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One-Step, No-Carrier-Added, Synthesis of a ^{18}F-Labelled Benzodiazepine Receptor Ligand

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SUMMARY

5-(2-Phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-fluorobenzoate, a non-classical benzodiazepine receptor ligand, has shown anticonvulsant activity against pentylenetetrazole-induced convulsion. In order to perform biological studies, we decided to label the compound with positron-emitting fluorine-18 ($t_{1/2}$=109.7 min). The latter compound was prepared in no-carrier-added form from [^{18}F]fluoride and 5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-*N,N,N*-trimethylanilinium triflate in one step. The best results were obtained using Kryptofix2.2.2/[^{18}F]fluoride with dimethylsulfoxide as the solvent at 90°C. Column chromatography afforded the desired compound in 15 min in an overall radiochemical yield of 70-75% corrected to the end of radionuclide production with a specific radioactivity of about 3000 Ci/mmol and a radiochemical purity of more than 95% and high chemical purity.

KEYWORDS: Fluorine-18, Benzodiazepines, Aromatic nucleophilic substitution

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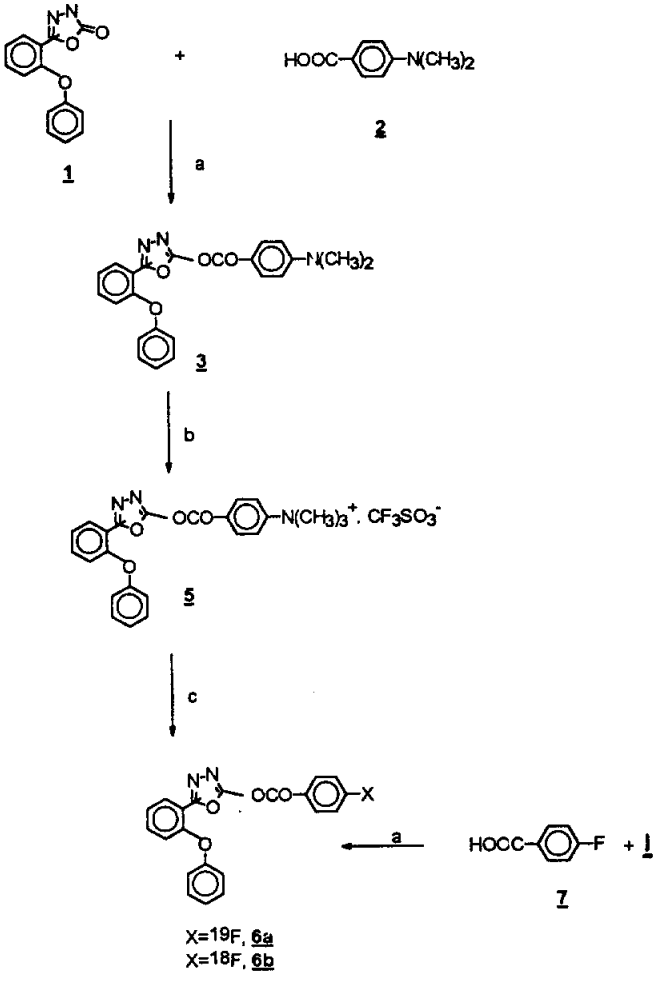
INTRODUCTION

