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Radiosynthesis and preclinical investigation of ^{11}C labelled 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime ([^{11}C]SZV 1287)

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Abstract: The radiosynthesis, as well as the *in vivo* and *ex vivo* biodistribution of the ^{11}C radiolabelled 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime (**6**, [^{11}C]SZV 1287) are reported. SZV 1287 is a novel SSAO inhibitor and a promising candidate to be a novel analgesic for the treatment of neuropathic pain. Its radiolabelling was developed via a four-step radiosynthesis which started from the reaction of Grignard reagent **2** with [^{11}C]CO_2 to produce [^{11}C]oxaprozin (**3**). In the next step this carboxylic acid **3** was directly reduced to yield aldehyde **4**, then it was converted to the oxime **6**. The [^{11}C]SZV 1287 was administered to male NMRI mice. The animals were examined with dynamic PET/MR imaging for 90 minutes. Biodistribution studies were performed at 10, 30, 60 and 120 minutes post injection. The accumulation of the labelled compound was observed in the brain of the animals. The main excretion pathway was found to be through the liver and intestines. These studies provide preliminary information for pharmacokinetic characterization of the SZV 1287.

1. Introduction

Semicarbazide-sensitive amine oxidase (SSAO) is a copper containing enzyme family which catalyses oxidative deamination of primary amines. The first molecularly defined member of the family was amine oxidase copper containing (AOC), originally known as vascular adhesion protein-1, which has also essential role in the transmigration of leukocytes from the bloodstream into inflamed tissues.[1,2] Thus, increased SSAO activity was observed in many inflammation-associated diseases, such as inflammatory liver diseases, diabetes, congestive heart failure, atherosclerosis, severe obesity and Alzheimer's disease.[3–6] Several studies described the anti-inflammatory properties[2,7–11] and the analgesic actions of SSAO inhibition.[12,13]

3-(4,5-Diphenyl-1,3-oxazol-2-yl)propanal oxime (SZV 1287) is a novel SSAO inhibitor. This compound is an oxime analogue of the cyclooxygenase (COX) inhibitor (called oxaprozin) which was developed and patented by Mátyus *et al*.[14,15] (Figure 1). In addition, SZV 1287 has dual antagonistic effect on transient receptor potential vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1) receptors which are expressed predominantly on capsaicin-sensitive peptidergic sensory neurons mediating pain and inflammation.[16]

The recently described Metabolism-Activated Multitargeting (MAMUT) concept[17] is based on the designed combination of synergistic pharmacological effects of a parent drug and its active metabolite(s). In order that a new type of broad-spectrum anti-inflammatory profile could be achieved through combination

of two anti-inflammatory mechanisms of action, the MAMUT concept was implemented for SZV-1287. In the case of SZV 1287 this concept means that it possesses a semicarbazide-sensitive amine oxidase (SSAO) inhibitory effect, while its metabolite, oxaprozin inhibits cyclooxygenase (COX) enzyme.
However, the unique pharmacodynamic profile of SZV 1287 is due to the fact that it has a significant analgesic effect besides its anti-inflammatory effect by antagonizing transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1) cationic channels on primary sensory neurons. Because of its unique mechanism of action, it is considered to be a promising analgesic drug candidate currently being developed for chronic neuropathic pain, which is an unmet medical need.[12,13,18]
Preclinical investigation of the pharmacokinetic properties of drug candidates is a very important part of the drug development process. Aldehyde oximes are generally metabolized fast in the blood, thus our goal was to study the biodistribution of radiolabelled SZV 1287 and its metabolites after intravenous administration to provide information about its site of action.
Positron Emission Tomography (PET) is a functional imaging technique which enables visualizing molecular events in living organisms. Its major fields of clinical application are diagnosing diseases, monitoring disease progression and detecting treatment respond by using a suitable radiopharmaceutical. In addition, PET is an effective tool for non-invasively monitoring the biodistribution and clearance of radiolabelled bioactive compounds and drugs. Due to its extremely high sensitivity and selectivity, this technique is able to determine the concentration of radiolabelled molecule at subpicomolar range, thus it has significant advantages in the initial phases of drug development process. PET imaging requires a radiotracer having a positron emitter isotope built into the molecule at low concentration (nanomolar range) and consequently it does not induce pharmacological effect. The biodistribution studies using the PET imaging could prove that the radiolabelled drug reaches the tissue of interest and it does not accumulate in non-target sites by causing potential toxicity. Therefore, this PET technique is suitable for initial drug candidate screening in humans[19a,b] and can contribute useful information to the conventional pharmacokinetic (PK) studies. Labelling with the short-lived positron emitting ^{11}C radionuclide ($t_{½}$ = 20.4 min) does not change the chemical structure as well as biochemical features of the molecule and results in a suitable radiotracer for PET imaging.

