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Use of 2-[^{18}F]fluoroethylazide for the Staudinger ligation – Preparation and characterisation of GABA$_A$ receptor binding 4-quinolones

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ABSTRACT

The labelling reagent 2-[^{18}F]fluoroethylazide was used in a traceless Staudinger ligation. This reaction was employed to obtain the GABA$_A$ receptor binding 6-benzyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (2-[^{18}F]fluoroethyl) amide. The radiotracer was prepared with a non-decay corrected radiochemical yield of 7%, a radiochemical purity >95% and a specific radioactivity of 0.9 GBq/μmol. The compound showed low brain penetration in normal rats. A series of fluoroalkyl 4-quinolone analogues with nanomolar to sub-nanomolar affinity for the GABA$_A$ receptor has been prepared as well.

The recently reported 2-[^{18}F]fluoroethylazide (**15**) has demonstrated its potential as a small labelling reagent for the copper(I) catalysed 1,3-dipolar cycloaddition with alkyne bearing substrates.[1–5] An application of **15** beyond 'click chemistry' would be the Staudinger ligation. This conjugation reaction offers a useful alternative approach to construct or label peptides.[6–8] The value of this chemistry has been already recognised for fluorine-18 labelling.[9] An elegant variant of the traceless Staudinger ligation, using an azide and auxiliary 2-(diphenylphosphino)ethanethiol, has been published recently by Soellner et al.[10]

Currently, the only efficient method to access [^{18}F]fluoroalkyl amides is a two-step labelling process using [^{18}F]fluoroalkyl amines as building blocks. This could be due to the tendency of prospective one-step sulphonate precursors to form 1,3-oxazoles under alkaline conditions.[11,12] Unfortunately, the present radiosynthesis of [^{18}F]fluoroalkyl amines is based on a low yielding method involving the use of protecting groups.[13] We have exemplified the traceless Staudinger ligation to access a new (2-[^{18}F]fluoroethyl) amide as potential radiotracer for the GABA$_A$ receptor.

The γ-aminobutyric acid (GABA) neurotransmitter is involved in a number of neurological conditions such as epilepsy, traumatic brain injury, anxiety and insomnia. For instance, in a model of Status Epilepticus a rapid internalisation of the subtype GABA$_A$ receptors, ligand gated ion channels, has been observed.[14] Similar changes have been reported in patients suffering from temporal lobe epilepsy.[15] The allosteric sites of the GABA$_A$ ion channel are the target of various drugs such as barbiturates and benzodiazepines (BZ).[16] The BZ allosteric site has been studied using positron emission tomography (PET). PET is a modern non-invasive imaging modality of superb sensitivity and it can be seen as the method of choice to study brain function in vivo. At the present, the PET gold standards for imaging of the GABA$_A$-BZ site are ^{11}C- and ^{18}F-labelled versions of Flumazenil (FMZ).[17–19] FMZ is a GABA$_A$ neutral allosteric modulator that binds to the BZ site with low nanomolar affinity. The radiosynthesis of ^{18}F analogues is either a low yielding nucleophilic aromatic fluorine substitution[18] or produces an alternative tracer of poor metabolic stability.[19] However in a clinical study, [^{18}F]-FMZ has recently shown similar binding and kinetic behaviour compared to [^{11}C]-FMZ.[20]

4-Quinolone derivatives with high affinity for the BZ site of GABA$_A$ have been reported by Lager et al.[21] Based on this work, we prepared a series of fluoroalkyl substituted 4-quinolones as potential new tracers for the GABA$_A$ receptor.

The synthesis of the 4-quinolone fluoroethyl amide **5** was achieved using the route described in Scheme 1. 4-Benzyl aniline **1** was reacted with diethyl ethoxymethylenemalonate to afford the desired intermediate **2** in quantitative yield. Compound **2** was heated with diphenylether under reflux conditions, to give **3** in good yield. The ester was hydrolysed to the corresponding acid **4** in quantitative yield. The desired amide **5** was synthesised using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) as coupling reagent. The same route

