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Radiosynthesis of novel ^{18}F-labelled derivatives of indiplon as potential GABA$_A$ receptor imaging tracers for PET

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The involvement of gamma amino butyric acid (GABA) receptors in a variety of neurological and psychiatric diseases has promoted the development and use of radiolabelled benzodiazepines (BZ) for brain imaging by PET. However, these radioligands are unable to distinguish between the various subtypes of GABA$_A$ receptors. Novel non-BZ such as the pyrazolo-pyrimidine indiplon proved to be selective for the α_1-subunit of the GABA$_A$ receptor. Here, we describe the syntheses of four novel ^{18}F-labelled indiplon derivatives. Radiosyntheses were performed via n.c.a. ^{18}F-nucleophilic substitution starting from the tosyl, bromo, and 4-nitrobenzoyl precursors to obtain fluorine substituted *N*-alkylamide side chain derivatives of indiplon, followed by multistep purification using semi-preparative high-performance liquid chromatography and solid phase extraction. Tosyl and bromo precursors were converted into ^{18}F-labelled indiplon derivatives with good and reproducible radiochemical yield (RCY) (35–70%, decay corrected), high radiochemical purity (⩾98.5%), and high specific activity (>150 GBq/μmol). By contrast, a low RCY (5–10%) and specific activity (10–15 GBq/μmol) were achieved for the 4-nitrobenzoyl precursor.

Keywords: GABA$_A$ receptor; ^{18}F-labelled indiplon derivatives; PET

Introduction

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the human brain and exerts its actions via ionotropic GABA$_A$ and GABA$_C$ receptors or metabotropic G-protein-coupled GABA$_B$ receptors.[1] Imbalances in the GABA-ergic system are involved in numerous neurodegenerative and psychiatric diseases such as epilepsy, anxiety, or insomnia. Therefore, drugs that target GABA$_A$ receptors, are used as therapeutics.[1,2] Benzodiazepines (BZ), which act as positive allosteric modulators at GABA$_A$ receptors, were introduced in the late 1950s and are still used as the treatment of choice for insomnia.[1,3] The specific BZ binding site consists of a variable α-subunit and the γ_2-subunit of the pentameric GABA$_A$ receptor. Therefore, 'classical' BZ (e.g. flunitrazepam, diazepam) do not discriminate between the various α-subunits.[1,3] Nevertheless, they were used as lead structures for the development of radioligands for brain imaging of neuropsychiatric diseases with PET or SPECT.[4,5] Hence, the clinically used PET and SPECT tracers [^{11}C]flumazenil,[6] [^{18}F]fluoroethylflumazenil,[7,8] [^{123}I]iomazenil,[9] and other related compounds such as [^{11}C]suriclone, [^{11}C]iomazenil, [^{18}F]fluoroflumazenil, [^{11}C]flunitrazepam, [^{11}C]fludiazepam, [^{11}C]Ro 154513, and [^{18}F]oxoquazepam (as previously reviewed[10–12]) also lack selectivity, which is a drawback for the investigation of subtype-related brain functions and diseases. Today, novel non-BZ with higher subtype selectivity are available,[3] among them the pyrazolo-pyrimidine indiplon (*N*-methyl-*N*-[3-[3-(2-thienylcarbonyl)-pyrazolo[1,5-*a*] pyrimidin-7-yl]phenyl]acetamide),[13] which is currently approved for the treatment of insomnia. Indiplon also acts as an allosteric agonist at the BZ binding site, but demonstrates pharmacological selectivity via high affinity to α_1-containing GABA$_A$ receptors.[14] It has been shown by radioligand binding studies on rat cerebellar and cerebral cortex membranes that [^{3}H]indiplon has a higher affinity compared with other related non-BZ such as zolpidem or zaleplon.[15] A first attempt of developing [^{11}C]zolpidem as PET radiotracer failed,[16] most probably due to low affinity *in vivo* and rapid, unfavourable metabolism.

To test the suitability of indiplon as a lead structure for α_1-subtype selective PET radiotracers, we have started with the development of fluorinated derivatives.[17,18]

In this article, we describe the radiosynthesis of four novel ^{18}F-labelled indiplon derivatives.

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Results and discussion

Based on the chemical structure of indiplon (Figure 1), novel derivatives with fluorine substituted *N*-alkylamide side chain **1b**–**5b** were synthesized (Table 1). Prior to considering specific ^{18}F-labelling procedures for the synthesis of radiotracers for PET applications, biological binding studies with compounds **1b**–**5b** were made to select appropriate candidates.[18] Their affinities (IC_{50} between 2.8 and 18 nM) are comparable to indiplon (IC_{50} = 3.3 nM).

The preparation of the precursors **1a**–**5a** focused on compounds suitable for one-step labelling to achieve high radiochemical yields (RCY) and to afford a simple separation of [^{18}F]**1b**–**5b**.

Non-radioactive syntheses

*Synthesis of **2a–3a**, **5a** and **1b–3b**, **5b***

In a first attempt we synthesized demethyl-indiplon (Figure 1) and used this molecule for further derivatizations. The latter was hampered by the low reactivity and the poor yields for the alkylation of the amide group. Thus, the introduction of the fluoroalkyl and hydroxyalkyl groups had to be performed at an earlier stage of the synthesis. Hence, several enaminones **II** were synthesized. Condensation of **II** with aminopyrazole **I** yielded the fluoroalkyl and hydroxyalkyl derivatives **III** of indiplon under acidic conditions. Finally, the hydroxyalkyl derivatives **IIIa** were converted to the corresponding tosylate precursors **IV** (Figure 2).[18]

The 4-nitro- and 4-fluorophenyl compounds **5a** and **5b** (with $R_1 = CH_3$, cf. Table 1) were obtained in a similar way. The detailed synthesis strategy is described elsewhere.[18]

*Synthesis of **4a**, **4b***

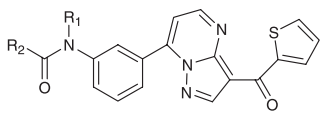
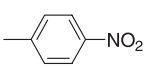
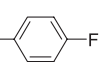
A second approach focused on a fluorinated derivative by modifying the acetyl group of indiplon. Such a strategy would allow a one-step radiolabelling process starting from the corresponding bromo precursor. The synthesis of the fluorinated reference compound **4b** and the bromo derivative **4a** is shown in Figure 3. Indiplon (**VI**) was prepared following a synthetic route described in the literature.[19] Both synthetic building blocks **I** and **V**, available via two convergent syntheses starting from 3-aminoacetophenone and 2-acetyl-thiophene, respectively, were assembled to obtain compound **VI** (Figure 3). The acetyl group of indiplon (**VI**) was removed under acidic conditions to give deacetyl-indiplon (**VII**). The latter compound served as the starting material for the synthesis of the fluoroacetamide **4b** and the bromoacetamide **4a** by applying a standard acylation under *Schotten– Baumann* conditions.

Radiosyntheses

For n.c.a. nucleophilic substitution, ^{18}F was transformed into the K[^{18}F]F-K222-carbonate or the K[^{18}F]F-18-crown-6-carbonate complex using a standard procedure.[20,21] A 2.3:1 molar ratio of K222 (or 18-crown-6) to potassium carbonate proved to be optimal. The nucleophilic insertion of ^{18}F is shown in Figure 4.