Benzodiazepine receptor ligands have previously been labelled with fluorine-18 (β^+, $t_{1/2}$=109.7 min) and administered to humans for imaging with positron emission tomography (PET) (1). Certain radioiodinated ligands are selectively absorbed in brain and used in the evaluation of blood flow, determination of benzodiazepine receptor distribution (2) and diagnosis of neuorologic disturbances like Alzheimer's disease, epilepsy and psychosis (3). Positron-emitters have advantages over gamma-emitters with respect to the accuracy of PET images versus SPET (single photon emission tomography). Fluorine-18 is an attractive positron-emitter due to its low positron energy (0.64 MeV), maximum range in tissue (2.4 mm), useful half-life (110 min) and steric similarity to hydrogen atom (Van der Waals radus: 1.2 A° for H and 1.35 A° for F). The increasing interests in the application of PET has engaged many research groups in preparing different ^{18}F-labelled receptor ligands for administration to humans. Radioligands for dopamine and serotonin receptors are good examples (4). The structure-activity relationship of benzodiazepine receptor ligands has been studied for the last two decades (5). Non-rigid derivatives of 1,3,4-oxadiazoles have been synthesized and tested to clarify the role of rigidity in benzodiazepine ligands (6). Some heterocyclic compounds have been shown to have good affinity for benzodiazepine receptors. New derivatives of 2-[2-(2-substituted-phenoxy)phenyl]-1,3,4-oxadiazole have been synthesized recently. These ligands can be labelled by positron emitters in order to show their similarity in distribution in comparison with standard benzodiazepine specific ligands (e.g. diazepam). Conformational analysis and superimposition of the above compounds showed that they were well matched with benzodiazepine pharmacophores (7). Compound **6a** showed a rather significant anticonvulsant activity against pentylenetetrazole-induced lethal convulsion in mice (minimum anticonvulsant concentration: 265-270 μmol/kg, LD50>3000 μmol/kg). In respect to new advances in the preparation and biological activity of non-classical benzodiazepine ligands we decided to prepare a ^{18}F-labelled

compound of this group, [^{18}F]5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-fluorobenzoate **6b** through a simple and fast method.

Scheme-1:Synthesis of **6a** & **6b**:



a) CH_2Cl_2, DCC, RT
b) CH_2Cl_2, RT, **4**
c) Kryptofix$_{222}$/F^-, K_2CO_3, DMSO

EXPERIMENTAL

All the chemicals were purchased from Aldrich Chemical Co., UK. Pentylenetetrazole was purchased from Acros Chemica Co.(Belgium). ^{1}H-NMR spectra were obtained on a FT-80 (80 MHz) or a Varian (400 MHz) instrument with tetramethylsilane as the internal standard. Infrared spectra were taken on a Perkin-Elmer 781 instrument (KBr disks). Thin-layer chromatography of non-radioactive products were performed on silica gel polymer backed (F 1500/LS 254, 20 x 20 cm, TLC Ready Foils Schleicher & Schuell™) or glass plates (25 x 35 cm, E-Merck). Dimethyl sulfoxide (DMSO) and acetonitrile used for labelling experiments, were of Sure-Seal™ grade (Aldrich). Analytical HPLC was performed on a Shimadzu SPD-10A instrument, equipped with two detectors, a flow scintillation analyzer (Packard™ 150TR) and a UV-visible detector (Shimadzu) using a Si Kromasil 100 column (5 μm; 250 x 4.6 mm M&W, Inchrom™). Radio-TLC was performed by a rotary motor armed with a Canberra™ germanium detector (model GC1020-7500SL) using polymer-backed silica gel papers. Chemical purity was assessed by HPLC. The purification of **6b** was performed on C_{18} Sep-Pak™ (Waters). Melting points were determined on a Reichert-Jung Bruker hot-stage microscope and were uncorrected. Elemental microanalyses were within ±0.4% of theoretical values for C, H, F, N.

Preparation of no-carrier-added [^{18}F]potassium fluoride from [^{18}O]water

[^{18}F]Fluoride was prepared by 18 MeV proton bombardment of [^{18}O]water (1.7 ml) (^{18}O-enrichment>95%, Cortec™, France) held in an all-silver target in the 30 MeV cyclotron at Nuclear Research Center for Agriculture and Medicine (NRCAM). After recovery of [^{18}O]water over an anion exchange resin (Dowex™), fluoride-18 anion was eluted by a 1% potassium carbonate solution (1 ml). The eluted solution was used directly in the radiosynthesis.

Preparation of 5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-dimethyl-amino benzoate **3**.