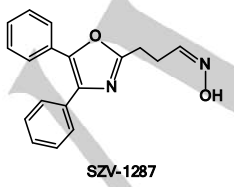


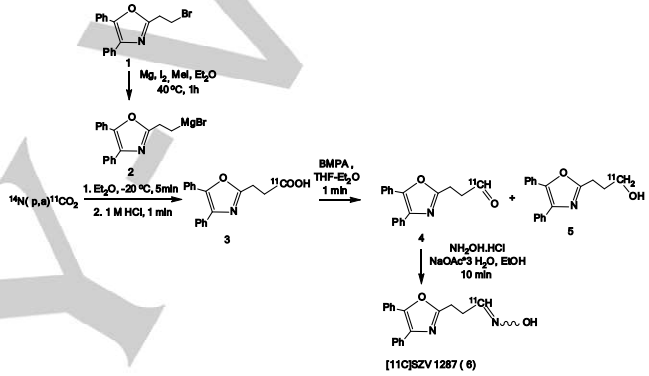
Figure 1. Chemical structure of SZV 1287.

In this work we report the radiosynthesis of the ^{11}C-labelled SZV 1287 and the evaluation of this PET probe distribution by in vivo dynamic PET/MR imaging and ex vivo biodistribution in mice at 10, 30, 60 and 120 minutes post injection.
The detailed PK analysis and toxicity studies of the analgesic candidate SZV 1287 for the preclinical dossier are currently being performed. These PET imaging studies provide important information for its distribution and help to understand its site of action.

2. Results and Discussion

2.1 Radiosynthesis

^{11}C isotope is an important positron emitter which is widely used for the preparation of PET radiopharmaceuticals. The short half-life (20.4 min) of this radionuclide makes its application challenging since the radiolabelling have to be performed as fast as possible. A five-step synthesis route was developed for the preparation of ^{11}C labelled 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime ([^{11}C]SZV 1287) (Scheme 1).



Scheme 1. Radiosynthesis of [^{11}C]SZV 1287.

The cyclotron produced [^{11}C]CO_2 is a common starting material for the radiosynthesis of different organic molecules such as carboxylic acids, aldehydes, and alcohols. The ^{11}C introduction was designed via carboxylation of a suitable Grignard reagent (organo-magnesium halide) with [^{11}C]carbon dioxide. First, the synthesis of the Grignard reagent **2** was carried out from 2-(2-bromomethyl)-4,5-diphenyl-1,3-oxazole (**1**) in argon atmosphere. In the next step Grignard reagent **2** was reacted with [^{11}C]CO_2 which was produced via $^{14}N(p,\alpha)^{11}C$ nuclear reaction by high-energy proton bombardment of nitrogen gas in cyclotron. The [^{11}C]CO_2 was trapped on 4Å molecular sieves. After heating, the released [^{11}C]CO_2 was reacted with the freshly prepared Grignard reagent at -20 °C. Then the reaction mixture was heated to room temperature and stirred for 5 minutes. Subsequently, hydrochloric acid (1 M) was added to the mixture to quench the reaction. Overall trapping efficiency varied between 33% and 63%. The reaction mixture was extracted with diethyl ether, then the organic phase containing the ^{11}C labelled propionic acid derivative **3** was transferred into another reactor and concentrated. Based on radio-HPLC chromatogram the residue contained approximately 60-70% [^{11}C]3-(4,5-diphenyl-1,3-oxazol-2-yl)propionic acid (**3**).
For the synthesis of aldehydes the most frequently used method is the reduction of different carboxylic acid derivatives (e.g. esters, acyl chlorides and nitriles) with mild reducing agents. However, there are only few reducing agents and methods for

the direct reduction of carboxylic acids into aldehydes. Since we intended to reduce the number of the synthetic steps as much as possible, therefore the previously described bis(N-methylpiperazinyl)aluminum hydride reagent (BMPA complex) by Hubert *et al*.[20] was applied for the conversion of carboxylic acid **3** into aldehyde **4**. The radio-HPLC chromatogram showed the crude product contained the ^{11}C labelled aldehyde derivative **4** and 10-20% alcohol byproduct **5**. It can be explained by the partial overreduction of the carboxylic acid **3**.

