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Current Radiopharmaceuticals, 2017, *10*, 1-10

RESEARCH ARTICLE

Synthesis of [^{18}F]2B-SRF101: A Sulfonamide Derivative of the Fluorescent Dye Sulforhodamine 101

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ARTICLE HISTORY

Received: July 07, 2017
Revised: September 20, 2017
Accepted: September 25, 2017

DOI:
10.2174/1874471010666170928112853

Abstract: ***Background*:** The red fluorescent dye Sulforhodamine 101 (SR101) has been used in neuroscience research as a useful tool for staining of astrocytes, since it has been reported as a marker of astroglia in the neocortex of rodents *in vivo*. The aim of this work is to label SR101 with positron emission radionuclides, in order to provide a radiotracer to study its biological behavior. This is the first attempt to label SR101 by [^{18}F], using a chemical derivatization *via* a sulfonamide-linker and a commercially available platform.

***Methods*:** The synthesis of SR101 *N*-(3-Bromopropyl) sulfonamide and SR101 *N*-(3-Fluoropropyl) sulfonamide (**2B-SRF101**) was carried out. The radiosynthesis of SR101 *N*-(3-[^{18}F]Fluoropropyl) sulfonamide (**[^{18}F]2B-SRF101**) was performed in a TRACERlab® FX-FN. Different labeling conditions were tested. Three pilot batches were produced and quality control was performed. Lipophilicity, plasma protein binding and radiochemical stability of **[^{18}F]2B-SRF101** in final formulation and in plasma were determined.

***Results*:** SR101 *N*-(3-Bromopropyl) sulfonamide was synthetized as a precursor for labelingradiolabeling with [^{18}F]. **2B-SRF101** was prepared for analytical purpose. **[^{18}F]2B-SRF101** was obtained with radiochemical purity of (97.0 ± 0.6%). The yield of the whole synthesis was (11.9 ± 1.7 %), non-decay corrected. **[^{18}F]2B-SRF101** was found to be stable in final formulation and in plasma. The octanol-water partition coefficient was (Log P_{OCT} = 1.88 ± 0.14). The product showed a high percentage of plasma protein binding.

Conclusions: *The derivatization of* ***SR101*** *via sulfonamide-linker and the first radiosynthesis of* ***[^{18}F]2B-SRF101*** *were performed.* It was obtained in accordance with quality control specifications. *In vitro* stability studies verified that [^{18}F]**2B-SRF101** was suitable for preclinical evaluations.

Keywords: Fluorine-18, Positron emission tomography, radiopharmaceutical, Sulforhodamine 101, sulfonamide, astrocyte marker.

1. INTRODUCTION

Rhodamines dyes are used extensively for conjugation with biomolecules because of their fluorescence properties. The dye is used in a free form but sometimes an activated form is necessary, attached to other molecule [1]. Sulforhodamine chlorides are among the most commonly used reactive dyes for amine labeling, particularly Sulforhodamine B (SRB) sulfonyl chloride and Texas Red sulfonyl chloride (derivative of Sulforhodamine 101, SR101). Both dyes have strong red fluorescence and are frequently used in combination with fluorescein for fluorescence analysis [2-4].

The red fluorescent dye SR101, has been used in neuroscience research as a tool for staining of astrocytes, since it has been reported as a specific marker of astroglia in the neocortex of rodents *in vivo* [5, 6]. A specific *in vivo* staining of astrocytes after intravenous injection of SR101, SRB and Sulforhodamine G [7,8] has been reported. However, the processes of uptake of the dyes remain unknown [9, 10]. On the other hand, some limitations of SR101 with respect to cell specificity have been raised. Different authors postulate that this dye labels not only astrocytes but also mature myelinating oligodendrocytes, diffusing via gap junctions from astrocytes to oligodendrocytes [11-14].

The labeling of SR101 with radionuclides used in Positron Emission Tomography (PET) might provide an interesting way to study its biological *in vivo* behavior. This molecular imaging technique produces three-dimensional images of functional processes at the molecular and cellular level. In this context, recently rhodamine derivatives labeled with [^{18}F] and [^{64}Cu] have been reported as PET radiotracers for myocardial perfusion [15, 16] and tumor mitochondria imaging [17], respectively.

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1874-4710/17 $58.00+.00

The first step to determine if PET-SR101 might be used as astrocyte marker agent was to develop a synthetic method for preparing a radiolabeled compound that could be evaluated *in vivo*. [^{18}F] has been used as the radionuclide of choice due to its relatively long half-life ($t_{1/2}$ = 109.8 min). Furthermore, its decay properties provide advantages compared to [^{11}C] concerning synthesis time and data acquisition for dynamic imaging studies.

The most widely used method for introducing [^{18}F] into target molecule is by a nucleophilic substitution (S_N2), where common leaving groups are Br, I and sulphonyl esters such as triflate, mesylate, tosylate or nosylate [18, 19]. To label SR101with [^{18}F], a chemical derivatization was carried out *via* a sulfonamide-linker.

The general synthetic approach used was to prepare SR101 *N*-(3-Bromopropyl) sulfonamide by a method analogous to the route used by Romieu *et al*. [20] for the preparation of *N*-(SR101)-L-glycine. In this work, we describe the radiosynthesis of SR101 *N*-(3-[^{18}F]Fluoropropyl) sulfonamide (**[^{18}F]2B-SRF101**) in the commercially available platform GE TRACERlab® FX-FN as well as the synthesis and characterization of the non-radiolabeled analogs. Studies were performed to evaluate the stability of the new labeled compound, both in the final formulation and in plasma. Lipophilicity and plasma protein binding were also determined.

2. EXPERIMENTAL

2.1. Organic Synthesis

2.1.1. General

All chemicals and reagents were purchased from Aldrich, Merck and Dorwil. Analytical TLC was performed on silica gel 60 F-254 plates and visualized with UV light (254 nm) and/or p-anisaldehyde in an acidic ethanol solution. Silica gel (Silica 60, 0.04 - 0.063mm, Macherey - Nagel) and Al_2O_3 (aluminium oxide 90 neutral, Macherey - Nagel) were used to carry out chromatographic columns. NMR spectra were recorded on a Bruker DPX-400 spectrometer. The chemical shifts assignment was based on standard NMR experiments (1H, 1H-COSY, HSQC, HMBC and 13C NMR). The chemical shift values were expressed in ppm relative to tetramethylsilane as the internal standard. Absorption spectra were recorded in a Shimadzu UV-1800-240 V spectrophotometer. Mass spectra were determined on an Applied Biosystem API 2000 (ESI-MS).