A mixture of 4-dimethylaminobenzoic acid **2** (165 mg, 1 mmol), 3*H*-2-oxo-5-(2-phenoxyphenyl)-1,3,4-oxadiazole **1** (254 mg, 1 mmol) and dicyclohexylcarbodiimide (DCC) (206 mg, 1 mmol) in. dry dichloromethane (10 ml) was stirred vigorously for 4d. The mixture was filtered and the filterant was evaporated. The residue was crystallized from ethyl acetate-hexane mixture (85:15) to yield 280 mg (70%) of **3** as a white light powder: m.p. 171–174°C; ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.88–8.01 (m, 4H, aromatic), 6.97–7.13 (m, 5H, aromatic), 6.39–6.60 (m, 4H, aromatic), 3.05 (s, 6H, $N(CH_3)_2$). IR (KBr) ν_{max} (cm^{-1}) 1808 (s, C=O), 1594 (s, aromatic), 1289 (s, N-CH_3). MS: m/z (%) 401 (M^+, 65), 254 (80), 197 (18), 181 (100), 152 (20), 77 (10).

Preparation of 5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-fluorobenzoate **6a**

Compound **6a** was prepared in 65% yield according to the procedure for **3**, but with equimolar portions of acid **7**, compound **1** and DCC in dry dichloromethane and subsequent recrystallization from ethyl acetate-hexane. m.p. 105–108°C; ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.94–7.90 (m, 4H, aromatic), 7.75–7.69 (m, 2H, aromatic), 7.56–7.52 (m, 2H, aromatic), 7.24–7.20 (m, 5H, aromatic). IR (KBr) ν_{max} (cm^{-1}) 2987 (C-H), 1728 (s, C=O), 1618 (aromatic), 1243 (s, C-F), 1106 (s, C-O). MS: m/z 376 (M^+, 37), 254 (82), 197 (100), 152 (15), 120 (35).

Preparation of 5-(2-phenoxy)phenyl-1,3,4-oxaadiazole-2-yl-4-N,N,N-trimethylanilinium trifluoromethanesulfonate **5**.

Compound **5** was synthesized by treating **3** with methyl trifluoromethanesulfonate **4** in dry dichloromethane at room temperature (8). **4** (25 µl, 0.1 mmol) was added to a stirred solution of **3** (40 mg, 0.1 mmol) in dry dichloromethane (10 ml), in one portion. After stirring for 4 h, the mixture

was filtered and the solid was crystallized from dichloromethane-ether (60:40 v/v) to yield 48 mg (86%) of **5** as white fine crystals: m.p. 192-195°C. ^{1}H-NMR (DMSO) δ (ppm) 8.25-8.02 (m, 4H, aromatic) 6.94–7.93 (m, 9H, aromatic), 3.65 (s, 9H, $N(CH_3)_3$). IR (KBr) ν_{max} (cm^{-1}) 1827 (s, C=O), 1478 (s, $N(CH_3)_3$), 1281 (brs, CF_3SO_3), 1263(C-N). MS: m/z (%) 565 (M^+, 100), 391 (45), 332 (74), 254 (80), 197 (60), 149 (43), 120 (30), 77 (13).

Fluorination of 5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-trimethyl-anilinium triflate **5** *with potassium fluoride and Kryptofix 2.2.2 .*

To a vial containing potassium fluoride (40 mg, 0.69 mmol) in 0.5 ml of water and Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8] hexacosane) (200 mg, 0.54 mmole) in acetonitrile (1 ml), was added acetonitrile (5 ml). The mixture was stirred and then dried by argon flow. Another portion of acetonitrile (5 ml) was added and dried again. The vial was cooled and a solution of **5** (125 mg, 0.22 mmol) in DMSO (5 ml) was added to the dried mixture. The vial was capped and heated at 90°C for 0.5 h. After cooling, the mixture was mixed with water (25 ml) and passed through a C_{18} column and washed with diethyl ether (10 ml). The organic layer was dried over anhydrous sodium sulfate and purified by preparative silica gel thin layer chromatography on glass using a mixture of chloroform-ethyl acetate (90%-10%, v/v) as the mobile phase. The desired fluoro compound was separated (R_f = 0.45). The white crystalline product was identified as **6a** by comparison with an authentic sample prepared before.