Finally, the mixture of aldehyde **4** and alcohol **5** was reacted with hydroxyl ammonium chloride and sodium acetate in ethanol. The reaction mixture contained 33-78% [^{11}C]3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime ([^{11}C]SZV 1287, **6**). Although the formation of the oxime **6** from the aldehyde **4** was quantitative, the yield was limited by the ratio of the aldehyde **4** and alcohol **5** formed in the reduction. First we intended to use the same type reverse phase columns for analytical and semipreparative HPLC methods too, but the application of the reverse phase column for the purification of ^{11}C labelled SZV1287 did not provide good separation. Therefore, we modified our initial concept and used normal phase silica column in the case of semipreparative HPLC purification. Thus the isolation of the desired ^{11}C labelled oxime **6** was performed on a normal phase silica column (WATERS NovaPak Silica) by using ethyl acetate and dichloromethane 30:70 as an eluent (see Figure 2). The [^{11}C]SZV 1287 (**6**) was collected between 9 and 11 minutes. Two peaks can be seen in this region of the radio-HPLC chromatogram because ^{11}C labelled oxime **6** is a mixture of E/Z stereoisomers and these stereoisomers were separated from each other on the normal phase silica column (see Figure 2), while both of them were co-eluated on the reverse phase column, Lichrospher 100 RP18 (see Figure 3 and Figure 4).

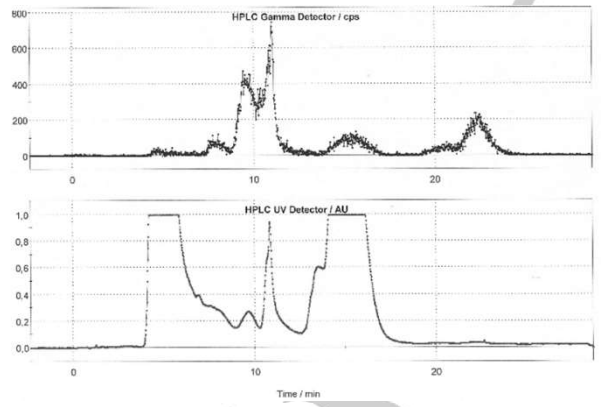


Figure 2. Preparative HPLC chromatogram of the final reaction mixture using normal phase silica column (WATERS NovaPak Silica).

The [^{11}C]SZV 1287 (**6**) was prepared under the optimized reaction conditions nine times starting from [^{11}C]CO_2 from 10 minutes irradiation with 44 µA beam current. The whole process took about 70 minutes and the radioactivity of the product varied between 8 and 70 MBq. Radiochemical purity was higher than 90% in every case. The analytical radio-HPLC chromatogram of the formulated [^{11}C]SZV 1287 (**6**) is shown in Figure 3. where the radiochemical purity was 91.4%.

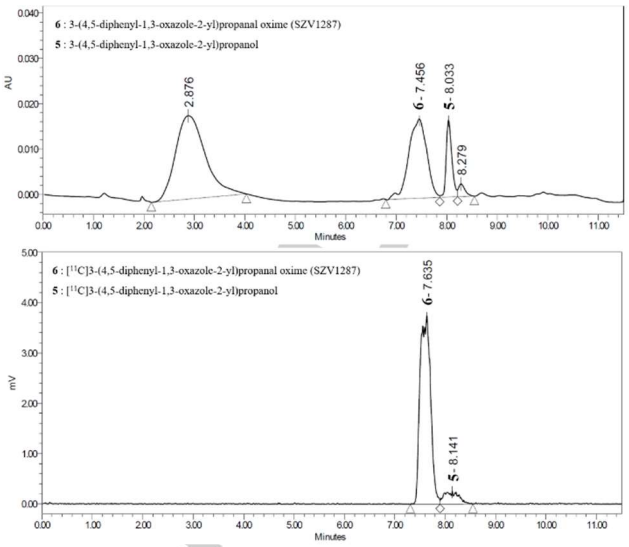


Figure 3. Analytical radio-HPLC chromatogram of the formulated [^{11}C]SZV 1287 using reverse phase column (Lichrospher 100 RP18). UV (254 nm); lower: RA.