Figure 1. Indiplon *N*-methyl-*N*-[3-[3-(2-thienylcarbonyl)-pyrazolo[1,5-*a*]pyrimidin-7-yl]phenyl]acetamide, (left) and demethyl-indiplon, *N*-[3-[3-(2-thienylcarbonyl)-pyrazolo[1,5-*a*]pyrimidin-7-yl]phenyl]acetamide (right).

Table 1. Fluorine derivatives of indiplon and precursors for ^{18}F-labelling

		Precursor		Fluorinated compound
R_1	**R_2**		**R_1, R_2**	
$-CH_3$	$-CH_3$	Indiplon		
$-(CH_2)_2OTos$	$-CH_3$	**1a**	R_1: $-(CH_2)_2F$	**1b**
$-(CH_2)_3OTos$	$-CH_3$	**2a**	R_1: $-(CH_2)_3F$	**2b**
$-(CH_2)_4OTos$	$-CH_3$	**3a**	R_1: $-(CH_2)_4F$	**3b**
$-CH_3$	$-CH_2Br$	**4a**	R_2: $-CH_2F$	**4b**
$-CH_3$		**5a**	R_2: 	**5b**

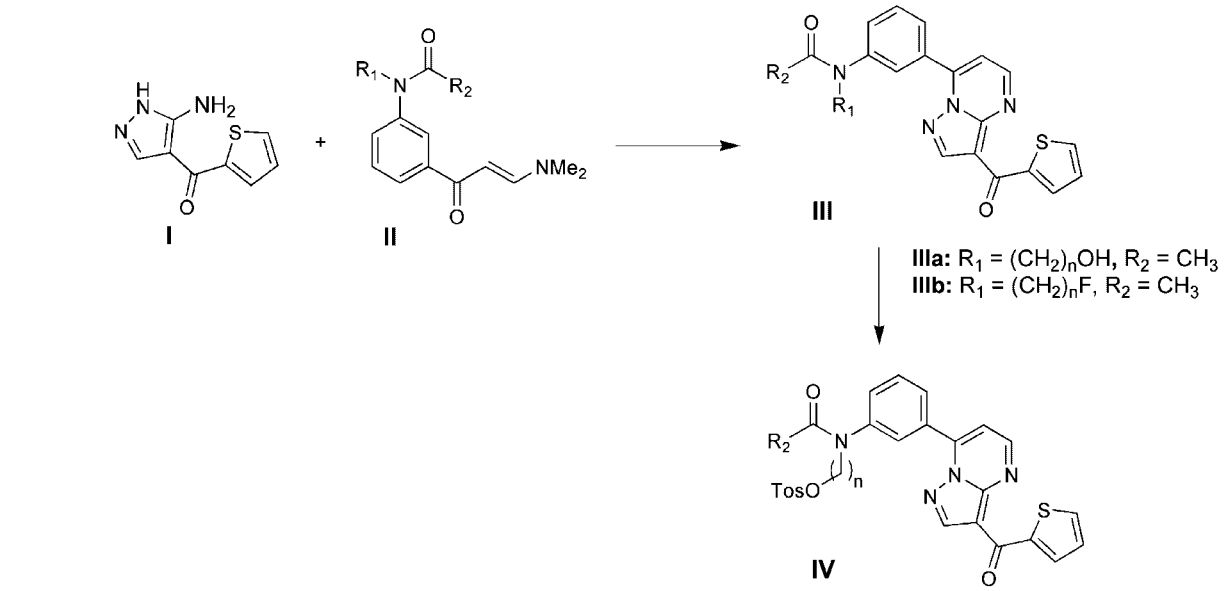


Figure 2. Strategy for the synthesis of the compounds **1b–3b** and the corresponding tosylate precursors **1a–3a** for ^{18}F-labelling.

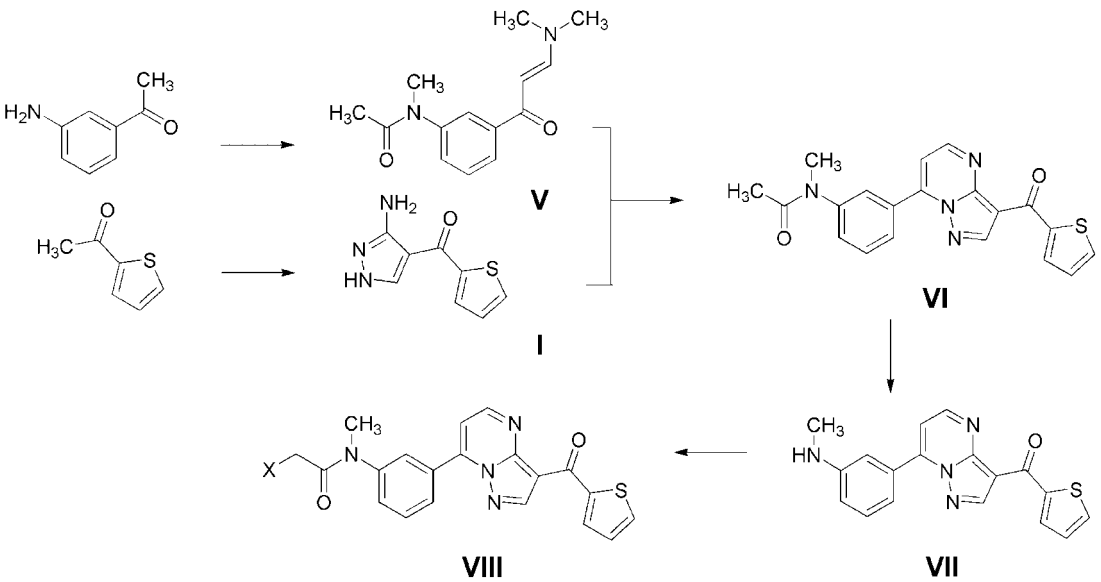


Figure 3. General scheme of synthesis of the compound **4b** (X = F) and the precursor **4a** (X = Br) for ^{18}F-labelling.

*Syntheses of [^{18}F]**2b–3b** via tosyl precursors **2a–3a***

Because an O-tosyl function is a good leaving group for the nucleophilic aliphatic exchange with ^{18}F,[22] the precursors **1a**–**3a** could readily be converted into the labelled products [^{18}F]**1b**–**3b**. However, the precursors themselves have only a limited stability. Depending on the length of the alkyl chain, precursor molecules gradually decomposed over months, even if stored in a deep freezer. This is probably due to the elimination of toluenesulphonic acid as indicated by analytical data (^{1}H-NMR, ESI-MS). Compound **1a** was quite unstable and decomposed rapidly within a few days. Therefore, a reasonable and reproducible radiosynthesis for [^{18}F]**1b** could not be established. For [^{18}F]**2b** (Figure 5a, 5b) and [^{18}F]**3b**, the optimum preparation conditions were achieved in dimethyl formamide (DMF) (approx. 140°C, 10 min) with reproducible labelling efficiencies of 55–65 and 56–75%, respectively. Compared with the propyl derivatives **2a** and **2b**, precursor **3a** and radiotracer [^{18}F]**3b** have a higher thermal stability; therefore, allowing the application of higher temperatures at reduced reaction time and precursor amounts. Labelling yields of [^{18}F]**2b** and [^{18}F]**3b** were lower in dimethyl sulphoxide (DMSO) than in DMF (Figure 5a). Almost no reaction was observed in acetonitrile (MeCN) and acetone. Thin-layer chromatography (TLC) analyses of the crude labelling product displayed a series of by-products and a highly intensive blue fluorescence for both reference substances **2b**, **3b** and the precursors **2a**, **3a**.