Preparation of [^{18}F]5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-fluoro-benzoate **6b**.

[^{18}F]Fluoride (6 mCi) eluted from the ion exchange resin by potassium carbonate (1 mg, 7 μmol) solution (1% w/v, 100 μl). The solution was transferred to a 2 ml-vial containing Kryptofix 222 (10 mg, 0.027 mmol) and dry acetonotrile (0.5 ml). The mixture was evaporated by slight heat and argon flow. Drying was repeated after addition of two more portions (0.5 ml)

of dry acetonitrile. A mixture of 5 (5 mg, 0.01 mmol) in dried DMSO (0.25 ml) was added to the dried [^{18}F]fluoride and heated at 90°C for 10 min. The mixture was cooled in ice bath and rapidly drained into a syringe containing water (5 ml). The mixture was passed through a C_{18} Sep-Pak column. The column was washed with diethyl ether (1 ml) and the eluate passed through a Si Sep-Pak column. The specific radioactivity of **6b** was calculated using a standard curve of absorbance peak area (λ=540 nm) versus injected mass of **6a**. The column was eluted with a mixture of acetonitrile-chloroform (65:35 v/v) at 2 ml/min (R_t of **6a**=5.4 min). Radio-TLC and HPLC revealed the [^{18}F]labelled product **6b** with a radiochemical purity of greater than 95%. The chemical purity was assessed by HPLC (>96%).

Table-1: Purification methods data in **6b** production:

Purification method	**radiochemical yield (%) (±SD)**	**Radiochemical purity (%)**	**Impurities (%)**
None	**80±6 (n=5)**	**82**	**10-20**
C18 Sep-Pak	**65±8 (n=5)**	**>95**	**None**
HPLC	**68±10 (n=5)**	**>97**	**None**

RESULTS AND DISCUSSION

Significant anticonvulsant activity had been observed for **6a** against pentylenetetrazole-induced convulsion. This observation suggested an anticonvulsant activity mediated probably by benzodiazepine receptors, but did not afford more information about biodistribution, metabolism and receptor subtype of this ligand category. We aimed therefore to label **6a** with fluorine-18 to provide a tracer for biological experiments in vivo.

Because fluorine-18 has a half life of only 110 min, it is important that labelling methods for this nuclide are fast and high yielding and involve only a few chemical steps. Therefore we designed a method for labelling **6b** in one

step based on the displacement of an aryl trimethylammonium group by [^{18}F]fluoride as reported by Haka *et al.* (1989).

We achieved the successful synthesis of [^{18}F]5-(2-phenoxy)phenyl-1,3,4-oxadiazole-4-fluorobenzoate **6b** utilizing 5-(2-phenoxy)phenyl-1,3,4-oxadiazole-2-yl-4-*N,N,N*-trimethylanilinium trifluoromethane sulfonate **5** as the precursor. Reference **6a** was prepared in two ways: a) direct fluorination by addition of **5** (dissolved in DMSO) to an azeotropic dried mixture of Kryptofix 222 and potassium fluoride, and b) conjugation of 4-fluorobenzoic acid **7** and **1**. Separation of the **6a** was achieved by silica gel chromatography on glass layer. The product was characterized by IR, ^{1}H NMR, mass spectroscopy and elemental analysis.

The radiolabelled target molecule **6b** was prepared according to Scheme. In the first step, the related 4-dimethylaminobenzoate ester was prepared in high purity by DCC-mediated condensation of related acid **2** with **1**.

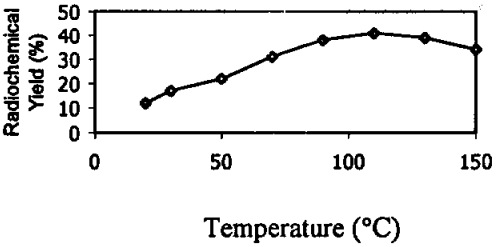
In order to obtain a good leaving group on the benzene ring, **3** was treated with methyl trifluoromethanesulfonate. Activated aryltrimethylammonium triflate salts had been shown to be the best substrates for nucleophilic aromatic substitution of fluoride anion in presence of reactive aminopoly ether, Kryptofix 222, in DMSO as solvent (8).The methylation reaction with methyl trifluoromethanesulfonate is tolerant of a wide range of substituents on the aromatic ring.