The radiochemical yield from trapped [^{11}C]CO_2 was 1.8 ± 0.1% (end of synthesis, decay corrected) and the molar activity of formulated [^{11}C]SZV 1287 was 4.3-6.5 GBq/µmol. To improve the radiochemical yield many reaction conditions were tested in the case of each step, but these experiments did not led to better results. Therefore, we increased the starting activity to meet the requirements of the animal experiments. The low radiochemical yield was caused by the four moderate yield steps and the partial overreduction of the carboxylic acid **3**. In addition, high activity loss was observed during transfer and in the course of the extraction steps and preparative HPLC purification (strong absorption to the column).

2.2. Chromatographic separation of SZV 1287 and related compounds

For the optimisation of the radiosynthesis we have developed a radio-HPLC method. Among the tested stationary phases, the non-endcapped Lichrospher 100 RP18 gave the best separation utilizing the differences in the polarity and hydrogen bond donor/acceptor ability of the functional groups of the analytes. The aim of the chromatographic development was to separate all compounds of interest from each other, namely 3-(4,5-diphenyl-1,3-oxazole-2-yl)propanal oxime (SZV 1287, **6**), the precursor used for the radiolabelling reaction, i.e. (2-(2-bromoethyl)-4,5-diphenyl-1,3-oxazole (**1**)), the intermediates of the radiosynthesis (3-(4,5-diphenyl-1,3-oxazole-2-yl)propionic acid (oxaprozin, **3**), 3-(4,5-diphenyl-1,3-oxazole-2-yl)propanal (**4**)) and the possibly formed side product (2-ethyl)-4,5-diphenyl-1,3-oxazole (**7**)). The synthesis and the characterization of compound **1** are described in Supplementary Material and the other compounds were synthesized according to a previously described method.[14]

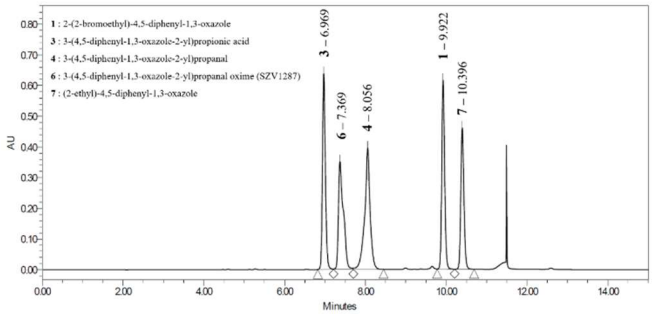


Figure 4. UV chromatogram (254 nm) of reference compounds using reverse phase column (Lichrospher 100 RP18).

The HPLC chromatogram of these reference compounds is shown in Figure 4. The compounds were baseline-separated with symmetrical peaks and without tailing.

2.3 In vivo imaging in mice

2.3.1. In vivo biodistribution of [^{11}C]SZV 1287

For the determination of the biodistribution of the [^{11}C]SZV 1287 molecule, dynamic PET scans were performed using healthy control animals. Representative decay-corrected PET/MRI images are shown in Figure 5. By the qualitative analysis of the PET images it was clearly visualized that [^{11}C]SZV 1287 was excreted through the liver and the intestines, and also the kidneys with the urine. Notable accumulation was also found in the Harderian-glands and moderate uptake was found in the heart and brain (Figure 5).

