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Rhodium-mediated [^{11}C]Carbonylation: a library of *N*-phenyl-*N*′-{4-(4-quinolyloxy)-phenyl}-[^{11}C]-urea derivatives as potential PET angiogenic probes

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As part of our ongoing investigation into the imaging of angiogenic processes, a small library of eight vascular endothelial growth factor receptor-2 (VEGFR-2)/platelet-derived growth factor receptor β dual inhibitors based on the *N*-phenyl-*N*′-4-(4-quinolyloxy)-phenyl-urea was labelled with ^{11}C (β^+, $t_{1/2}$ = 20.4 min) in the urea carbonyl position via rhodium-mediated carbonylative cross-coupling of an aryl azide and different anilines. The decay-corrected radiochemical yields of the isolated products were in the range of 38–81% calculated from [^{11}C]carbon monoxide. Starting with 10.7 ± 0.5 GBq of [^{11}C]carbon monoxide, 1-[4-(6,7-dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-fluoro-phenyl)-[^{11}C]-urea (2.1 GBq) was isolated after total reaction time of 45 min with a specific activity of 92 ± 4 GBq μmol^{-1}.

Keywords: carbonylation; carbon-11; angiogenesis; VEGFR-2; PET

Introduction

Angiogenesis, the process by which new capillaries are created from pre-existing blood vessels, is essential for the growth of solid tumors.[1,2] The vascular endothelial growth factor receptor-2 (VEGFR-2/KDR/flk-1) plays a key role in angiogenic processes and overexpression of both the VEGFR-2 protein and mRNA was found in tumor-associated endothelial cells, yet not in the vasculature surrounding normal tissues.[3–5] Inhibition of the VEGFR-2 has been shown both to induce tumor regression and reduce metastatic potential in preclinical models.[6] The platelet-derived growth factor receptor β (PDGFR-β) has also been associated with angiogenic processes. The pericytes that coat the angiogenic blood vessels confer stability to the capillary walls and participate in assembling the basal lamina. These pericytes require PDGFR-β signalling, which, when inactivated, reduces pericyte coverage of tumor blood vessels, render endothelial cells more sensitive to the damaging effect of cytotoxic drugs and can inhibit tumor growth.[7] In addition, therapeutic regimes involving both receptor tyrosine kinase (RTKs) inhibitors functioning in synergism were found to be more efficacious than selective inhibition of either receptor alone.[8] Since angiogenesis plays a central role in the growth of most solid tumors, both VEGFR-2 and PDGFR-β appear to be promising imaging targets for positron emission tomography (PET).

To perform *in vivo* quantitative molecular imaging using PET, a 'high-resolution' PET biomarker is required. The prerequisites for adequate *in vivo* imaging of biological/physiological processes vis-à-vis the labelled biomarker and its respective target have been widely discussed in the literature.[9] Some of these prerequisites deal with the different considerations that should be taken into account regarding the labelling position within the chemical structure of the suggested tracer and the selection of the radioisotope.[10–13] Recently, the design and synthesis of a series of *N*-phenyl-*N*′-4-(4-quinolyloxy)-phenyl-urea derivatives as VEGFR-2/PDGFR-β selective and potent inhibitors was reported.[14] These derivatives offer different labelling approaches at various positions and with different positron emitters such as ^{18}F (β^+, $t_{1/2}$ = 109.8 min) or ^{11}C (β^+, $t_{1/2}$ = 20.4 min) (Figure 1). General ^{18}F-labelling procedure of diaryl-urea containing molecules using either 4-nitrophenyl chloroformate[15] or triphosgene[16] was developed. In the latter article, complete chemical characterization, evaluation of receptor phosphorylation inhibitory potency and selectivity toward other RTKs were described. One of the advantages of labelling with ^{18}F is that it offers a longer *in vivo* surveillance time period than with ^{11}C; on the other hand, however, the shorter half-life of ^{11}C offers unique opportunities for multiple *in vivo* examinations in the same subject over a shorter period of time. Moreover, since ^{11}C, as opposed to ^{18}F, can be incorporated into various positions of the chemical

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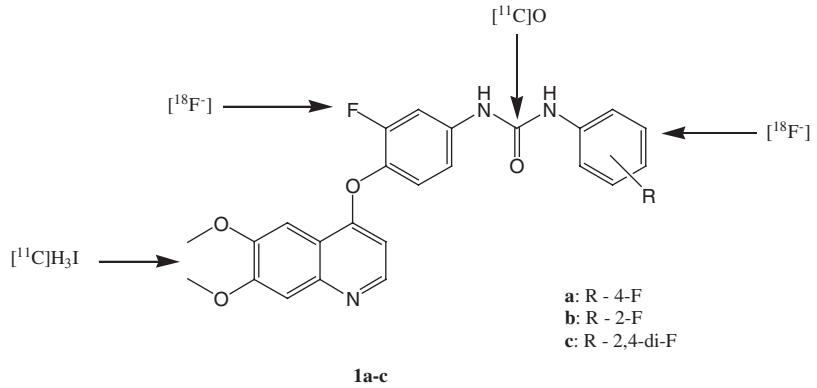
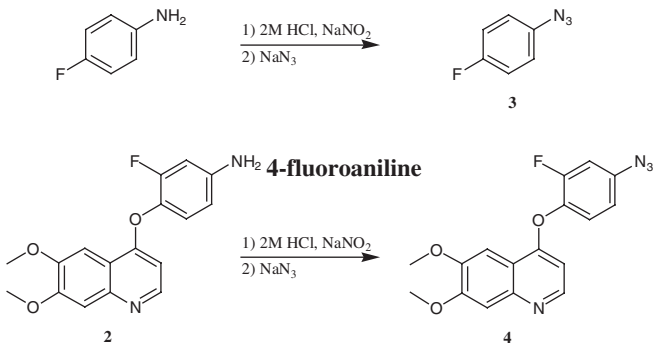


Figure 1. VEGFR-2/PDGFR-β inhibitors and potential positron emitter labelling positions via available labelled precursors.