The labile 4-*N,N,N*-trimethylammonium triflate salt affords a very suitable leaving group, because of the non-nucleophilicity of the triflate anion in comparison with K222/^{18}F. The purification of labelled compound from the starting triflate salt was however performed easily by reversed phase C_{18} short column followed by separation on silica column in order to remove ionic impurities.

Total synthesis and purification of ^{18}F-compound took about 15 to 20 min with a yield of about 70 to 75% (decay corrected). Compound **5** has two active sites, which are susceptible to nucleophillic attack. Higher temperature and an excess amount of base lead to hydrolysis of ester functional group.

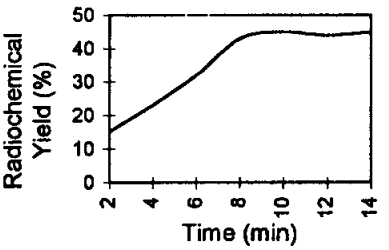
Thus, in order to obtain the best labelling conditions, we performed many experiments to optimize temperature, time, solvent and Kryptofix/base ratio. Heating the reaction mixture to 90°C, increased the yield and this remained constant for reactions up to 130°C. Further heating of reaction mixture reduced the yield of synthesis due to decomposition or ester cleavage of precursor and/or product (**Figure-1**).

Figure-1: Radiochemical yield of **6b** as a function of temperature in DMSO



At the optimum temperature reaction yield was maximal after 10 min and then stayed constant (**Figure-2**).

Figure-2:Radiochemical yield of **6b** as a function of time in 90°C:



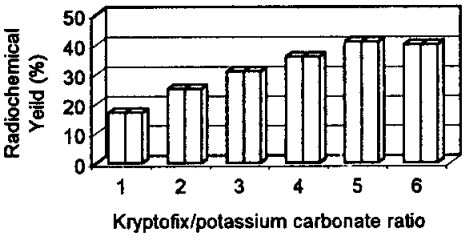
DMSO proved to be the best aprotic solvent used. On the other hand, tetrahydrofuran (THF) and dimethylacetamide (DMA) did not afford good results because of low solubility of the starting triflate salt **5** and Kryptofix222. Dimethylformamide (DMF) showed a moderate result (**Table-2**).

Table-2: Yield of **6b** in 0.25 ml of different solvents at 90°C:

Solvent	DMA	THF	DMSO	DMF
Radiochemical yield (%)	23	31	45	35

Increasing the ratio of Kryptofix222 to potassium carbonate increased the labelling yield by making [^{18}F]fluoride more available in solution (**Figure-3**).

Figure-3: Yield of **6b** as a function of Kryptofix222/K_2CO_3 molar ratio at 90°C:



A high specific radioactivity can be achieved via displacement of -NMe_3^+ by [^{18}F]fluoride ion (9). In the final compound, we did not observe other unlabelled products (aryl fluorides or trimethylammonium salts) upon TLC or HPLC analysis of the final preparations. [^{19}F]labelled compound prepared from **5** was examined by different chromatographic methods repeatedly and carefully and had shown a consistent final specific activity in excess of 2900–3100 Ci/mmole (limit of detection). The value was consistent with the use of high specific activity, n.c.a. [^{18}F]fluoride **6b**. Furthermore, it was indicated that there was little (if any) dilution of the specific activity by fluoride ion in the reagents or precursors, nor an exchange of the fluorines between the [^{18}F]fluoride ion and the trifluoromethyl group of the triflate counter ion.

This is an efficient and rapid synthetic route to prepare [^{18}F]5-[2-(2-substitutedphenoxy)phenyl]-1,3,4-oxadiazole-2-yl-4-fluorobenzoate which can be easily automated in order to synthesize higher radioactivities. The latter compounds were obtained in higher than 95% of radiochemical and chemical purity without application of HPLC.

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