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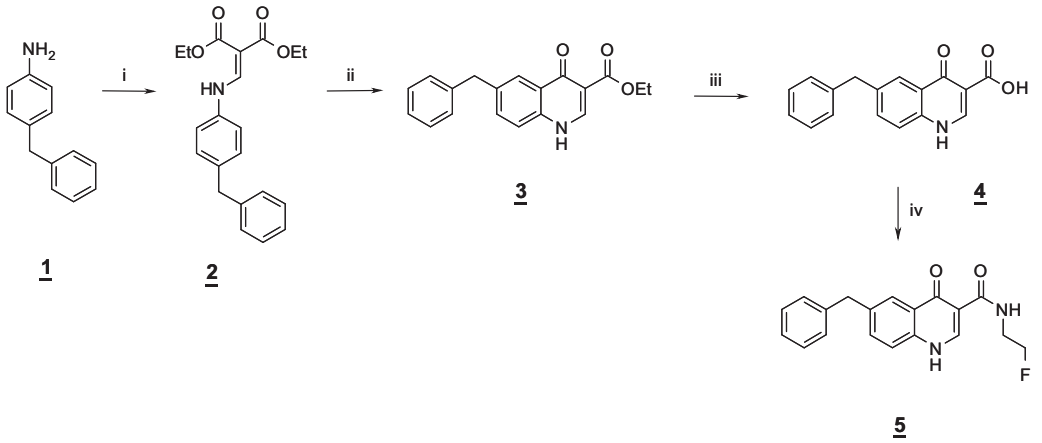


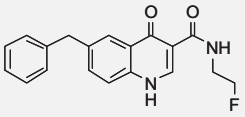
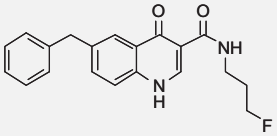
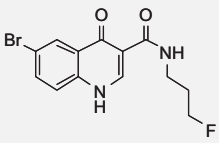
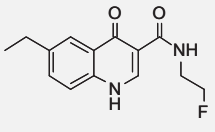
Scheme 1. Synthesis of quinolone **5**. Reagents and conditions: (i) ethoxymethylene malonate, 3 h, 120 °C; (ii) diphenylether, 1 h, reflux; (iii) NaOH (2 N), ethanol, 2 h, reflux; (iv) 2-fluoroethylamine, HATU, DIPEA, DMF, 12 h, rt.

was applied for the synthesis of analogues **6**–**8** (Table 1), using 4-bromo aniline and 4-ethyl aniline, respectively.

Compound **5** was identified in vitro as a potent ligand for the $GABA_A$ receptor with a K_i of 0.70 ± 0.66 nM (Table 1). Further variation at the 6-position of the quinolone system did not significantly alter the affinity of the test compounds. The same applied for extending the 2-fluoroethyl chain to a 3-fluoropropyl function. Quinolone **5** was selected for in vivo evaluation.

In order to obtain quinolone **5** via Staudinger ligation, the required precursor **10** was generated from commercially available thioester **11**. This was achieved in two high-yielding steps following a previously reported approach (Scheme 2).[10,22,23] Initially, **13** was employed with the quinolone acid **4** using literature conditions[22] to form the desired quinolone thioester. Unfortunately, this method failed to give the desired product, with no reaction observed. This was most likely due to the low reactivity of the quinolone acid **4** under the standard coupling conditions. However, treatment of quinolone acid **4** with 1 equiv of oxalyl chloride gave quantitative conversion to the acyl chloride **9**. The latter was characterised by NMR to confirm the formation of the desired intermediate and then successfully converted to compound **10** in good overall yield (28%) using the conditions described above.

Table 1
Structures of fluoroalkyl 4-quinolones **5**–**8** and measured K_i data using inhibition of [^{3}H]Ro151788 (FMZ) binding to $GABA_A$ receptors on rat cerebellar membrane preparations.

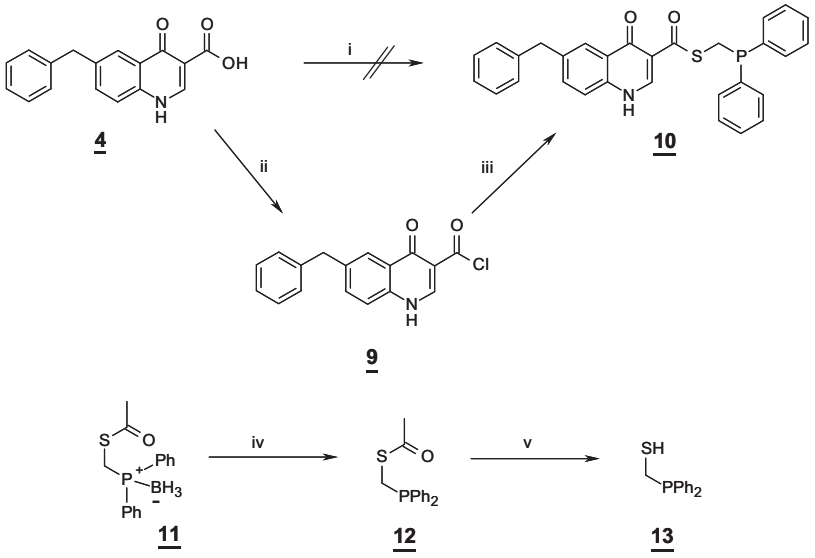
Compound		K_i (nM)
5		0.70 ± 0.66
6		0.13 ± 0.09
7		1.30 ± 0.87
8		3.60 ± 2.24

Initial attempts to radiolabel compound **5** by one-step labelling of the ethyl tosylate precursor using fluorine-18 failed. We hypothesised that this was caused by the acidic nature of the tautomeric quinolone proton, effectively quenching the ^{18}F-fluoride during the labelling reaction.