2.1.2. Synthetic Procedures

2.1.2.1. SR101 N-*(3-Bromopropyl) sulfonamide (1)*

The synthesis was carried out following the technique reported by Romieu *et al.* [20]. SR101 (300 mg, 0.49 mmol) was dried under high vacuum for 2 hours. The resulting dried powder was mixed with 1.8 mL of phosphorus oxychloride ($POCl_3$, 20.0 mmol). The mixture was stirred at room temperature under nitrogen atmosphere for 21 hours. Thereafter, $POCl_3$ was removed under vacuum, and the resulting residue was dried under high vacuum. 3-bromopropylamine (252 mg, 1.1 mmol) and triethylamine (TEA, 348 µL, 2.5 mmol) were dissolved in dry dimethyl sulfoxide (DMF, 3.6 mL), under nitrogen atmosphere. The mixture was cooled with ice bath and a DMF solution of the crude SR101 sulfonyl chloride (8.4 mL) was dropwise added over 10 min. The resulting reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. After 21 hours, the mixture was evaporated under reduced pressure. The resulting residue was dissolved in dichloromethane (CH_2Cl_2) and washed with deionized water. The organic layer was dried over sodium sulfate (Na_2SO_4) and evaporated under reduced pressure. Purification and isolation of the two isomers (**1A** and **1B**) was achieved by chromatography on an aluminium oxide (Al_2O_3) column, with 5% and 10% methanol in CH_2Cl_2 sequentially as eluent (isomer 1A eluted first and then isomer 1B), to give 57.7 mg (16%) of SR101 *N*-(3 -Bromopropyl)-sulfonamide isomer **1A** and 20.0 mg (6%) of isomer **1B**. *Para* isomer **1A**: ^{1}H NMR (400 Hz, DMSO-d_6) δ (ppm): 8.41 (1H, d, *J* = 2 Hz, ArH), 7.99 (1H, t, *J* = 5.6 Hz, NH), 7.91 (1H, dd, *J* = 2.0 Hz, 8.0 Hz, ArH), 7.37 (1H, d, *J* = 8.0 Hz, ArH), 6.53 (2H, s, CHAr), 3.65 (2H, t, *J* = 6 Hz, CH_2Br), 3.50 (8H, m, CH_2NCH_2), 3.01 (6H, m, $NHCH_2$, $ArCH_2$), 2.62 (4H, t, *J* = 6 Hz, $ArCH_2$), 2.02 (4H, m, $ArCH_2CH_2$), 1.86 (6H, m, CH_2CH_2Br, $ArCH_2CH_2$). ^{13}C-NMR (100 Hz, DMSO-d_6) δ (ppm): 154.98, 151.74, 150.92, 148.58, 141.38, 134.26, 131.37, 129.0, 127.62, 127.04, 126.37, 122.95, 113.14, 104.67, 50.68, 50.20, 42.86, 36.25, 32.41, 27.40, 20.64, 19.73, 19.71. *Ortho* isomer **1B**: ^{1}H-NMR (400 Hz, MeOH-d_6) δ (ppm): 8.59 (1H, s, ArH), 8.22 (1H, dd, *J* = 1.2 Hz, 8.0 Hz, ArH), 8.03 (1H, s, NH), 7.46 (1H, d, *J* = 8.0 Hz, ArH), 6.63 (2H, s, CHAr), 3.70 (2H, t, *J* = 6 Hz, CH_2Br), 3.55 (8H, m, CH_2NCH_2), 3.10 (6H, m, $NHCH_2$, $ArCH_2$), 2.75 (4H, m, $ArCH_2$), 2.15 (4H, m, $ArCH_2CH_2$), 1.98 (6H, m, CH_2CH_2Br, $ArCH_2CH_2$). ^{13}C-NMR (100 Hz, MeOH-d_6) δ (ppm): 153.15, 152.01, 150.32, 147.95, 141.01, 134.76, 131.20, 129.2, 127.62, 127.04, 125.5, 122.95, 114.7, 105.5, 50.15, 49.75, 44.01, 41.12, 35.2, 27.80, 21.30, 20.80, 19.95. ESI-MS, *m/z*: 724 (M+•), 747 (M+• + Na), 706.5 (M+• - H_2O).

2.1.2.2. SR101 N-*(3 -Bromopropyl) Sulfonamide: Isomers Identification*

Both isomers were identified by UV-spectroscopy, following the assay reported by Marchesini *et al.* [21]. The absorption spectra were recorded in a spectrophotometer in the 500-700nm, at different pH solutions (pH: 3, 7 and 11). Solutions concentrations: 0.1 mg/mL isomer **1A** or **1B** in H_2O/MeOH (1:1, v/v). A solution of HCl 1N or NaOH 3N was used to reach acid or basic pH, respectively.

2.1.2.3. Synthesis of 3-Fluoropropylamine

The synthesis was carried out in 4 steps, following the technique reported by Gilissen *et al.* [22], with some modifications.

***N-tert*-Boc-[3-(*p*-toluensulfonyloxy)propylamine]:** A solution of propanolamine (0.77 mL, 10.0 mmol) in 15 mL CH_2Cl_2 was cooled to 0ºC and TEA (1.5 mL, 11.0 mmol) was added. Di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) dissolved in 2 mL CH_2Cl_2 was added and the solution was stirred at room temperature for 22 hours. The solution was washed with water (10 mL), dried over Na_2SO_4 and evaporated under reduced pressure to afford *N-tert*-Boc-3-aminopropanol, which was used in the next reaction without further purification. Next, a solution of *N-tert*-Boc-3-aminopropanol (1.75g) in 10 mL CH_2Cl_2 was cooled to 0ºC

and TEA (0.88 mL, 6.3 mmol) was added. After addition of *p*-toluensulfonyl chloride (1.09 g, 5.7 mmol) the solution was stirred for 22 hours at room temperature, washed with water (20 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2) with hexane/ethyl acetate (7:3 v/v) as eluent to yield 0.89g (27%) of *N*-*t*-Boc-[3-(p-toluensulfonyloxy)propylamine]. ^{1}H NMR (400 Hz, $CDCl_3$): δ_H: 7.81 (2H, d, *J* = 8.4Hz), 7.37 (2H, d, *J* = 8.0Hz), 4.63 (1H, bs), 4.10 (2H, t, *J* = 6.0Hz), 3.18 (2H, t, *J* = 6.4Hz), 2.47 (3H, s), 1.86 (2H, q, *J* = 6.4Hz), 1.44 (9H, s). The proton NMR signals are consistent with data previously published for this compound [23].