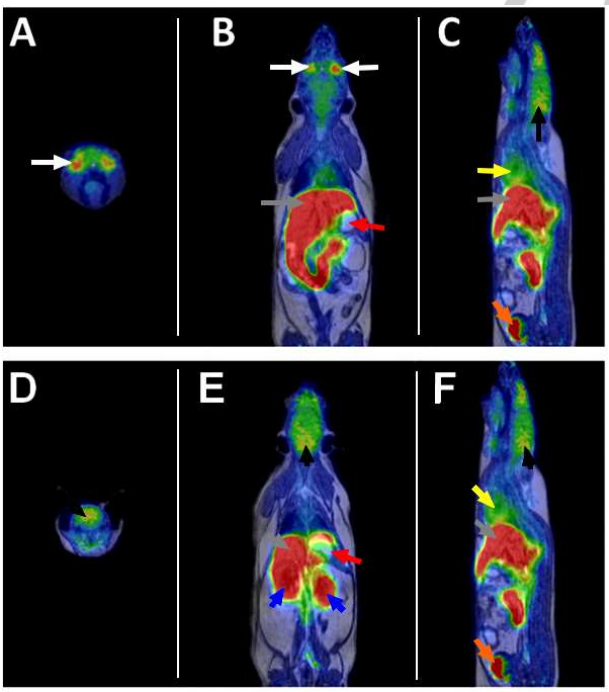


Figure 5. Representative axial (A,D), coronal (B,E) and sagittal (C,F) PET/MRI images of a healthy control mouse after intravenous injection of [^{11}C]SZV 1287 (90 min summ.). White arrows: Harderian glands; grey arrows: liver; red arrow: stomach; blue arrows: kidney; orange arrow: urine; yellow arrow: heart; black arrow: brain.

After quantitative SUV analysis of the dynamic PET images (Figure 6) we found that the radiotracer accumulation in the liver is constantly increasing in the first 4-9 minutes post injection (SUVmean: 5-6) and then decreasing with time. In parallel the radioactivity of the intestines increased from 30 to 90 min post injection (SUVmean: approx. 3.5 at 90 min). Another route of elimination from the body was observed through the urinary system.

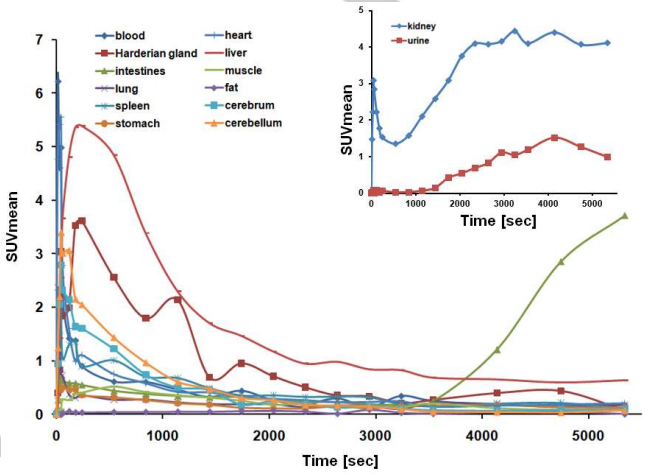


Figure 6. Representative mean time-activity curve (TAC) of [^{11}C]SZV 1287 in selected organs and tissues of a healthy control NMRI mouse.

The elimination was faster via the kidneys than the liver. Radioactivity appeared in the urine 25 minutes after the injection and increased exponentially (Figure 6). No significant accumulation was observed in other organs in the chest or in the abdomen.

Figures 6. and 7. show that the [^{11}C]SZV 1287 is able to cross the blood-brain-barrier. Significant accumulation was observed in the Harderian glands behind the eyes and in the brain in the first minutes post injection, thereafter the radiotracer accumulation decreased rapidly (Figure 6). It was earlier reported that high accumulation of different radiotracers (e.g.: ^{18}FDG, ^{11}C-PIB, ^{18}F-fallypride) was observed in the Harderian glands of rodents during PET investigation,[21] which makes the evaluation of the PET images of the brain difficult. The cause of this uptake is not explained yet.

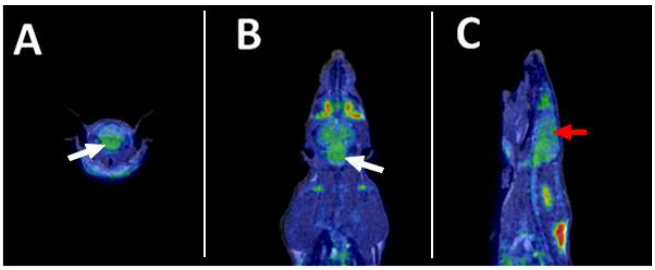


Figure 7. Representative PET/MRI images of a healthy control mouse after intravenous injection of [^{11}C]SZV 1287 (90 min summ.). White arrows: cerebellum; red arrow: cerebrum.