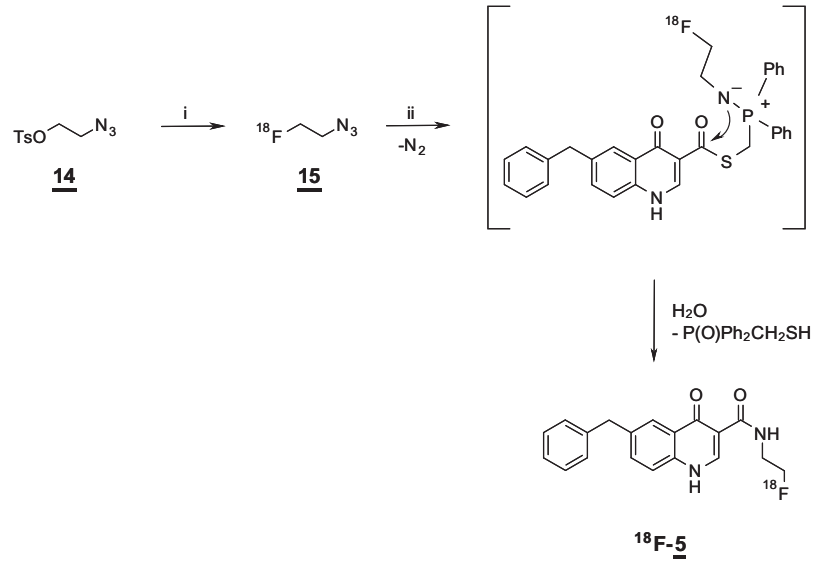
An alternative approach, based on the use of 2-[^{18}F]fluoroethylazide **15** for the Staudinger ligation, was explored for the labelling of this class of compounds. The optimised synthetic route to suitable Staudinger precursors is exemplified in Scheme 3. Reagent **15** was prepared from tosylate **14** according to a published method[1] and subsequently reacted with phosphine **10**. The desired product ^{18}F-**5** was formed in 75% radiochemical incorporation as observed by analytical HPLC. The tracer was subsequently isolated using preparative HPLC with a non-decay-corrected radiochemical yield of 7% (based on starting [^{18}F]fluoride) after 105 min (see Supplementary information, Fig. 1).[24] The radiochemical purity of the formulated product (30–50 MBq) was >95% with a specific radioactivity of 0.9 GBq/μmol. The PBS formulated compound was found to be stable at room temperature for at least 2 h (see Supplementary information, Figs. 5 and 6). This chemistry has therefore provided a convenient method to introduce a 2-[^{18}F]fluoroethylamide functionality into a molecule of interest.

The biodistribution of ^{18}F-labelled **5** was evaluated in naïve Sprague–Dawley rats (Table 2). The results show that only a modest fraction of the compound crossed the blood–brain barrier, with peak uptake no greater than 0.12% of the injected dose (0.11% ID/g). In the periphery, there was high initial uptake to the muscle with clearance over the 60-min study period. Excretion was primarily via the hepatobiliary rather than urinary system, as shown by increasing levels in the small intestines with time. There was no increase in the radioactivity in the bone that would indicate de-fluorination. Ex vivo autoradiographic analysis of prefrontal cortex, striatum and thalamus slices indicated significant differential binding of ^{18}F-**5** consistent with the distribution of $GABA_A$ receptors (Fig. 1, and Supplementary information, Fig. 8). The specific tracer binding in these areas was high as found by blocking with cold Flumazenil (~75% in high expressing regions and ~60% in low expressing regions). Thus, ^{18}F-**5** demonstrated affinity for the BZ sites (see Supplementary information, Table 1).

The *c* log *D* value of **5** at pH 7.0 was found to be 2.1 ± 1.0. Therefore, the tracer would be expected to penetrate the blood–brain barrier.[25] Further biological evaluation will be required to investigate the metabolic profile of ^{18}F-**5** and whether the compound is a PgP substrate.



Scheme 2. Synthesis of quinolone **10**. Reagents and conditions: (i) HOBt, DCC, DMF, **13**, 3 h, rt; (ii) oxalyl chloride, DCM, cat DMF, 1 h, rt; (iii) **13**, DCM, 12 h, rt; (iv) DABCO, toluene, 3 h, 40 °C; (v) NaOH, methanol, 2 h, rt.



Scheme 3. Radiosynthesis of ^{18}F-**5**. Reagents and conditions: (i) [^{18}F]KF/Kryptofix/K_2CO_3, MeCN, 20 min, 80 °C, distillation; (ii) **10**, MeCN, DMF, 15 min, 130 °C.