***N*-*t*-BOC-(3-fluoropropylamine):** A tetrabutylammonium fluoride solution (1 M in THF, 2.6 mL, 2.5 mmol) was added to a solution of *N*-*t*-BOC-[3-(p-toluensulfonyloxy) propylamine] (0.4 g, 1.3 mmol) in 15 mL acetonitrile. The solution was heated at 70ºC, under nitrogen atmosphere, for 30 minutes and then concentrated under reduced pressure. The reaction product was purified by column chromatography (SiO_2) with hexane/ethyl acetate (8:2 V/V) as the eluent to yield the desired product as an oil (0.15g, 66%). ^{1}H NMR (400 Hz, $CDCl_3$): δ_H: 4.72 (1H, s), 4.54 (2H, dt, *J* = 47.2 Hz, 5.6 Hz), 3.29 (2H, m), 1.90 (2H, dq, *J* = 27.2 Hz, 6.0 Hz), 1.40 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.66, 84.10 (d, *J* = 208.0 Hz), 76.67, 37.08, 29.69, 29.17. The proton and carbon NMR signals are consistent with data previously published for this compound [24].

3-Fluoropropylamine: the amino group was deprotected by addition of 0.75 mL trifluoroacetic acid (TFA) to a solution of *N*-*t*-BOC-(3-fluoropropylamine) in 3mL CH_2Cl_2. The solution was stirred for 1 hour at room temperature and then concentrated under reduced pressure to yield 0.16g of a yellow oil of 3-fluoropropylamine trifluoroacetate salt (99%). ^{1}H NMR (400 Hz, DMSO-d_6): δ_H: 7.89 (3H, s), 4.54 (2H, dt, *J* = 47.2, *J* = 5.6Hz), 2.91 (2H, m), 1.94 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.8 (q, *J* = 35.8 Hz, C=O), 116.2 (q, *J* = 285.4 Hz, CF_3), 81.7 (d, *J* = 154.7 Hz, C3), 36.2 (d, *J* = 5.1 Hz, C1), 28.5 (d, *J* = 21.2 Hz, C2).

2.1.2.4. SR101 N-*(3 -Fluoropropyl) sulfonamide (2)*

The synthesis was carried out following the technique described before for SR101 derivatization. In this case, 3-fluoropropylamine trifluoroacetate salt (160 mg, 0.84 mmol) was used. Purification and isolation of the two isomers (**2A** and **2B**) were achieved by column chromatography (SiO_2) with 2.5%, 3.5%, 4%, 5% and 10% methanol in CH_2Cl_2 sequentially as eluent (isomer 2A eluted first and then isomer 2B). A second purification by semipreparative HPLC was done, with a Macherey-Nagel C_{18} column (10 x 250 mm), 5 µm, a mixture of acetonitrile (MeCN):H_2O (47:53) as eluent, a flow of 4,0 mL/min and UV detection at λ=200 nm (retention time (t_R) isomer **2B**: 12 min and isomer **2A**: 23 min). Finally, 9.7 mg (3%) of SR101 *N*-(3 -Fluoropropyl)-sulfonamide isomer **2A** and 3.5 mg (1%) of isomer **2B** were obtained. *Para* isomer **2A**: ^{1}H NMR (400 Hz, DMSO-d_6) δ (ppm): 8.41 (1H, d, *J* = 1.6 Hz, ArH), 8.00 (1H, t, *J* = 6.0 Hz, NH), 7.92 (1H, dd, *J* = 1.6 Hz, 7.6 Hz, ArH), 7.38 (1H, d, *J* = 8.0 Hz, ArH), 6.52 (2H, s, CHAr), 4.53 (2H, dt, *J* = 6 Hz, *J* = 47 Hz, CH_2F), 3.52 (8H, m, CH_2NCH_2), 3.00 (6H, m, $NHCH_2$, $ArCH_2$), 2.63 (4H, t, *J* = 5.6 Hz, $ArCH_2$), 2.03 (4H, m, $ArCH_2CH_2$), 1.85 (6H, m, CH_2CH_2Br, $ArCH_2CH_2$). ^{13}C-NMR (100 Hz, DMSO-d_6) δ (ppm): 153.80, 151.90, 151.05, 148.82, 141.64, 134.22, 131.55, 127.60, 127.15, 125.97, 122.95, 113.13, 104.66, 82.33 (d, *J*=158Hz), 50.68, 39.44, 27.37, 20.64, 19.87, 19.68. O*rtho* isomer **2B**: ^{1}H-NMR (400 Hz, MeOH-d_6) δ (ppm): 8.38 (1H, s, ArH), 8.00 (1H, dd, *J* = 1.2 Hz, 8.0 Hz, ArH), 8.01 (1H, s, NH), 7.37 (1H, d, *J* = 8.0 Hz, ArH), 6.54 (2H, s, CHAr), 4.43 (2H, dt, *J* = 5.2 Hz, *J* = 45.6 Hz, CH_2F), 3.51 (8H, m, CH_2NCH_2), 3.01 (6H, m, $NHCH_2$, $ArCH_2$), 2.65 (4H, m, $ArCH_2$), 2.02 (4H, m, $ArCH_2CH_2$), 1.84 (6H, m, CH_2CH_2Br, $ArCH_2CH_2$). ^{13}C-NMR (100 Hz, DMSO-d_6) δ (ppm): 153.45, 151.29, 151.09, 148.76, 141.35, 134.35, 131.30, 130.01, 126.75, 125.35, 122.15, 113.65, 104.01, 82.15 (d, *J*=145Hz), 50.16, 39.87, 27.67, 21.15, 20.98, 19.87.

ESI-MS, *m/z*: 688.4 (M+• + Na), 668.5 (M+• + 2H), 647.7 (M+• - F).