The separation of the remaining [^{18}F]fluoride from the reaction mixtures by using an anion exchanger Acell Plus

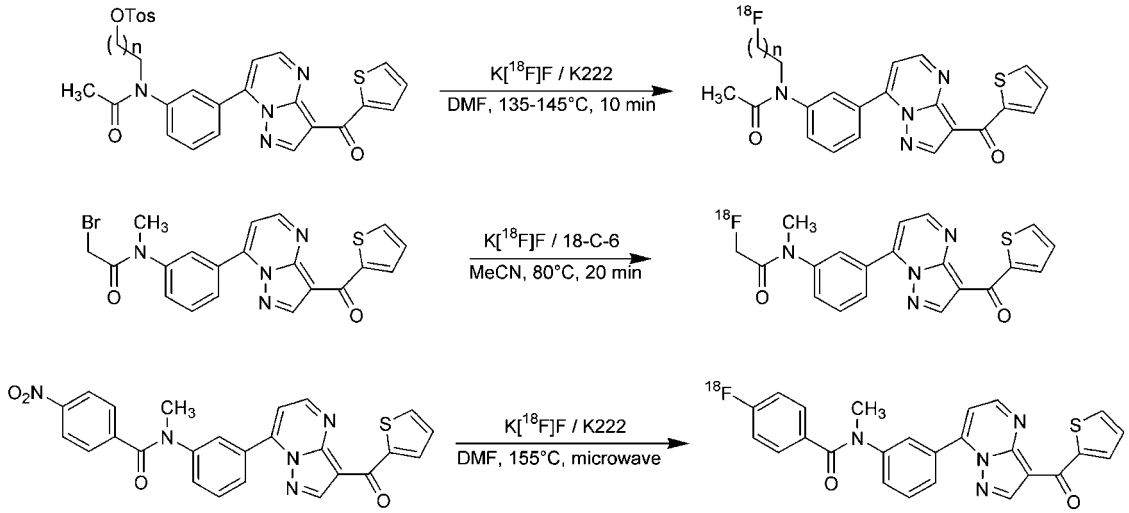


Figure 4. Radiosyntheses of ^{18}F-labelled indiplon derivatives **1b–5b**.

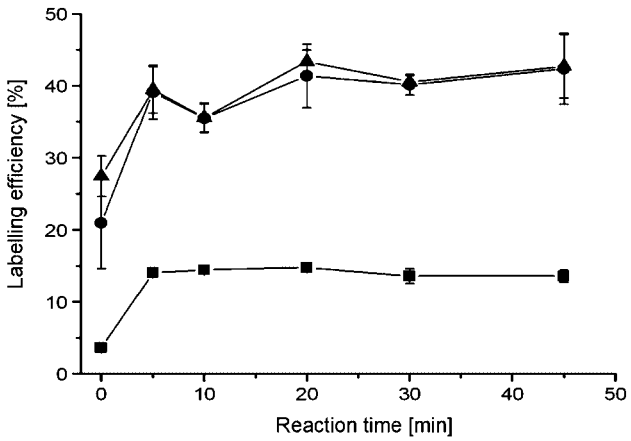


Figure 5a. Labelling efficiency of [^{18}F]**2b** over time in dependence on solvent and precursor concentration **2a** (approx. 140°C). ■ 1 mg/0.5 ml DMSO, ● 1 mg/0.5 ml DMF, ▲ 2.4 mg/0.5 ml DMF, $n = 5–7$ for each point.

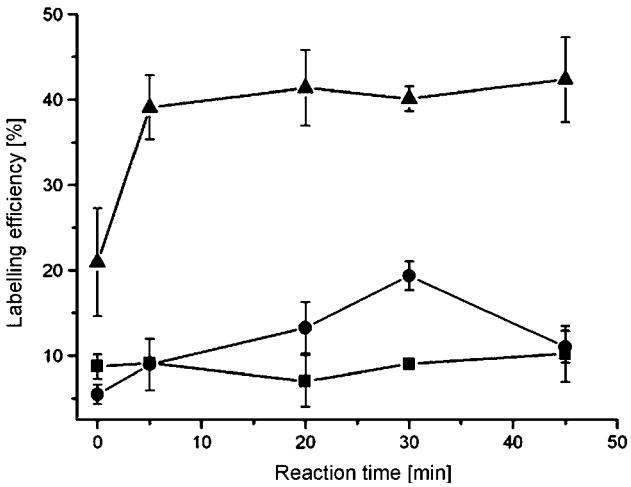


Figure 5b. Labelling efficiency of [^{18}F]**2b** over time in dependence on reaction temperature (1 mg precursor **2a** in DMF). ■ 80°C, ● 110°C, ▲ 140°C, $n = 3–5$.

QMA, or PS-HCO_3, or Sep-Pak Alumina N Plus cartridges was accompanied by a considerable loss (about 12–28%) of the final products. On the other hand, the adsorption of [^{18}F]**2b** and

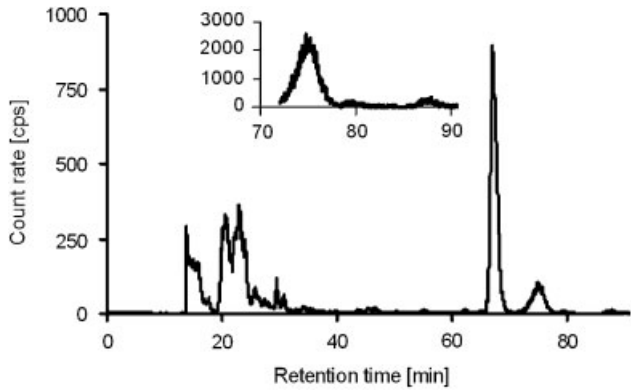


Figure 6. Semi-preparative HPLC separation of crude ^{18}F-labelling product [^{18}F]**2b**. The final product elutes at ~63 min; inset: [^{18}F]**2b** is well separated from impurities (25-fold magnification of the *y*-axis). To obtain [^{18}F]**2b** free from any impurities, a high retention time has to be accepted (see text).

[^{18}F]**3b** on solid phase extraction (SPE), for example, an RP-18 cartridge, was incomplete due to their hydrophilic properties and was associated with the enrichment of by-products. Hence, the mixture was directly purified via semi-preparative radio-high-performance liquid chromatography (radio-HPLC) using an RP column with MeCN/water mixtures including ammonium acetate as eluent. By means of isocratic radio-HPLC, a very good separation of [^{18}F]**2b** and **3b** was obtained (Figure 6). However, further improvement of this method is needed to reduce the retention time. Figure 7 shows that high radiochemical and chemical purity of [^{18}F]**2b** (after solvent change (EtOH) with SPE) was achieved as analysed by HPLC.

It has been demonstrated that the syntheses can be transferred into a commercially available automated synthesis module (see Experimental).