The detectable radioactivity using in vivo PET imaging reached its maximum at the first minute post injection (SUVmean approx.: 3) in mouse brain, then decreased with time (Figure 6). The highest uptake of [^{11}C]SZV 1287 was found in the cerebellum (SUVmean: 3.18-4.79) 1 minute post injection.

2.3.2 Ex vivo biodistribution studies

The ex vivo biodistribution of [^{11}C]SZV 1287 in healthy control animals was assessed by gamma-counter measurements. Higher accumulation of [^{11}C]SZV 1287 was found in the liver and in the small intestine than in other organs at each investigated time point (see Supplementary material Figure 1). These results suggested that [^{11}C]SZV 1287 was excreted mainly through the liver and intestines due to its lipophilic property. Furthermore, high activity was found in the kidney, which implies that some unidentified polar metabolites was rapidly washed out through the urinary system. [^{11}C]SZV 1287 concentration of all organs decreased from 10 to 120 minutes (see Supplementary material Figure 1). Similar results were found by Malfatti et al.[22], where the biodistribution and pharmacokinetics of a new oxime (RS194B) were investigated. They also found higher liver and kidney accumulation and rapid renal clearance in guinea pigs after intravenous administration of the radiolabelled oxime, furthermore, this observation was dose-dependent.

2.3.4. Determination of metabolites

The investigation of metabolization of [^{11}C]SZV 1287 was attempted by radio-HPLC separation of the blood- and urine samples taken during biodistribution experiments. Radio-HPLC analysis of the samples is described in the Supplementary Material. The low injected activity did not allow repeated measurements. The sample with the highest activity was examined in every case.
Unfortunately the administered low activity did not make possible the correct identification of metabolites arising from the short half-life of the ^{11}C isotope and the low activity of the HPLC samples. According to the obtained radio-HPLC chromatograms the [^{11}C]SZV 1287 and its metabolite, the carboxylic acid **3** (oxaprozin) were identified in the blood (see Supplementary material Figure 2), whereas unidentified polar metabolites were detected in the urine (see Supplementary material Figure 3).

3. Conclusion

We have successfully developed and accomplished a radiolabelling procedure for the preparation of ^{11}C labelled 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime ([^{11}C]SZV 1287, **6**) in four active synthesis steps. The radiosynthesis process took about 70 minutes and resulted in 8-70 MBq labelled product with >90% radiochemical purity. The [^{11}C]SZV 1287 was administered to NMRI mice to investigate its in vivo properties with non-invasive dynamic PET/MR imaging and ex vivo biodistribution studies. These experiments showed that the labelled compound was accumulated moderately in the brain and excreted through mainly the liver and the intestines.
The metabolic stability of [^{11}C]SZV 1287 was examined with radio-HPLC technique, but the administered low activity did not allow precise identification of metabolites. However the [^{11}C]SZV 1287 and its metabolite (oxaprozin, **3**) could be discovered in the blood, while unidentified polar metabolites could be found in the urine.
SZV 1287 rapidly enters the brain, and it is quickly metabolized, but its irreversible SSAO inhibitory effect in the central and peripheral nervous systems can explain its potent analgesic effects. These preclinical studies reveal valuable details about the *in vivo* behavior of this novel analgesic drug candidate.

4. Experimental Section

4. Materials and methods

4.1 General

All reagents and solvents were obtained from Sigma-Aldrich and VWR, and used without further purification. For the HPLC system HPLC-MS grade acetonitrile, methanol (Fisher Solutions) and deionized water (Milli-Q, 18.2 MΩcm−1) were used. 3-(4,5-diphenyl-1,3-oxazole-2-yl)propanal oxime (SZV 1287, **6**), 2-(2-bromoethyl)-4,5-diphenyl-1,3-oxazole (**1**), 3-(4,5-diphenyl-1,3-oxazole-2-yl)propionic acid (**3**), 3-(4,5-diphenyl-1,3-oxazole-2-yl)propanal (**4**) and 2-ethyl)-4,5-diphenyl-1,3-oxazole (**7**) were provided by Institute of Organic and Medicinal Chemistry, University of Pécs and synthesized as described previously.[14] The synthesis and the characterization of compound **1** are described in Supplementary Material.