2.1.2.5. SR101 sulfonamide (3)

SR101 *N*-(3 -Bromopropyl) sulfonamide isomer mixture (**1A**: **1B**, (85:15); 8.0 mg, 11 µmol) was dissolved in dry DMF (1.2 mL) and TBAF (176 µL, 0.61 mmol) was added under nitrogen atmosphere. The solution was heated at 130 ºC for 45 minutes and then the solvent was evaporated under reduced pressure. The crude was analysed by 1H-RMN. Compound **3**: ^{1}H-NMR (400 Hz, MeOH-d_6) δ (ppm): 8.34 (1H, s, ArH), 7.95 (2H, bs, NH, ArH), 7.48 (1H, d, *J* = 8.0 Hz, ArH), 6.54 (2H, s, CHAr), 3.83 (4H, t, *J* = 7,6 Hz, SO_2NCH_2), 3.50 (8H, m, CH_2NCH_2), 3.01 (4H, m, $ArCH_2$), 2.63 (4H, t, *J* = 6 Hz, $ArCH_2$), 2.06 (6H, m, $SO_2NCH_2CH_2$, $ArCH_2CH_2$), 1.86 (4H, m, $ArCH_2CH_2$). ^{13}C-NMR (100 Hz, DMSO-d_6) δ (ppm): 154.76, 151.73, 150.93, 148.58, 140.6, 135.28, 131.76, 129.7, 128.40, 127.61, 123.02, 113.09, 104.67, 99.98, 51.45, 50.70, 50.19, 36.24, 31.22, 27.42, 20.80, 19.78.

2.2. Radiosynthesis

2.2.1. Materials

All chemicals and solvents were purchased from commercial sources (Merck, Sigma-Aldrich, Carlo Erba). They were of analytical grade and used without further purification. The QMA Chromafix 30-PS-HCO_3^- cartridge was purchased from Machery-Nagel. The Sep-Pak C_{18} light cartridge and 0.2 µm hydrophilic sterilizing filters were purchased from Waters. The semipreparative HPLC column was a VP 250/10 mm Nucleosil 100-5 C_{18} (Macherey-Nagel). The analytical HPLC column was an EC 250/4.6 mm Nucleodur 100-5 C_{18}ec (Macherey-Nagel). The analytical GC column was a DB-WAX 30 m long, 0.53 mm in diameter and with 1.00 mm film thickness (Agilent). The Molecular exclusion columns were MicrospinTM G-50, GE Healthcare. *In vitro* studies were carried out using pool human plasma donated by a registered clinical laboratory.

2.2.2. Instruments

[^{18}F]Fluoride was produced in a PET Trace 16.5 MeV cyclotron (GE Healthcare). Radiosynthesis was carried out using the TRACERlab® FX-FN module (GE Healthcare). HPLC analyses were performed with a Shimadzu UFLC equipped with diode array and gamma detectors. The GC analyses of ethanol and residual solvents were done with a

Shimadzu GC-2010 Plus equipped with an FID detector. Activity measurements were performed with a dose calibrator Capintec CRC 25R, CRC 25 PET or a 3"x3" well type NaI (Tl) solid scintillation detector coupled to a multichannel analyzer ORTEC. Stability studies were conducted using a Thermo Scientific Sorvall ST 16R centrifuge.

2.2.3. Fully Automated Radiosynthesis

2.2.3.1. Production and Azeotropic Drying of [^{18}F]Fluoride

The synthetic process was performed in the automated synthetic platform GE TRACERlab® FX-FN, with [^{18}F]fluoride (solubilized in $H_2{}^{18}O$) produced in the cyclotron via the nuclear reaction $^{18}O(p,n)^{18}F$. To remove the enriched water, the aqueous [^{18}F]fluoride was trapped on an anion exchange cartridge. The fluoride anion was then eluted into a reactor using a solution of aqueous potassium carbonate (3.5 mg in 100 µL of water) mixed with a solution of Kryptofix [2.2.2] (15 mg in 900 µL of acetonitrile). The solution was concentrated to dryness to remove both the acetonitrile and the water. The drying was carried out at 65ºC for 5 minutes, followed by 95ºC for 3 minutes under reduced pressure and a stream of helium. After removal of the solvents, vacuum was applied for 30 seconds at 60 ºC and helium was injected at atmospheric pressure.

2.2.3.2. Radiosynthesis of SR101 **N-*(3-[^{18}F]Fluoropropyl) sulfonamide ([^{18}F]2B-SRF101)***

A solution of the precursor SR101 *N*-(3-Bromopropyl) sulfonamide (isomer **1A**, **1B** or the mixture) was added to the activated [^{18}F]fluoride in anhydrous aprotic solvent. Different labeling conditions were tested during the procedure, varying precursor mass (1, 2 and 5 mg), solvent (DMF and DMSO) and temperature (50, 100, 130 and 160 ºC). The reaction time was 10 minutes in all cases. After the labeling time, the crude reaction mixture was cooled to 40 ºC. To evaluate the presence of the desired [^{18}F]labeled compounds and the radiochemical purity by means of analytical HPLC, the crude mixture was diluted with 2 mL of the solvent reaction and a sample was taken and analyzed (by-passing the HPLC system of the module).

2.2.3.3. Purification and Formulation

The crude solution (using isomer **1B** as precursor) was transferred to a pre-injection vial and diluted with 2 mL of the semipreparative HPLC eluent. It was then transferred to a 5 mL injection loop and purified by semipreparative HPLC, using MeCN:H_2O (47:53) as a mobile phase with an isocratic flow of 4.0 mL/min. Chromatograms were registered using an UV detector (λ: 200 nm; t_R precursor **1B** 16.0 min, t_R **2B-SRF101** 12.5 min) and a gamma detector in series. The fraction corresponding to [^{18}**F]2B-SRF101** (eluting between 10 and 13 minutes) was collected and diluted with 50 mL of water for injection into a collection flask. The diluted solution was passed through a C_{18} light-SPE cartridge (preactivated with 1 mL of absolute ethanol followed by 10 mL of water for injection). The cartridge was washed with 10 mL of water for injection. The trapped product was eluted with 1 mL of absolute ethanol and collected in a two-neck vial containing 6 mL of 0.9% NaCl solution. Finally, formulation was done passing 3 mL of 0.9% NaCl solution through the cartridge and collecting it into the mentioned vial through a 0.2 µm hydrophilic sterilizing filter.

