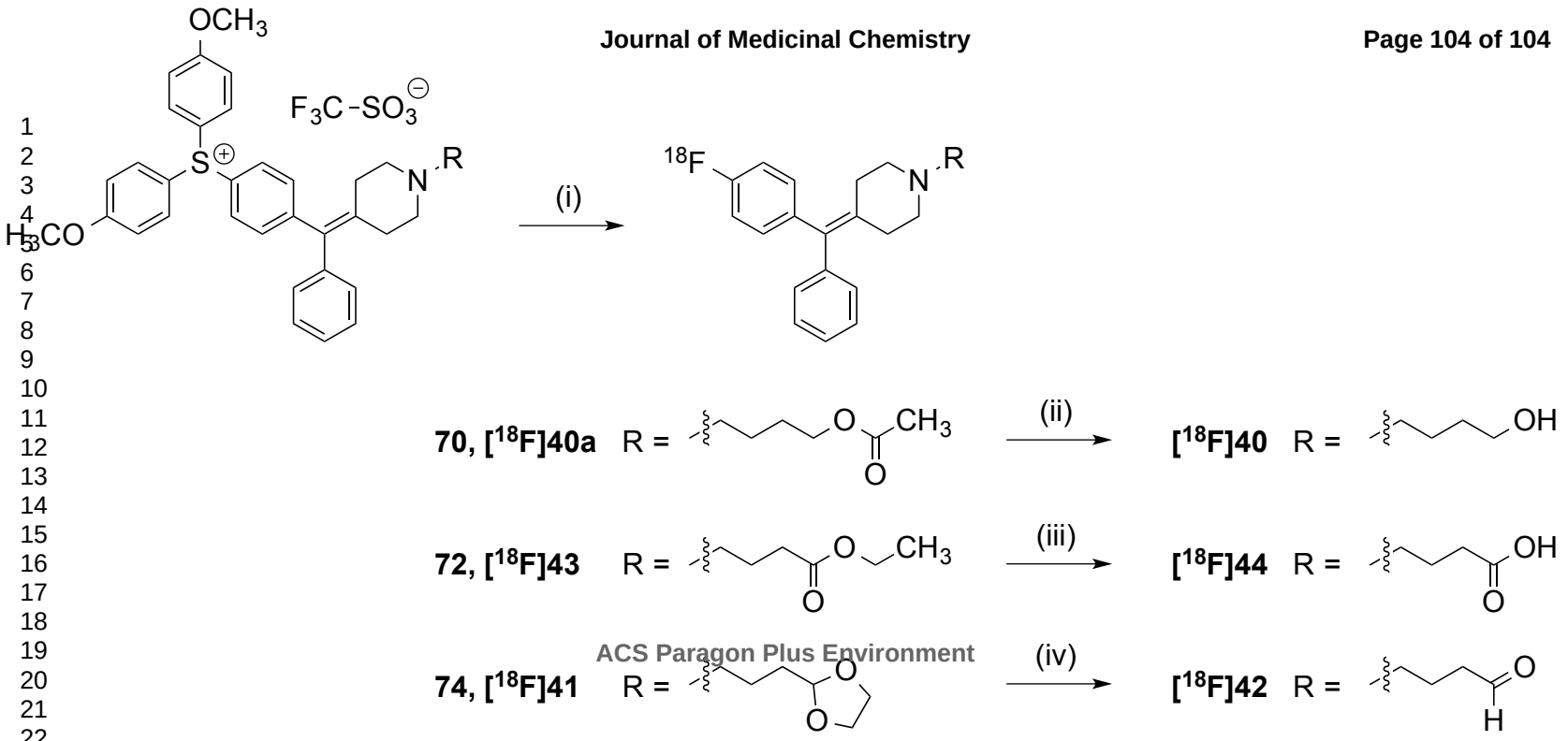
R' =

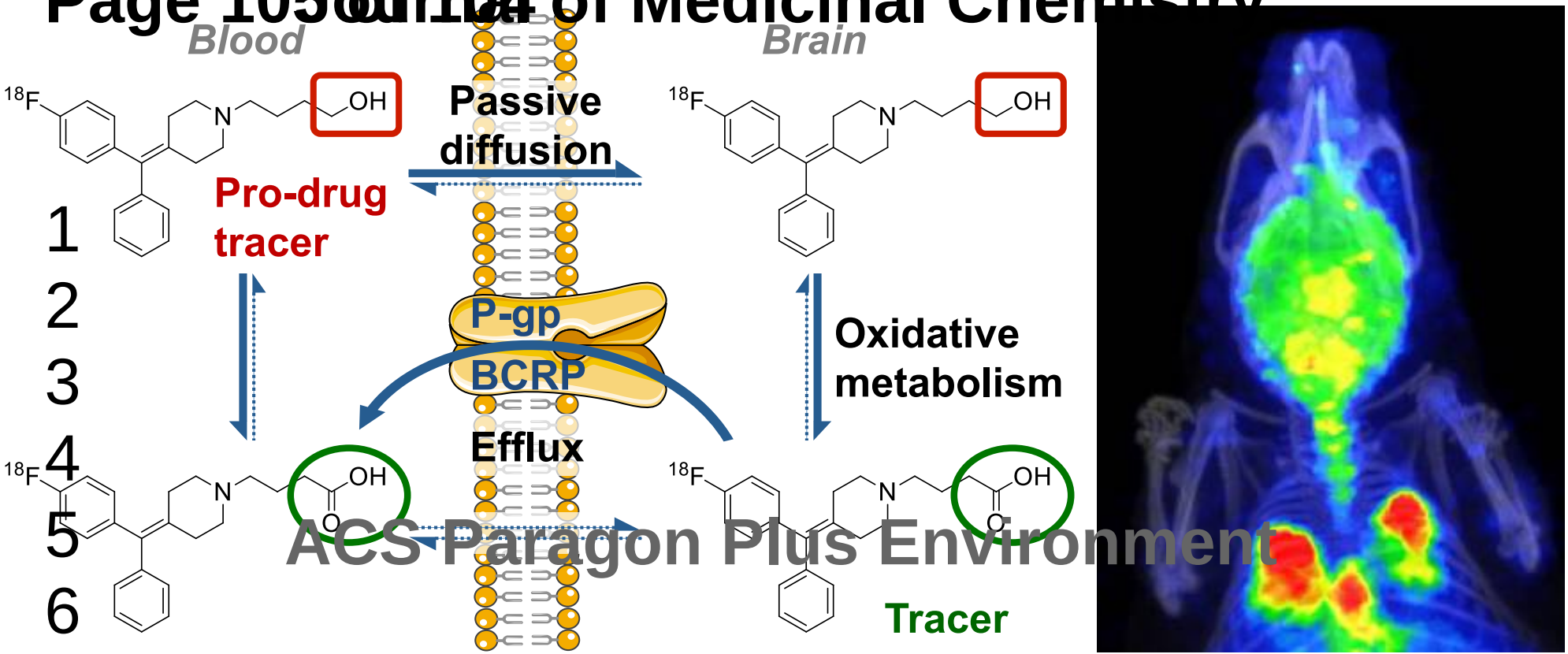


64, [^{18}F]47

R' =






Blood
Brain
Passive diffusion
Pro-drug tracer
P-gp
BCRP
Efflux
Oxidative metabolism
Tracer
ACS Paragon Plus Environment