*Synthesis of [^{18}F]**4b** via bromo precursor **4a***

Because both compounds, [^{18}F]**4b** and its bromo precursor **4a** (Figure 4), are alkali sensitive, the phase transfer catalyst (PTC) Kryptofix K222 was replaced by 18-crown-6.[23] A change of the carbonate was not necessary. The nucleophilic ^{18}F substitution succeeded in MeCN at 80°C with good labelling yields (50–60%). Higher temperatures and application of other solvents led to

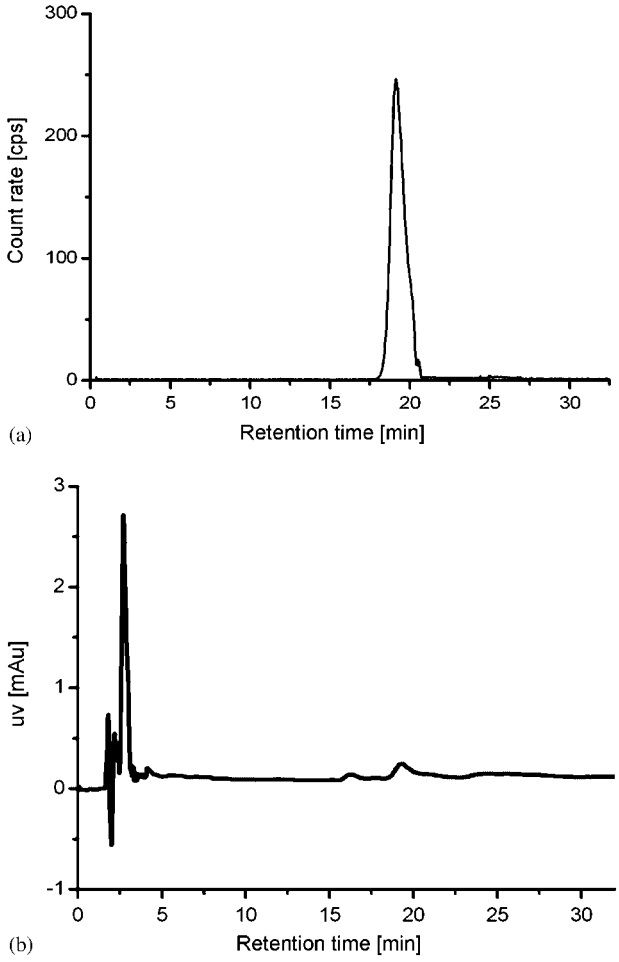


Figure 7. Radiochemical and chemical purity of [^{18}F]**2b**, suitable for animal experiments: (a) radio-chromatogram and (b) UV (228 nm) (isocratic HPLC, 35% MeCN+20 mM ammonium acetate).

both decreased labelling efficiency and decomposition of [^{18}F]**4b** due to the limited chemical stability of the precursor molecules. Purification of the crude reaction mixture on Sep-Pak Alumina N and C18 Plus cartridges permitted the removal of radioactive impurities without significant loss of labelled radiotracer. Nonetheless, non-radioactive by-products were simultaneously enriched. Therefore, processing of [^{18}F]**4b** was performed with an isocratic semi-preparative HPLC (Figure 8) as described above.

*Synthesis of [^{18}F]**5b** via nitro precursor **5a***

For the preparation of the ^{18}F-labelled benzoyl derivative **5b**, the easily accessible nitro precursor **5a** (Figure 4) was used. The nucleophilic aromatic substitution of the nitro group by ^{18}F is difficult to achieve by conventional thermal heating.[24,25] Here, the microwave-assisted synthesis in DMF under defined reaction conditions (140–150 W, 155°C, 12 min) afforded [^{18}F]**5b** in low yields of up to 14%. Numerous radioactive products were found under such harsh conditions making a time-consuming purification of the reaction mixture necessary (Figure 9). A decrease in the labelling yield with increasing reaction time was observed. HPLC analyses show an increasing

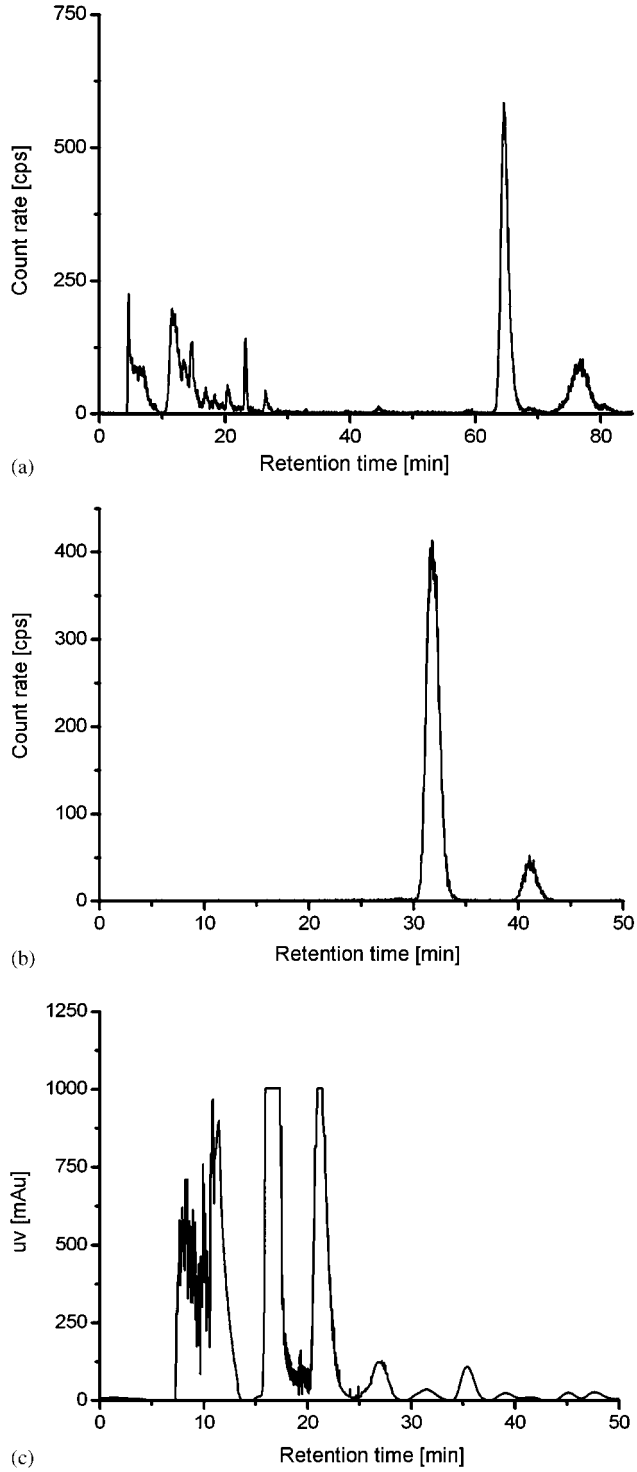


Figure 8. Semi-preparative HPLC separation of [^{18}F]**4b**. Radio-chromatogram (a) of crude ^{18}F-labelling mixture without SPE purification, final product elutes at ~65 min, and after SPE purification with Sep-Pak Alumina N/C18 Plus cartridges: radio-chromatogram (b), corresponding UV chromatogram (c).

content of more lipophilic components with low retention time. One of them is consistent with [^{18}F]fluoride (proved by [^{18}F]fluoride spike) and therefore, a strong evidence for the defluorination process of [^{18}F]**5b**. The application of solvents with better dielectric properties (e.g. DMSO, ethylene glycol) and an increase in reaction temperature did not result in higher