2.2.3.4. Quality Control

Chemical and radiochemical impurities were detected and quantified using radio-HPLC on a C_{18} column. The mobile phases consisted of Na_2HPO_4 100mM, pH 6.8 (A) and MeCN (B); gradient: 0-10 min: from 0% to 100% B, 10-15min: 100% B; flow rate: 1.0 mL/min; UV detection at 578 nm. The whole HPLC analysis was completed within 20 minutes. The retention time of the precursor and the product were 13.1 and 12.8 minutes, respectively. The chemical identity of [^{18}**F]2B-SRF101** was determined by comparing the retention time of the unlabeled reference compound. The radiochemical purity was calculated considering the portion of [^{18}**F]2B-SRF101** in relation to the total radioactivity. Specific activity was determined considering the total radiopharmaceutical activity at the end of synthesis and the amount of the unlabeled product. The residual solvents (such as acetone, acetonitrile and DMSO) and the ethanol were analyzed by gas chromatography (GC) in accordance with USP general chapter < 467 > [25]. The appearance of the solution was checked by visual inspection, and pH was determined using a calibrated pH-metre. The radionuclidic purity was assessed by recording the corresponding gamma spectrum and the identity by measuring the physical half-life. The concentration of Kryptofix [2.2.2] in the final product was assessed with an iodine spot test.

2.3. *In vitro* Studies

2.3.1. Radiochemical Stability of [^{18}F]2B-SRF101 in Final Formulation

The radiochemical stability of [^{18}**F]2B-SRF101** was checked in the final formulation at room temperature for 4 hours. Four final formulation samples of [^{18}**F]2B-SRF101** were taken at different times (30 minutes, 2, 3 and 4 hours). These samples were analyzed by analytical radio-HPLC using the conditions described for quality control.

2.3.2. Radiochemical Stability of [^{18}F]2B-SRF101 in Plasma

The radiochemical stability of [^{18}**F]2B-SRF101** was checked in plasma at 37 °C for 2 hours. Human plasma (1000 µL) was incubated with [^{18}**F]2B-SRF101** (100 µL) at 37 ºC for 2 hours. Four plasma samples (100 µL) were taken at different incubation times (25, 60, 90 and 120 minutes). Plasma samples were extracted with absolute ethanol (100 µL, at -15ºC) and mixed with vortex to denaturalize the proteins. To precipitate proteins the mixtures were centrifuged (2 minutes, 5000 rpm at 4 ºC). The supernatants were injected in the radio-HPLC (method described for quality control) and the radiochemical purity of [^{18}**F]2B-SRF101** was determined.

2.3.3. Plasma Protein Binding Determination

Molecular exclusion columns were prepared by centrifugation (2 minutes, 3300 rpm at 4ºC). Human plasma (1000 µL) was incubated with [^{18}**F]2B-SRF101** (100 µL) at 37 ºC for 120 minutes. Plasma samples (50 µL) were applied to the columns at different incubation times (35, 60 and 120 minutes) and incubated for 2 minutes. The columns were centrifuged (2 minutes, 3300 rpm at 4ºC) and eluates were collected. The radioactivity of columns and eluates was measured by gamma counter. A blank was carried out at 120 min-

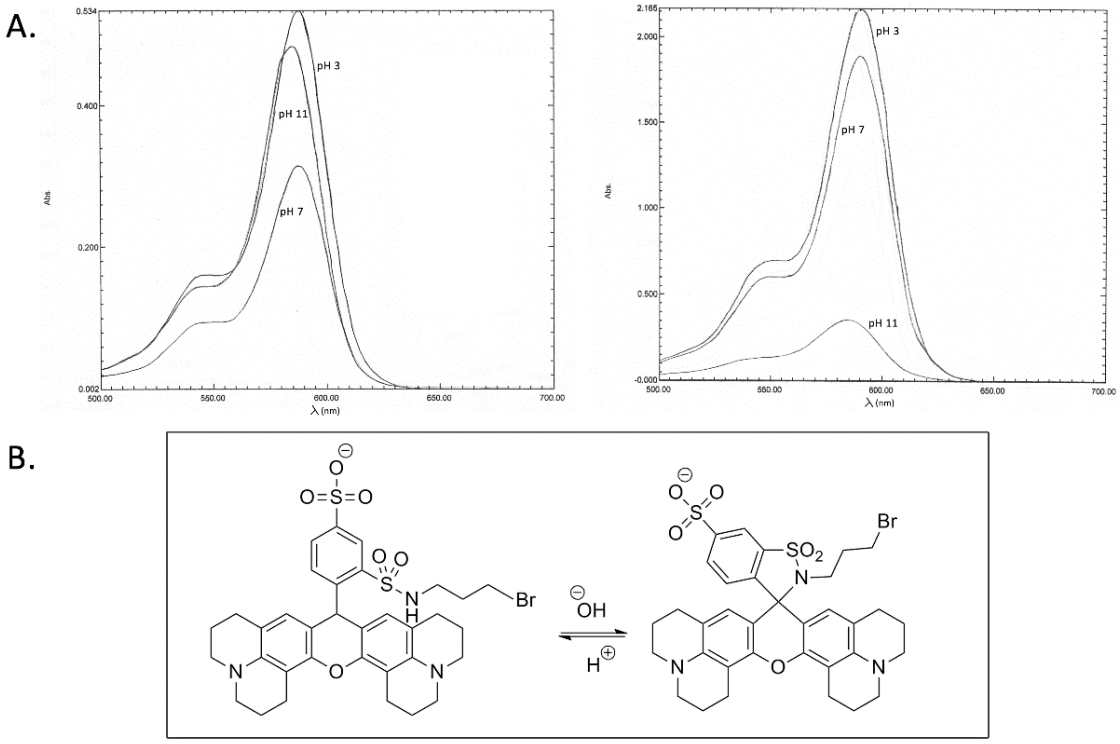


Fig. (1). A. Absorption UV spectra of isomer **1A** (left) and **1B** (right), recorded at different pH. **B.** Cyclization of Isomer 1B to the isothiazoline dioxide at alkaline pH.

utes of incubation, replacing plasma for 0.1 M phosphate buffer.