4.2 Chemistry

4.2.1 Cyclotron production of [^{11}C]CO_2

[^{11}C]CO_2 was produced in a GE PETtrace cyclotron by ^{14}N(p, α)^{11}C nuclear reaction using 16.5 MeV proton bombardment of nitrogen gas containing 1 % oxygen. The beam current (44 μA) was kept constant, and the produced activity was adjusted by changing the irradiation time between 1 and 10 minutes. The obtained activity of [^{11}C]CO_2 was approximately 3.4 GBq/minute.
The [^{11}C]CO_2 from the cyclotron was trapped quantitatively on 4Å molecular sieves (Alltech, 80-100 mesh) at room temperature. The molecular sieves was heated to 350 °C and the released [^{11}C]CO_2 was transferred with helium sweep gas (10 ml/min) to the reactor for entrapment.

4.2.2 Radiosynthesis of [^{11}C]SZV 1287 (6)

Radiolabelling was performed on a Scansys TracerMaker system in a Von Gahlen hotcell. The Grignard reagent was prepared by suspending magnesium turnings (5 mg, 0.20 mmol) in anhydrous diethyl ether (0.5 mL, freshly distilled from sodium) in the presence of catalytic amount of iodine and methyl-iodide under argon atmosphere. Then 2-(2-bromo-ethyl)-4,5-diphenyl-1,3-oxazol (**1**) (10 mg, 0.03 mmol) in anhydrous diethyl ether (0.5 mL) was added. The mixture was heated at 40 °C for 1 hour. The [^{11}C]CO_2 from the cyclotron was trapped quantitatively on 4Å molecular sieves (Alltech, 80-100 mesh) at room temperature. The molecular sieves was heated to 350 °C and the released [^{11}C]CO_2 was transferred with helium sweep gas (10 ml/min) to the reactor for entrapment. The [^{11}C]CO_2 was bubbled into the Grignard reagent solution at -20 °C with a low (10 ml/min) helium flow. When the activity in the reactor reached a plateau it was heated to room temperature and left to react for 5 min with constant helium bubbling. The reaction was stopped with the addition of 1 mL of 1 M hydrochloric acid. [^{11}C]Carbon dioxide waste was collected on an Ascarite column connected after the reactor.

Diethyl ether (2 mL) was added two times to the reaction mixture, using a syringe pump and mixed thoroughly with bubbling helium. The organic phase, containing ^{11}C labelled propionic acid derivative (**3**) was separated and transfered into another reactor through a needle using helium pressure. During the transfer, the ether phase was passed through a drying cartridge, filled with dried magnesium sulphate and 4Å molecular sieves. The collected ethereal solution was concentrated in the second reactor with 100 ml/min He flow at 70 °C.

After cooling, the residue was dissolved in a solution of BMPA complex[20] in the mixture of tetrahydrofurane and diethyl ether 1:1 (0.5 mL). After one minute reaction time saturated NaCl (1 mL) was added to quench the reduction process. The reaction mixture was extracted with diethyl ether (2x1 mL) manually. The ethereal solution was concentrated to dryness to give the crude product, containing [^{11}C]3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal (**4**).

After cooling the residue was dissolved in 96% (0.5 mL) ethanol and mixed with a solution of 20 mg (0.29 mmol) hydroxyl ammonium chloride and 20 mg (0.15 mmol) sodium acetate in 200 µL water. After 10 minutes the [^{11}C]3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime ([^{11}C]SZV 1287, **6**) was isolated with preparative HPLC. Purification of the labelled oxime **6** was performed on a WATERS NovaPak Silica 6 µm, 7.8 x 300 mm column with ethyl acetate and dichloromethane 30:70, 4 mL/min flow with UV (254 nm) and radioactivity detection. The product was eluted between 9 and 11 minutes. After collection and evaporation to dryness it was reconstituted with 5% ethanol in saline.

4.2.3 Radio-HPLC method

Standard samples were prepared as follows: 1 mg material (**1**, **3**, **4**, **6** and **7**) were dissolved in a 1.5 mL of a mixture 0.1 % phosphoric acid and acetonitrile (60:40, v/v). The sample was obtained by mixing these standard solutions. This aliquot was filtered with of 0.45 µm PTFE syringe filter.