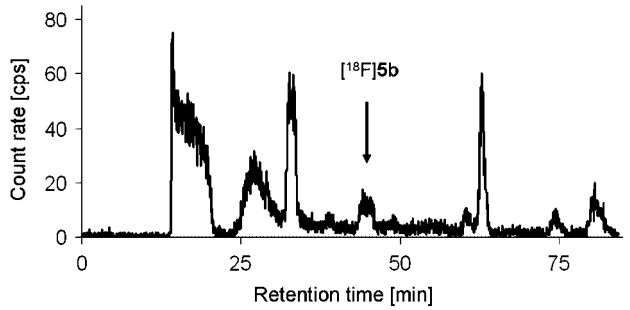


Figure 9. Semi-preparative HPLC separation of crude [^{18}F]**5b**. The chromatogram displays numerous major radioactive by-products. [^{18}F]**5b** appears at 45.5–47.5 min.

yields. One of the major decomposition products, deacetyl-indiplon, resulting from cleavage of the nitro-benzoyl moiety, could be identified by electrospray ionization (ESI-MS) and was confirmed by ^{1}H-NMR.

Stability

The stability of [^{18}F]**2b**–**5b** was tested in 0.9% NaCl, phosphate-buffered saline, and inactivated plasma at 37°C for up to 5 h. According to TLC and analytical HPLC, [^{18}F]**2b**–**5b** proved to be very stable (impurities $<$1.2%). No decomposition or defluorination was observed.

Experimental

General

Solvents were purchased from Merck and Fisher Scientific. Chemicals were obtained from Merck, Fisher Scientific, Johnson Matthey, and Sigma-Aldrich. All chemical reagents were of highest commercially available quality and applied without further purification.

TLC was performed on silica gel precoated plates (Polygram® SIL G/UV$_{254}$), and spots were visualized under UV light (254, 366 nm).

^{1}H- and ^{19}F-NMR spectra were recorded with a Varian Gemini 300BB, Bruker DRX-400 or Bruker AV500 Ultra Shielded spectrometer. Chemical shifts are reported as δ values. Coupling constants are denoted in Hertz.

Elemental analyses were performed with a vario EL analyser (Elementar Analysensysteme, Germany).

Low-resolution mass spectra were recorded on a Mariner Biospectrometry Workstation (Applied Biosystems) using ESI.

Aqueous [^{18}F]fluoride was generated on a PETtrace® 16.5 MeV cyclotron (GE Healthcare-Siemens, Germany) and used directly or after recovery on an anionic exchange resin.[26]

^{18}F-labelling was performed in 5 mL silicon-coated glass vials sealed by a rubber septum under controlled Ar atmosphere.

Microwave-assisted radiosyntheses were done in a closed standard 10 mL vial using a research microwave reactor equipment Discover® (CEM, Germany).

First experiments towards an automated manufacturing were performed using a slightly modified TraceLab FxN® synthesis module (GE Healthcare-Nuclear Interface, Germany).

For SPE, Sep-Pak® cartridges Plus (Waters, USA), and alternatively Chromafix® cartridges (MACHEREY-NAGEL, Germany), were used.

The crude labelling product was separated on a semi-preparative radio-HPLC consisting of pump S1021 (SYKAM Chromatographie, Germany), UV detector (WellChrom K-2001; KNAUER, Germany), NaI(Tl)-counter, and data acquisition by Nina Chromatografix system (Nuclear Interface, Germany) using Kromasil 100-5 C18 (20 × 4 mm i.d.) and Kromasil 100-5 C18 (250 × 8 mm) columns with a particle size of 5 μm; solvent: various mixtures of MeCN/water with 20 (40) mmol ammonium acetate and a flow gradient from 0.75 to 1.75 mL/min.

Radioluminescence thin-layer chromatograms were recorded using a BAS-1800 II system Bioimaging Analyzer (Fuji Film, Japan) and images were evaluated with AIDA 2.31 software (raytest).

Analytical radio-HPLC was performed on a Merck-Hitachi LaChrome device (Merck, Darmstadt) with a binary pump, UV detector, and a GINA Radio-Chromato-Graphik-System (raytest); column: Multisorb RP 18-5 (250 × 4 mm), particle size 5 μm; solvent: various mixtures of MeCN and water with 20 mM ammonium acetate and a flow rate of 1.0 mL/min. Chromatographic data (UV absorption at wavelengths 228, 254, 220 nm, radioactivity in cps) were recorded using a D-7000 HPLC System Manager version 3.1 (Merck, Hitachi).

Chemical syntheses

*N-(2-Fluoro-ethyl)-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidin-7-yl]-phenyl}-acetamide (**1b**)*

The synthesis of **1b** was carried out as described in detail for **2b**. Yield: 44% of a yellow solid. m.p. = 179–180°C. TLC: R_f (EtOAc/ CH_2Cl_2/ MeOH 10:10:1) = 0.39. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.02 (s, 3H, CH_3), 4.05 (td, 1H, J_{3F} = 26 Hz, J = 4.8 Hz, N-CH_2), 4.69 (td, 2H, J_{2F} = 47 Hz, J = 4.7 Hz, CH_2F), 7.16 (d, 1H, J = 4.4 Hz, H_{Ar}), 7.21 (m, 1H, H_{Ar}), 7.52 (m, 1H, H_{Ar}), 7.66–7.73 (m, 2H, H_{Ar}), 8.03 (m, 2H, H_{Ar}), 8.10 (m, 1H, H_{Ar}), 8.72 (s, 1H, H_{Ar}), 8.84 (d, 1H, J = 4.4 Hz, H_{Ar}). ^{19}F-NMR ($CDCl_3$, 470 MHz) δ −222.24 (1F). Anal. ($C_{21}H_{17}FN_4O_2S$): Calculated C, 61.75; H, 4.20; N, 13.72; S, 7.85. Found C, 61.49; H, 4.06; N, 13.50; S, 8.12.

*N-{3-[3-(Thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-phenyl}-N-[4-(toluene-4-sulphonyloxy)-propyl]-acetamide (**2a**)*

N-(3-Hydroxy-propyl)-*N*-{3-[3-(thiophene-2-carbonyl)-pyrazolo [1,5-a]-pyrimidin-7-yl]-phenyl}-acetamide[18] (1 g, 2.4 mmol) and pyridine (0.57 mL, 7.1 mmol) were dissolved in 100 mL abs. CH_2Cl_2 under argon atmosphere, and the mixture was cooled in an ice bath. Toluene sulphonic acid anhydride (1.0 g, 3.1 mmol) was added, and stirring was continued for 30 min. The ice bath was removed and stirring was continued for 45 min. The mixture was washed with 100 mL 1 N hydrochloric acid solution, half-saturated $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4 and the solvent was evaporated (bath temperature $<$30°C). The product was purified by flash chromatography (silica gel, EtOAc). After evaporation of the solvent, the product was washed with diethyl ether and pentane, and dried in high vacuum. Yield: 0.88 g (65%) of a yellow, temperature-sensitive solid (handle $<$25°C, storage at −20°C). m.p. = 123–127°C (decomp.). TLC: R_f (EtOAc) = 0.40. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.92–2.05 (m, 5H, CH_2, CH_3), 2.43 (s, 3H, CH_3), 3.83 (m, 2H, CH_2), 4.11 (t, 2H, J = 6.3 Hz, CH_2), 7.22 (m, 2H, H_{Ar}), 7.32 (d, 1H, J = 8.1 Hz, H_{Ar}), 7.42 (m, 1H, H_{Ar}), 7.66–7.76 (m, 4H, H_{Ar}), 7.96–8.06 (m, 2H, H_{Ar}), 8.12 (m, 1H, H_{Ar}), 8.73 (s, 1H, H_{Ar}), 8.86 (d, 1H, J = 4.4 Hz, H_{Ar}). Anal. ($C_{29}H_{26}N_4O_5S_2$): Calculated C, 60.61; H, 4.56; N, 9.75; S,