2.3.4. Lipophilicity

The [^{18}**F]2B-SRF101** partition coefficient was determined in 1-octanol and 0.1 M phosphate buffer at pH 7.0. The two phases were pre-saturated with each other. The 1-Octanol (2.0 mL) and the phosphate buffer (2.0 mL) were pipetted into two test tubes. Next, [^{18}**F]2B-SRF101** (100 µL) was added in each tube, the mixture was shaken by vortex for 2 minutes and centrifuged (5 minutes, 5000 rpm at 4ºC). After phase separation, three samples (100 µL) of each phase were taken and the amount of radioactivity was measured by gamma counter. The extraction was done two times with each tube. The partition coefficient was calculated using:

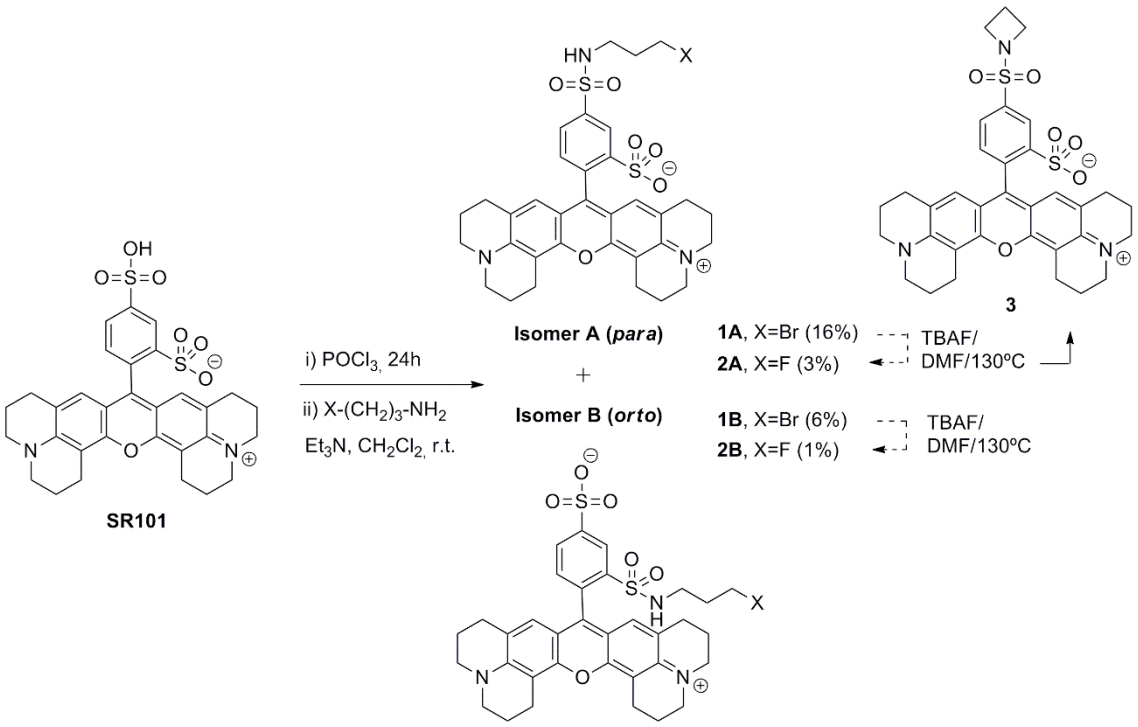
Log P_{OTC}: Log (counts in octanol/ counts in buffer).

3. RESULTS AND DISCUSSION

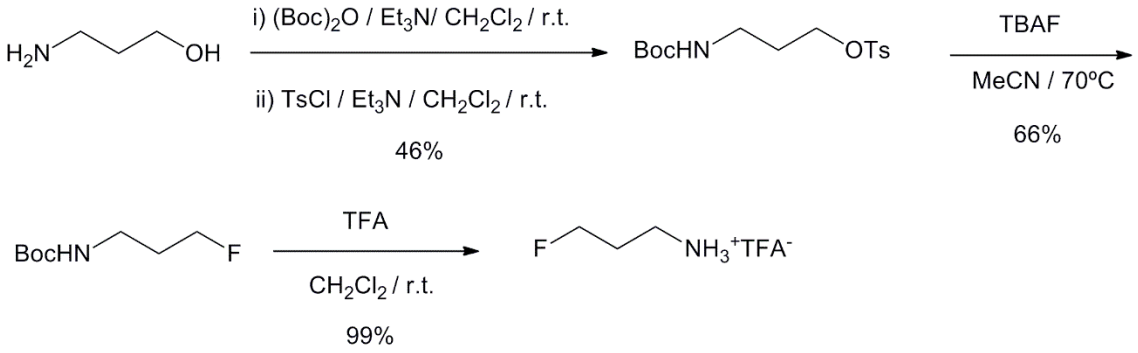
3.1. Organic Synthesis

A sulfonamide-SR101 (**1**) was synthetized to be used as a precursor for labelingradiolabeling with [^{18}F]. Sulforhodamine 101 was first converted to SR101 sulfonyl chloride by treatment with a large excess of phosphorus oxychloride (Scheme **1**). After removal of $POCl_3$, the crude sulfonyl chloride derivative was directly used in the next step without chromatographic purification, since it is known to be unstable to moisture during storage and prone to hydrolysis in the conjugation reactions [20]. Chemical derivatization of the SR101 sulfonate group was performed, reacting SR101 sulfonyl chloride with 3-bromopropylamine, resulting in the formation of two regioisomers of SR101 *N*-(3-Bromopropyl)-sulfonamide (*para* isomer **1A** and *ortho* isomer **1B**, Scheme **1**) [20, 21]. Different column chromatography conditions were tested to purify and isolate both isomers. The isomers were purified using silica gel as stationary phase (isomer **1A** + **1B** as a 85:15 mixture) but they were not isolated. The isolation was achieved employing an Al_2O_3 column to give sulfonamide **1A** (*para* isomer) in 16% yield and **1B** (*ortho* isomer) in 6% yield. Sulfonamide-SR101 isomers (**1A** and **1B,** Scheme **1**) were identified by UV-spectroscopy because of their different pH behavior. Isomer **1B** had a maximal UV absorption variable with the pH, due to a reversiblecyclization to the isothiazoline dioxide at alkaline pH, whereas isomer **1A** was not pH-sensitive (Fig. **1**) [21].

SR101 *N*-(3-Fluoropropyl)-sulfonamide **(2)** was prepared to be used as a standard for analytical purpose. First, through nucleophilic substitution of the bromine atom in sulfonamide **1** by fluorine, a reaction of the isomer mixture (**1A + 1B**) was carried out with TBAF, heating up to 130ºC for 45 minutes. In this case, only compound **3** was obtained (Scheme 1). This derivative was formed due to an internal cyclization of **1A**, which we hypothesize could be promoted by F^- acting as base, high temperature or a combination of both. This pathway did not take place with **1B**, since fluoride could initially promote isothiazoline dioxide cyclization (Fig. **1B**), leaving the bromine atom available to be replaced by fluoride. In this context, 3-Fluoropropylamine was synthetized (Scheme **2**), to be used in the synthesis of compound **2** (Scheme 1). Furthermore, 3-Fluoropropylamine was reacted with SR101 sulfonyl chloride to obtain compound **2** as a regioisomer mixture (**2A** + **2B,** Scheme **1**). Isomer purification and isolation were achieved by column chromatography (SiO_2) followed by semipreparative HPLC. The low synthe-



Scheme 1. Synthesis of sulfonamide-SR101 derivatives.