HPLC measurements were performed on a Waters Acquity UPLC I-Class System equipped with a Binary Solvent Manager, a Sample Manager (Flow-Through-Needle with 100 µL loop), a Column Manager, a Photodiode Array Detector. A photomultiplier tube (Hamamatsu Photonics), equipped with a plastic scintillator was used as radioactivity detector. Data were evaluated by Empower 3 chromatography software. The following four reversed-phase columns were tested for the separation of the reference compounds: Kinetex XB-C18 (50 mm x 4.6 mm, 2.6 µm, 100 Å, Phenomenex), Nucleosil C18 (150 mm x 4.6 mm, 5 µm, 100 Å, Macherey-Nagel), Symmetry C18 (150 mm x 4.6 mm, 3.5 µm, 100 Å, Waters), Lichrospher 100 RP18 (250 mm x 4.6 mm, 5 µm, 100 Å, Merck). The best separation was achieved with the Lichrospher 100 RP18 column, with the following gradient: A: 0.1 % phosphoric acid, B: acetonitrile, from 40% B to 100% B in 8 minutes, followed by an isocratic hold for 1 minute. The separation took 13 minutes. The UV chromatograms were integrated at λ=254 nm. Measurements were carried out with a flow rate of 0.8 mL/min and injection volume 5-20 µL.

4.3 Biology

4.3.1 Animals

NMRI mice (male, 12 weeks old; 20-22 g; n=15) (Charles River Laboratories) were housed under sterile conditions in IVC cages at a temperature of 26±2 °C, with 50±10% humidity and artificial lighting with a circadian cycle of 12 h. Semi-synthetic diet (VRF1; Akronom Ltd., Budapest, Hungary) and drinking water were available ad libitum to all the animals. Laboratory animals were kept and treated in compliance with all applicable sections of the Hungarian Laws and regulations of the European Union. Ethical license number: 6/2018/DEMÁB.

4.3.2 In vivo PET/MRI imaging

For in vivo PET imaging studies NMRI mice (n=3) were injected with 5.44±1.28 MBq of [^{11}C]SZV 1287 via the lateral tail vein. Animals were anaesthetized by 3% isoflurane (Forane) with a dedicated small animal anesthesia device (Tec3 Isoflurane Vaporizer) and dynamic (90 min) PET scans were performed (mouse whole body) using the preclinical nanoScan PET/MRI system (Mediso Ltd., Hungary). Reconstructed images were analyzed using the InterView™ FUSION (Mediso Ltd., Hungary) image analysis software. Radiotracer uptake was expressed in terms of standardized uptake values (SUVs). SUV was calculated as follows: SUV = [VOI activity (Bq/mL)]/[injected activity (Bq)/animal weight (g)], assuming a density of 1 g/mL.

4.3.3 Ex vivo biodistribution studies

For ex vivo biodistribution studies NMRI mice (n=12) were injected intravenously with 5.62±1.21 MBq of [^{11}C]SZV 1287. After 10, 30, 60 and 120 min incubation time mice were euthanized with 5% Forane. Tissue samples were taken from selected organs and the weight and the radioactivities of the samples were measured with calibrated analytical lab balance and gamma counter. The uptake was expressed as decay corrected %ID/g tissue.

4.3.4. Investigation of metabolites

Samples were taken during biodistribution experiments and were extracted with ethyl acetate and a 10 µL aliquot was injected onto the column (see Supplementary material Figure 2 and Figure 3). Chromatography system: Waters Acquity UPLC I-class liquid chromatograph with a Berthold LB513 radioactivity detector, equipped with a plastic scintillation cell.

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Keywords: carbon-11• radiochemistry • imaging agents • *in vivo* biodistribution • positron emission tomography

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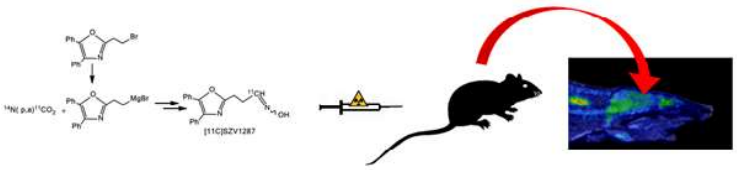

A radiosynthesis has been developed for the preparation of [^{11}C]SZV 1287. This radiolabelled compound was evaluated by *in vivo* dynamic PET/MR imaging and *ex vivo* biodistribution studies. The [^{11}C]SZV 1287 was accumulated in the brain of NMRI mice and excreted through the liver. These studies provide preliminary details for the development of analgesic drug candidate SZV 1287.