11.16. Found C, 60.28; H, 4.57; N, 9.76; S, 11.28. LRMS (ESI pos.) *m/z* 575.16 $[M+H]^+$.

(N-(3-Fluoro-propyl)-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidin-7-yl]-phenyl}-acetamide) (**2b**)

N-[3-(3-Dimethylamino-acryloyl)-phenyl]-*N*-(3-fluoro-propyl)-acetamide (800 mg, 2.74 mmol) and (5-amino-1H-pyrazol-4-yl)-thiophen-2-yl-methanone (530 mg, 2.74 mmol) were suspended in 20 mL acetic acid, and the suspension was heated to 65°C for 1.5 h. After cooling to room temperature, the mixture was added to ice–water and the pH value was adjusted to 8 with $NaHCO_3$. The mixture was extracted three times with CH_2Cl_2. The combined organic phases were dried with Na_2SO_4, and the solvent was evaporated. Purification of the product was achieved by flash chromatography (silica gel, EtOAc) to yield 535 mg (46%) of a yellow solid. m.p. = 194–195°C. TLC: R_f (EtOAc) = 0.45. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.95–2.09 (m, 5H, CH_2, CH_3), 3.92 (t, 2H, J = 7.3 Hz, N-CH_2), 4.53 (dt, 2H, J_{2F} = 47 Hz, J = 5.8 Hz, CH_2F), 7.17 (d, 1H, J = 4.4 Hz, H_{Ar}), 7.21 (m, 1H, H_{Ar}), 7.47 (m, 1H, H_{Ar}), 7.67–7.73 (m, 2H, H_{Ar}), 8.00 (m, 2H, H_{Ar}), 8.10 (m, 1H, H_{Ar}), 8.72 (s, 1H, H_{Ar}), 8.84 (d, 1H, J = 4.4 Hz, H_{Ar}). ^{19}F-NMR ($CDCl_3$, 470 MHz) δ −219,99 (1F). Anal. ($C_{22}H_{19}FN_4O_2S$): Calculated C, 62.54; H, 4.53; N, 13.26; S, 7.59. Found C, 62.42; H, 4.57; N, 13.15; S, 8.09. LRMS (ESI pos.) *m/z* 423.12 $[M+H]^+$.

N-{3-[3-(Thiophene-2-carbonyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-phenyl}-N-[4-(toluene-4-sulphonyloxy)-butyl]-acetamide (**3a**)

The synthesis of **3a** was performed in analogy with **2a**. Yield 55%; yellow, temperature-sensitive solid (handle <25°C, storage −20°C), m.p. = 83–85°C (decomp.). TLC: R_f (EtOAc) = 0.28. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.61–1.76 (m, 4H, CH_2), 1.95 (m, 3H, CH_3), 2.43 (s, 3H, CH_3), 3.78 (t, 2H, J = 6.9 Hz, CH_2), 4.04 (t, 2H, J = 5.9 Hz, CH_2), 7.22 (m, 2H, H_{Ar}), 7.31 (d, 1H, J = 8.1 Hz, H_{Ar}), 7.42 (m, 1H, H_{Ar}), 7.67–7.76 (m, 4H, H_{Ar}), 7.95–8.04 (m, 2H, H_{Ar}), 8.12 (m, 1H, H_{Ar}), 8.74 (s, 1H, H_{Ar}), 8.86 (d, 1H, J = 4.4 Hz, H_{Ar}). Anal. ($C_{30}H_{28}N_4O_5S_2$): Calculated C, 61.21; H, 4.79; N, 9.52; S, 10.89. Found C, 60.54; H, 4.82; N, 9.29; S, 10.84. LRMS (ESI pos.) *m/z* 589.14 $[M+H]^+$.

(N-(4-)Fluoro-butyl)-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidin-7-yl]-phenyl}-acetamide) (**3b**)

The synthesis of **3b** was performed according to **2b**. Yield 47%, yellow solid, m.p. = 151–152°C, TLC: R_f (EtOAc) = 0.26. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.68–1.81 (m, 4H, CH_2), 1.97 (s, 3H, CH_3), 3.84 (t, 2H, J = 7.1 Hz, N-CH_2), 4.47 (dt, 2H, J_{2F} = 41.5 Hz, J = 5.7 Hz, CH_2F), 7.18 (d, 1H, J = 4.3 Hz, H_{Ar}), 7.23 (m, 1H, H_{Ar}), 7.45 (m, 1H, H_{Ar}), 7.66–7.73 (m, 2H, H_{Ar}), 7.99–8.03 (m, 2H, H_{Ar}), 8.11 (m, 1H, H_{Ar}), 8.73 (s, 1H, H_{Ar}), 8.87 (d, 1H, J = 4.3 Hz, H_{Ar}). ^{19}F-NMR ($CDCl_3$, 470 MHz) δ −218.57 (1F). Anal. ($C_{23}H_{21}FN_4O_2S$): Calculated C, 63.29; H, 4.85; N, 12.84; S, 7.35. Found C, 63.17; H, 4.87; N, 12.68; S, 7.82. LRMS (ESI pos.) *m/z* 437.13 $[M+H]^+$.

2-Bromo-N-methyl-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidin-7-yl]-phenyl}-acetamide (**4a**)

Bromoacetylbromide (66 mg, 28.5 µl, 0.325 mmol) was reacted with VII (100 mg, 0.3 mmol) according to the procedure described for **4b** to give a yellowish solid (125 mg) after aqueous work-up and trituration with MeOH. The solid was further purified by flash chromatography (EtOAc) to afford **4a** (112 mg, 82%) as pale yellow powder. m.p. = 160–165°C. TLC: R_f (EtOAc) = 0.39. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.39 (s, 3H, NCH_3), 3.80 (s, 2H, –N(CO)CH_2Br), 7.18 (d, J = 3.9 Hz, 1H_{Ar}), 7.20 (d, J = 3.9 Hz, 1H_{Ar}), 7.56 (d, J = 7.8 Hz, 1H_{Ar}), 7.68–7.73 (m, 2H_{Ar}), 8.00–8.10 (m, 2H_{Ar}), 8.09 (dd, J = 3.9, 1.6 Hz, 1H_{Ar}), 8.71 (s, 1H_{Ar}), 8.83 (d, J = 4.7 Hz, 1H_{Ar}). Anal. ($C_{20}H_{15}BrN_4O_2S$): Calculated C, 52.76; H, 3.32; N, 12.30; S, 7.04. Found C, 52.86; H, 3.20; N, 12.35; S, 7.29. LRMS (ESI pos.) *m/z* 455.00 $[M+H]^+$.