Scheme 2. Synthetic scheme of 3-Fluoropropylamine.

sis yield of both compounds **1** and **2** was attributed to instability of SR101 sulfonyl chloride [20].

With this procedure, all the standards and precursors required for labelingradiolabeling and quality control, which were not commercially available, were obtained.

3.2. Radiosynthesis

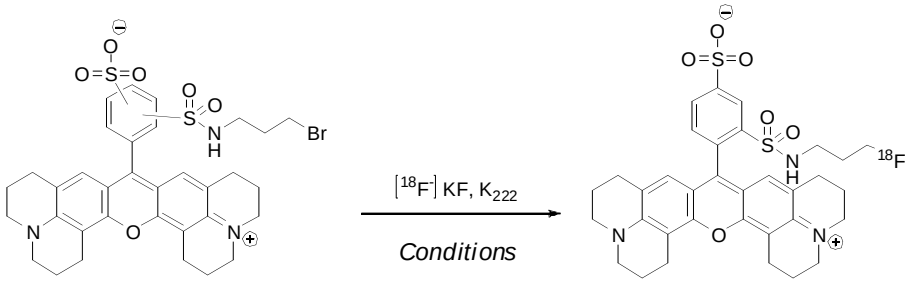
Initially, different labeling conditions were tested using the isomer mixture (**1A+1B**) as precursor. No product was obtained with low temperature (50 ºC), employing DMF as solvent, whereas a labeled compound was produced at 130 ºC. However, its radiochemical purity was very low (Table **1**, Entry 1 and 2). DMSO was also tested as solvent reaction, with different mass precursor (1.0 and 5.0 mg), obtaining a single major product in both cases (Table **1**, Entry 3 and 4). This means that only one isomer was reacting with [^{18}F].

Once both isomers were isolated, different labeling conditions were tested with each one, considering the previous results obtained with the mixture. Labeling with [^{18}F] was achievable only with isomer **1B**, whereas isomer **1A** suffered the internal cyclization described above (Table 1, Entry 5-8). Two different temperatures were tested with the isomer **1B** (100 and 160 ºC). The desired product ([**^{18}F]2B-SRF101**) was obtained with optimal radiochemical purity at 160ºC (Table **1**, Entry 6 and 7). There were also used 1.0 and 2.0 mg of precursor **1B**, producing the labeled compound with similar radiochemical purity. Because of that, the mass precursor selected for the labeling was 1.0 mg (Table **1**, Entry 7 and 8).

Finally, the optimum labeling conditions for isomer **1B** were 1 mg of precursor in 1 mL of DMSO, 10 minutes of reaction time and heating at 160ºC. These conditions were chosen as the most suitable to perform a complete synthesis (including HPLC separation, SPE purification, and the formulation steps) (Fig. **2**).

In the complete synthesis, SR101 *N*-(3-[^{18}F]Fluropropyl)-sulfonamide ([**^{18}F]2B-SRF101**) was obtained in 10 mL, with radiochemical purity of (97.0 ± 0.6) % (n=3), in accordance with the established quality control specifications. Quality controls of three different batches are summarized in Table **2**. The specific activity of [**^{18}F]2B-SRF101** ranged from 23

Table 1. Radiochemical purity of [^{18}F]2B-SRF101 in reaction crude under different labeling conditions.



	Precursor	Mass Precursor (mg)	Solvent	Labeling Temperature (ºC)	Radiochemical Purity of [^{18}F]2B-SRF101 in Reaction Crude
1	Isomer mixture	1.0	DMF	50	nl [a]
2	Isomer mixture	1.0	DMF	130	3.6 [a]
3	Isomer mixture	1.0	DMSO	160	11.2 [a]
4	Isomer mixture	5.0	DMSO	160	19.5 [a]
5	Isomer **1A**	1.0	DMSO	160	nl [a]
6	Isomer **1B**	1.0	DMSO	100	0.5 [a]
7	Isomer **1B**	1.0	DMSO	160	44.1 [b]
8	Isomer **1B**	2.0	DMSO	160	41.3 [a]

nl: not labeling
[a] (n =1)
[b] (n=2)

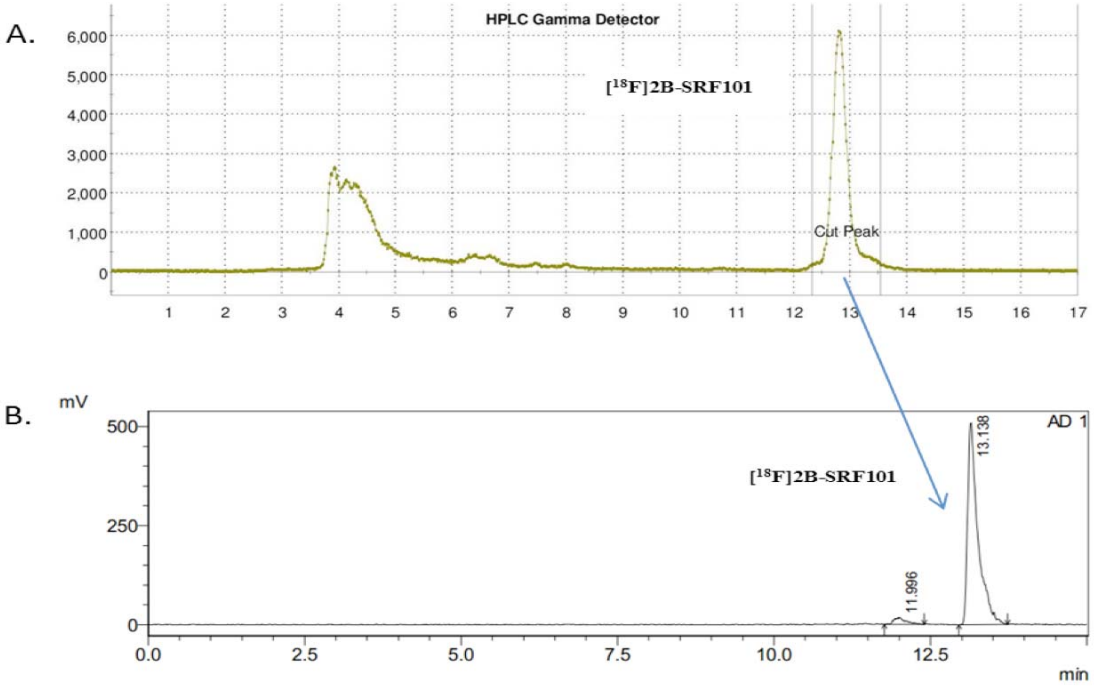


Fig. (2). An example of a typical semi-preparative (**A**) and analytical (**B**) HPLC gamma chromatogram of [^{18}**F]2B-SRF101**.

to 69 GBq/μmol (end of synthesis, EOS) and the overall yield synthesis was (11.9 ± 1.7) %, non-decay corrected (ndc) (n=3). The entire process was completed within no more than 55 min.