Table 2
Biodistribution data of ^{18}F-**5** in naïve Sprague–Dawley rats (n = 3)

	Radioactivity levels ± SD (% injected dose)				
	0.5 min pi	2 min pi	10 min pi	30 min pi	60 min pi
Blood	8.7 ± 1.8	4.2 ± 0.6	2.8 ± 0.2	1.5 ± 0.1	0.7 ± 0.1
Brain	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Heart	2.0 ± 0.4	1.1 ± 0.2	0.3 ± 0.2	0.2 ± 0.0	0.1 ± 0.0
Bone	3.0 ± 0.3	3.0 ± 0.4	1.7 ± 0.2	1.5 ± 0.2	1.4 ± 0.2
Liver	14.6 ± 8.2	28.5 ± 4.1	33.7 ± 1.7	18.7 ± 2.7	4.8 ± 0.9
Lung	2.9 ± 0.3	1.5 ± 0.7	0.6 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
Spleen	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
Muscle	56.1 ± 14.1	47.3 ± 10.3	27.2 ± 1.6	16.5 ± 1.3	4.6 ± 0.8
Kidneys	4.0 ± 1.7	3.6 ± 0.5	2.1 ± 0.1	1.5 ± 0.2	0.5 ± 0.1
Stomach	0.8 ± 0.3	1.2 ± 0.5	1.0 ± 0.3	0.5 ± 0.2	0.2 ± 0.0
Small intestine	4.7 ± 2.8	7.0 ± 0.5	24.5 ± 3.1	50.3 ± 4.1	74.5 ± 0.5
Large intestine	0.9 ± 0.6	1.3 ± 0.1	1.7 ± 0.4	1.7 ± 0.1	1.9 ± 0.4
Bladder and Urine	0.2 ± 0.3	0.0 ± 0.0	0.8 ± 0.2	4.2 ± 0.8	7.3 ± 1.4
Fat	0.6 ± 0.9	1.8 ± 0.9	1.8 ± 0.5	1.7 ± 0.2	0.3 ± 0.6

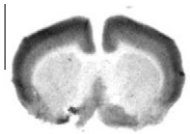


Figure 1. Autoradiography of striatal brain slice using [^{18}F]-**5**: indicates highest receptor expression in outer cortex and lower expression in the inner cortex. The laminar distribution is consistent with the high alpha1 subunit distribution in lamina III. Lowest expression observed in striatal areas (and thalamic) consistent with lower expression levels of GABA receptor in these regions.

In conclusion, the reagent 2-[^{18}F]fluoroethylazide is starting to show versatility as a building block for PET radiochemistry beyond the established field of 'Click Labelling'. Here, the traceless Staudinger ligation proved to be compatible with the half-life of fluorine-18. This reaction can thus be seen as useful tool to readily obtain 2-[^{18}F]fluoroethylamides without the need for protective groups. The investigated series of fluoroalkyl 4-quinolones demonstrated high $GABA_A$ receptor affinity. However, the selected ^{18}F-**5** showed a brain uptake in rats that was not suitable for PET imaging.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.106.

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24. Preparation of 6-benzyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (2-[^{18}F]fluoroethyl) amide (^{18}F-**5**) – [^{18}F]fluoride was transferred to a 3 mL Wheaton vial containing Kryptofix K222 (5 mg, 13.4 μmol), MeCN (0.5 mL), and potassium carbonate (50 μL, 0.1 M). The solution was dried at 100 °C under a flow of N_2 (0.3 L/min) for 20 min and cooled to room temperature. To the dried [^{18}F]fluoride was added 2-azidoethyl toluenesulfonate **14** (3 μL, 15.0 μmol)[1] in MeCN (0.2 mL), the Wheaton vial sealed and heated to 80 °C for 20 min. The temperature was increased to 130 °C and [^{18}F]fluoroethylazide **15** was allowed to distil into a 1 mL Wheaton vial that was chilled in ice using a stream of nitrogen (0.1 mL/min). To the Staudinger precursor **10** (2 mg, 4 μmol) in a mixture of water/DMF (1:9 v/v, 100 μL) was added **15** in MeCN (0.2 mL). The reaction mixture was heated at 130 °C for 15 min and allowed to cool to room temperature. Water (100 μL) was added and the resulting colourless precipitate was removed by filtration (0.45 μm, PALL ACRODISK CR13) to give the crude product. The reaction mixture was diluted into water (3 mL) and was purified by preparative HPLC (Luna C18(2) 100 × 10 mm, A: 50 mM ammonium acetate, B: MeCN, gradient: 30–40% B over 25 min, 4.0 mL/min, 254 nm).
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