2-Fluoro-N-methyl-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidin-7-yl]-phenyl}-acetamide (**4b**, *representative procedure*)

Indiplon (**VI**) and deacetyl-indiplon (**VII**) were synthesized utilizing the methods reported.[18,19]

Fluoroacetylchloride (10 mg, 7.2 µL, 0.103 mmol) in CH_2Cl_2 (1.5 mL) was added in the course of 20 min to a stirred mixture of **VII** (30 mg, 0.09 mmol) in CH_2Cl_2 (3.5 mL) and an aqueous solution of $NaHCO_3$ (0.35 M, 3 mL) at 0–2°C. The mixture was warmed to ambient temperature, and after being stirred for 3 h at 20°C, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 3 mL). The combined organic phases were dried (Na_2SO_4), filtered, and evaporated to leave a yellowish film (39 mg). This was triturated with MeOH (1 mL) to yield **4b** (26 mg, 73%) as pale yellow powder, m.p. = 171–173°C. TLC: R_f (EtOAc) = 0.27. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.39 (s, 3H, NCH_3), 4.76, 4.92 (2s [rotamers], 2H, N(CO)CH_2F), 7.18 (d, J = 4.4 Hz, 1H_{Ar}), 7.21 (dd, J = 4.9, 3.8 Hz, 1H_{Ar}), 7.49 (d, J = 7.1 Hz, 1H_{Ar}), 7.67–7.75 (m, 2H_{Ar}), 8.00 (d, J = 8.2 Hz, 1H_{Ar}), 8.05 (dd, J = 2.2, 1.6 Hz, 1H_{Ar}), 8.08 (dd, J = 3.8, 1.1 Hz, 1H_{Ar}), 8.71 (s, 1H_{Ar}), 8.84 (d, J = 4.4 Hz, 1H_{Ar}). ^{19}F-NMR ($CDCl_3$, 282 MHz) δ −47.46 (t, J = 48.8 Hz, 1F). Anal. ($C_{20}H_{15}FN_4O_2S$): Calculated C, 60.90; H, 3.83; N, 14.20; S, 8.13. Found C, 60.65; H, 3.80; N, 14.10; S, 8.36. LRMS (ESI pos.) *m/z* 395.08 $[M+H]^+$.

(4-Nitro-N-methyl-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidin-7-yl]-phenyl}-benzamide) (**5a**)

A mixture of (E)-*N*-(3-(3-(dimethylamino)acryloyl)phenyl)-*N*-methyl-4-nitrobenzamide (915 mg, 2.59 mmol) and (5-amino-1H-pyrazol-4-yl)-thiophen-2-yl-methanone (500 mg, 2.59 mmol) was refluxed in 10 mL acetic acid for 4 h. After cooling, the mixture was poured into ice–water, neutralized with $NaHCO_3$ solution (pH 8), and extracted three times with CH_2Cl_2. The combined organic phases were dried, evaporated, and purified by column chromatography (silica gel; EtOAc/petroleum ether 4:1) to give 989 mg (89%) **5a** as yellow solid, R_f (silica gel, EtOAc/petroleum ether 4:1) = 0.28. m.p. = 160.5–162.5°C. TLC: R_f (EtOAc) = 0.52. ^{1}H-NMR (DMSO-d6, 500 MHz) δ 3.48 (s, 3H, NCH_3), 7.30 (dd, 1H_{Ar}, J = 3.9, 4.9 Hz), 7.42 (d, 1H_{Ar}, J = 4.4 Hz), 7.54 (m, 1H_{Ar}), 7.63 (d, 1H_{Ar}, J = 8.2 Hz), 7.93 (m, 1H_{Ar}), 8.01 (s, 1H_{Ar}), 8.05 (dd, 1H_{Ar}, J = 1.0, 4.9 Hz), 8.14 (d, 1H_{Ar}, J = 8.5 Hz), 8.19 (dd, 1H_{Ar}, J = 1.0, 3.8 Hz), 8.74 (s, 1H_{Ar}), 8.87 (d, 1H_{Ar}, J = 4.4 Hz). Anal. ($C_{25}H_{17}N_5O_4S$): Calculated C, 62.10; H, 3.54; N, 14.48; S, 6.63. Found C, 62.11; H, 3.37; N, 14.40; S, 7.02. LRMS (ESI pos.) *m/z* 484.2 $[M+H]^+$.

(4-Fluoro-N-methyl-N-{3-[3-(thiophene-2-carbonyl)-pyrazolo[1,5-a]-pyrimidine-7-yl]-phenyl}-benzamide) (**5b**)

Compound **5b** was prepared according to **5a**. (E)-*N*-(3-(3-(dimethylamino)acryloyl) phenyl)-*N*-methyl-4-flurobenzamide (843 mg,

2.59 mmol) and (5-amino-1H-pyrazol-4-yl)-thiophen-2-yl-methanone (500 mg, 2.59 mmol) were reacted to yield 918 mg (78%) of a yellow solid, R_f (silica gel, EtOAc) = 0.30. m.p. = 170.7–171.8°C. TLC: R_f (EtOAc) = 0.57. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.57 (s, 3H, NCH_3), 6.92 (m, $3H_{Ar}$), 7.20 (dd, $J = 4.9$, 3.8 Hz, $1H_{Ar}$), 7.30 (m, $1H_{Ar}$), 7.39 (m, $2H_{Ar}$), 7.50 (t, $J = 7.9$ Hz, $1H_{Ar}$), 7.71 (dd, $J = 4.9$, 1.1 Hz, $1H_{Ar}$), 7.80 (m, $2H_{Ar}$), 8.07 (dd, $J = 3.7$, 1.0 Hz, $1H_{Ar}$), 8.65 (s, $1H_{Ar}$), 8.77 (d, $J = 4.4$ Hz, $1H_{Ar}$). Anal. ($C_{25}H_{17}FN_4O_2S$): Calculated C, 65.78; H, 3.75; N, 12.27; S, 7.02. Found C, 65.68; H, 3.76; N, 12.01; S, 7.49. LRMS (ESI pos.) *m/z* 457.1 $[M+H]^+$.

Radiochemistry

RCY are decay-corrected data based on EOB. The radiochemical purity and specific activity are calculated to EOS.

Procedure for [^{18}F]F transfer to organic phase

[^{18}F]Fluoride (typically approx. 0.8–2.5 GBq) was trapped on an anion exchanger (Acell Plus QMA or 30-PS-HCO_3 cartridges), eluted with aqueous K_2CO_3 solution, and transferred into the reaction vial with a septum that contains K222 in MeCN. The mixture was completely dried under reduced pressure in argon atmosphere at 85–105°C, and [^{18}F]F^- was converted to the reactive K[^{18}F]F-K222–carbonate complex. For a standard protocol, 11.2 mg (29.7 μmol) K222 (or 7.86 mg = 29.7 μmol of the 18-crown-6 complex) and 1.78 mg (12.9 μmol) K_2CO_3 were used.