3.3. *In vitro* Studies

In vitro studies are a prerequisite for *in vivo* preclinical evaluations, being used to predict the *in vivo* behavior of the labeled compound. Several studies were conducted to char-

Table 2. Quality control results of three consecutive preparation batches of [^{18}F]2B-SRF101.

Test	Specifications	Batch 1	Batch 2	Batch 3
Appearance	Clear, pink and particle free solution	Ok	Ok	Ok
pH	4.5-8.5	6.3	6.0	6.0
Kryptofix	< 50µg/mL	< 50µg/mL	< 50µg/mL	< 50µg/mL
Radiochemical Purity	> 90%	96.3 %	97.4 %	97.4 %
Radiochemical Identity	RT difference of radioactivity and reference peaks ≤ 10 %	3.2 %	1.5%	2.3%
Residual solvent:				
Acetonitrile	< 0.05%	0.0064%	0.0035%	0.003%
Acetone	< 0.5%	0.0014%	0.0012%	ND
DMSO	< 0.5%	0.0029%	0.0006%	0.0002%
Ethanol	< 10%	9.7 %	9.9 %	9.6 %
Radionuclidic Identity ($t_{1/2}$)	105 - 115 min	111 min	109 min	107 min

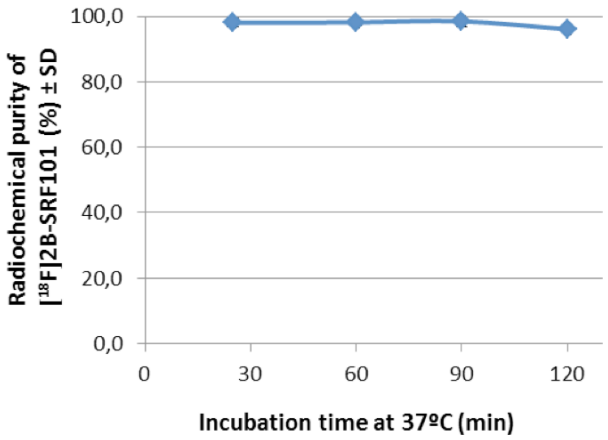


Fig. (3). Plasma stability study: radiochemical purity of **[^{18}F]2B-SRF101** at different incubation times in plasma (n=3).

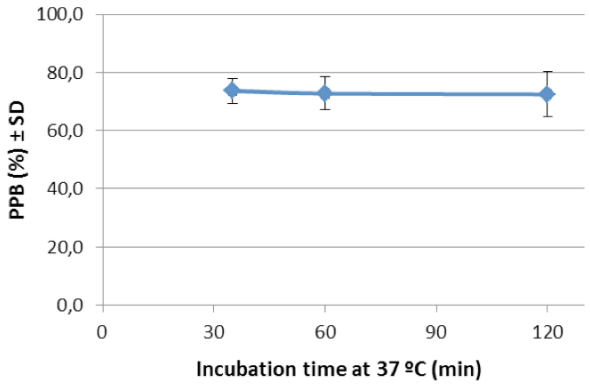


Fig. (4). Plasma protein binding of [^{18}F]**2B-SRF101** at different incubation times in plasma (n=3).

acterize the novel radiopharmaceutical: stability evaluation in the final formulation as well as in contact with plasma; lipophilicity and plasma protein binding.

The stability studies were performed by measurement of the radiochemical purity over time. **[^{18}F]2B-SRF101** was stable in final formulation for 4 hours, with a radiochemical purity > 90%. The studies in plasma showed that [^{18}**F]2B-SRF101** was stable at all incubation times (Fig. **3**). This assay was done up to 120 minutes, which is the time required to perform preclinical evaluations such as *in vivo* imaging or biodistribution studies.

Plasma protein binding determination of [^{18}**F]2B-SRF101** was performed by gel filtration. The product showed a high PPB percentage, which was constant over time (Fig. **4**). This could affect the clearance, as it is, in general, the unbound fraction which undergoes hepatic clearance. Similarly, renal clearance is reduced for compounds with high PPB. So, it is a factor that must be studied through biodistribution studies in rodents.

Lipophilicity evaluation could assess the ability of a compound to cross the blood-brain barrier (BBB), which is a requirement for a brain imaging PET tracer. The octanol-water partition coefficient (log P_{OCT}) is an indicator of tracer lipophilicity. It has been reported that the log P_{OCT} value desirable in these cases is between 1 and 3[26]. In our study, the log P_{OCT} value for [^{18}**F]2B-SRF101** was (1.88 ± 0.14) (n=4). This value states that the compound is lipophilic and is expected to cross the blood-brain barrier.

CONCLUSION

A synthetic method for labelingradiolabeling the fluorescent dye Sulforhodamine 101 (SR101) with [^{18}F] was developed to evaluate the compound as a possible PET tracer for astrocytes. The derivatization of SR101 *via* a sulfonamide-linker was done and the first radiosynthesis of [^{18}**F]2B-SRF101** was achieved with the commercially available platform GE TRACERlab® FX-FN. The [^{18}F] labeled compound was obtained with a high radiochemical purity (>96%) and an overall yield synthesis of (11.9 ± 1.7) %, ndc (n=3). The entire process was completed within no more than 55 min.

The novel compound was obtained in accordance with quality control specifications. *In vitro* stability studies verified that [**^{18}F]2B-SRF101** was suitable for preclinical evaluations. Additional studies are currently underway for the *in vivo* characterization of this compound.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

National Research and Innovation Agency (ANII) for a grant to Ingrid Kreimerman as research fellowship; PEDECIBA; Andrea Boné for her support in quality control analysis; Pablo Buccino for his collaboration in drafting this paper.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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PMID: 28956517