*Representative procedure for [^{18}F]**2b** and [^{18}F]**3b***

To the anhydrous K[^{18}F]F-K222–carbonate complex, 2 mg precursor **2a** in 0.5 mL DMF was added and the pale yellow solution was reacted at 135°C for 8 min. The labelling yield, determined by radio TLC, was 55–65%. After dilution with 3.5 mL water, the crude product was directly applied to semi-preparative HPLC under isocratic conditions (column: Kromasil 100-5 C18 7 μm (20 × 4 mm i.d.) +(250 × 4.6 mm); eluent: 37% MeCN+20 mM ammonium acetate; flow gradient: 0.75–1.5 mL/min. A solvents change is necessary for future animal experiments. Hence, combined separated fractions of [^{18}F]**2b** were highly diluted with water, adsorbed on a Sep-Pak C18 Plus cartridge, and almost quantitatively desorbed with ethanol. The final product was analysed by HPLC and TLC. RCY of 30–40% (total synthesis time: 90–115 min), radiochemical purity of ⩾99.0%, and a specific activity of ⩾250 GBq/μmol were achieved with good reproducibility ($n = 17$). Chemical purity was sufficient for pharmaceutical requirements.

The radiosynthesis of [^{18}F]**3b** (*N*-(3-[^{18}F]fluorobutyl)-*N*-{3-[3-(thiophen-2-carbonyl)-pyrazolo[1,5-a]pyrimidin-7-yl]-phenyl}-acetamide) was done according to [^{18}F]**2b** (labelling yield: 56–75%, RCY: 32–43%, time: 90–115 min, radiochemical purity ⩾98.5%, specific activity of ⩾250 GBq/μmol, $n = 13$).

*Representative procedure for [^{18}F]**4b***

The radiosynthesis of [^{18}F]**4b** was performed using 18-C-6 as PTC (7 mg, 26.5 μmol) under optimized conditions with a mixture of 2 mg of **4a** in 0.8 mL MeCN at 80°C for 20 min to achieve labelling efficiencies of 50–60%. The crude product was immediately applied to semi-preparative HPLC: Kromasil 100-5 C18 column (20 × 4 mm i.d.)+Kromasil 100-5 C18 pre-column (250 × 8 mm), particle size 7 μm; eluent: 37% MeCN +20 mM ammonium acetate; flow gradient: 0–10 min: 0.75 mL/min, 10–17.5 min: 1.25 mL/min; 17.5–30 min: 1.5 mL/min, 30–60 min: 1.75 mL/min; t_R(final product) = 37.5–40.5 min. Combined separated fractions of [^{18}F]**4b** were highly diluted with water, adsorbed on a Sep-Pak C18 Plus cartridge, and almost quantitatively desorbed with diethyl ether. Before injection, diethyl ether was removed under reduced pressure. The purified final product was obtained by semi-preparative HPLC, SPE and formulation (overall synthesis time 100 min), and analysed by HPLC and TLC: RCY of [^{18}F]**4b** 38–43%, radiochemical purity of >99%, and a specific activity of >150 GBq/μmol ($n = 21$).

*Representative procedure for [^{18}F]**5b***

To the anhydrous K[^{18}F]F-K222–carbonate complex, 4 mg precursor and 0.75–1 mL DMF were added and stirred for complete dissolution (5 min, 75°C). The reaction mixture was transferred to a 10 mL standard vial sealed by a rubber septum. The microwave-assisted radiosynthesis was carried out with an optimized temperature program under continuous PC controlling system (heat-up phase: 1.5 min, 175 W; reaction: 13 min, 145–150 W, 150–155°C). The colour of the reaction solution turned to yellow. After cooling, the solution was diluted with 3.0 mL 20% MeCN/water and applied to semi-preparative HPLC (column: Kromasil, solvent: 48% MeCN and 20 mmol ammonium acetate, flow gradient 0.5–1.5 mL/min). The collected final fractions (t_R = 45.5–47.5 min) were diluted with 150 mL water and transferred to a Sep-Pak C18 Plus cartridge. After trapping and washing (about 10 mL water), [^{18}F]**5b** was eluted with 1.25 mL EtOH. RCY of 5–10% (total preparation time ~2 h), radiochemical purity of >98.5%, and a specific activity of 10–15 GBq/μmol were achieved ($n = 7$).

The complex crude labelling mixture was also investigated by analytical HPLC. One of the isolated compounds could be identified as deacetyl-indiplon (ESI-MS: $[M+H]^+ = 335.10$), a major non-radioactive decomposition product.

*Representative automated module synthesis of [^{18}F]**2b***

The following solutions were prepared and filled into reagent vials: (a) 1.78 mg K_2CO_3 (12.9 μmol) in 0.4 mL water, (b) 11.2 mg K222 (29.7 μmol) in 0.75 mL anhydrous MeCN, (c) 3.0 mL MeCN, (d) 2 mg precursor **2a** in 0.75 mL anhydrous DMF, (e) 3 mL 25% MeCN/water, (f) 100 mL water (150 mL flask), and (g) 2 mL absolute EtOH.

The automated radiosynthesis was initiated and started on program-controlled equipment and online recording of process parameters.

No-carrier-added [^{18}F]fluoride was trapped on an anion exchange resin (30-PS-HCO_3 cartridge) and eluted into the reaction vessel as K[^{18}F]F by adding 1.78 mg K_2CO_3 in 0.4 mL water (solution (a)). After the addition of solution (b), water was removed by heating (80 and 95°C), reduced pressure, and He carrier gas flow. Three azeotropic distillations by repeated addition of MeCN (1 mL each) were necessary to remove trace amounts of residual water. Solution (d) was transferred into the reaction vessel. Labelling reaction afforded [^{18}F]**2b** with 25–42% efficiency at 135°C in 10 min. The reaction mixture was dissolved in 3.0 mL of 25% aqueous MeCN (solution (e)), injected onto semi-preparative HPLC (column: Kromasil, as described above), and eluted with 40% MeCN/water containing 20 mmol ammonium acetate at a flow rate of 1.5 mL/min. [^{18}F]**2b** eluted at 63 min and was collected in a flask containing 100 mL water. The

mixture was transferred onto a Sep-Pak C18 Plus cartridge and [^{18}F]**2b** was eluted with solution (g). After adding 15 mL sterile saline, the solution was passed through a sterile filter. Radiochemical purity of $>98.5\%$, specific activity >150 GBq/μmol, an overall RCY of about 15–23% (without decay correction), and synthesis time of about 85 min ($n = 5$) were achieved.

Conclusion

Four novel indiplon analogues, fluorinated either at the modified *N*-acyl or *N*-alkyl group, were synthesized as reference compounds and primarily used for the selection of appropriate candidates for ^{18}F-labelling. ^{18}F-labelling approaches were focused on tosyl or bromo precursors to obtain high RCY in a one-step radiolabelling procedure followed by similar multiple-stage purification using semi-preparative HPLC and SPE. The ^{18}F-labelled indiplon derivatives [^{18}F]**2b**–**4b** were reproducibly synthesized in good RCY (35–70%), high radiochemical purity ($\geqslant 98.5\%$), and high specific activity (>150 GBq/μmol) under optimized conditions. In addition, [^{18}F]**5b** was obtained from the 4-nitro-benzoyl precursor by microwave-assisted synthesis. However, poor labelling yields, defluorination, and numerous decomposition products were found. Furthermore, it has been shown that the synthesis for [^{18}F]**2b** can be performed in a commercially available automated module. For biological experiments, the stability of radiotracers [^{18}F]**2b**–**5b** was sufficient in saline, phosphate-buffered saline, and inactivated plasma.

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