Scheme 1. Synthesis of the azide precursors **3** and **4**.

structure of a certain class of compounds, it may yield a superior PET agent.

[^{11}C]Carbon monoxide has proven to be a valuable and versatile precursor in labelling chemistry and its chemistry has been extensively explored.[17] Rhodium-catalyzed carbonylation of phenyl azides and amines to form the corresponding ureas has been used in laboratory-scale synthesis.[18–21] Recently, a method using a rhodium-mediated reaction for labelling *N,N′*-diphenyl-[^{11}C]-urea in the carbonyl position was reported.[22] Herein, we describe the importance of choosing an appropriate radiosynthetic pathway for the urea-labelling reaction and the ^{11}C-labelling at the urea position of eight different *N*-phenyl-*N′*-4-(4-quinolyloxy)-phenyl-urea derivatives.

Results and discussion

Labelling chemistry

Reaction of carbon monoxide with an organic azide and an amine nucleophile to form a urea moiety has been shown to occur both at the mass and tracer levels under high temperatures and pressures even without the presence of any catalyst.[23] However, mild conditions are desirable in order to avoid unwanted side reactions with sensitive functional groups present in a complex drug/tracer-like chemical skeleton. With the use of rhodium-mediated carbonylative cross-coupling, the temperature could be significantly decreased while simultaneously increasing the radiochemical yield.[22] While the mechanism of this reaction is not yet fully understood, it may involve the formation of a nitrene–metal complex via loss of molecular nitrogen from the azide.[18,24] The [^{11}C]-urea bond formation may then proceed via the reaction between [^{11}C]carbon monoxide and the coordinated nitrene. In laboratory scale, in the absence of a nucleophile, the isocyanate is the major product, which upon treatment with an amine forms the corresponding urea. Obviously, a one-step reaction is desirable in ^{11}C-labelling chemistry due to the short half-life of the radionuclide. When the starting solution contains an amine, the amine can react either directly with the metal–carbonyl complex or with the *in situ* produced isocyanate to form the urea.

The presence of two nitrogen atoms in the urea moiety, one originating from the amine nucleophile and the other from the azide, opens up two possible routes for the synthesis of unsymmetrical ^{11}C-labelled ureas. Azides **3** and **4** were synthesized from 4-fluoroaniline and **2**, respectively, using sodium nitrite and sodium azide under conventional conditions (Scheme 1).

In the first route, we used the less sterically hindered 4-fluoro-phenyl moiety **3** as the azide and the more sterically hindered 4-(6,7-dimethoxy-quinolin-4-yloxy)-3-fluoro-phenylamine **2** as the amine nucleophile (Scheme 2). Within the ranges tested, the concentrations of azide, catalyst and ligand did not have any significant impact on the radiochemical yield of **5** and low yields were obtained (Table 1, entries 1–4). Unfortunately, it was not possible to increase the concentration of the nucleophile **2** to more than approximately 50 mM due to its low solubility in THF, thus an alternative nucleophilic derivative of **2** was explored. The use of an organic lithium base such as *n*-BuLi to form the lithium salt of an amine has previously been a successful approach when using weak nucleophiles in transition

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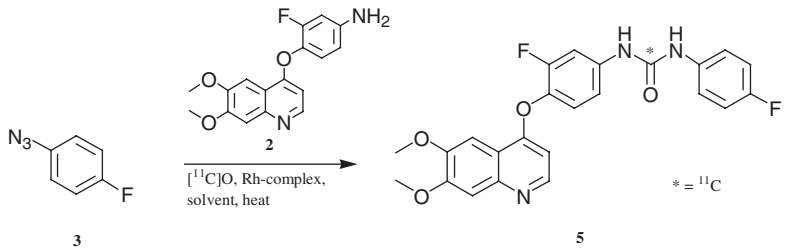
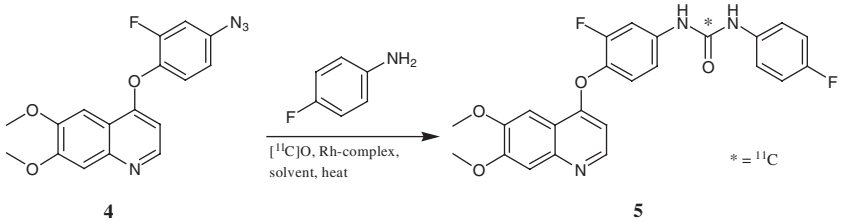


Scheme 2. Rhodium-mediated carbonylative cross-coupling between 4-fluoro-phenylazide and an amine **2** using [^{11}C]carbon monoxide.

Table 1. [^{11}C]-urea labelling of a VEGFR-2/PDGFR-β inhibitor using **2** as nucleophile

Entry	Azide **3** (mM)	Aniline **2** (mM)	$[Rh(cod)Cl]_2$ (mM)	Ligand (mM)	T (°C)	Conversion of [^{11}C]O (%)[a]	Isolated rcy (%)[bc]
1	36	48	0.39	dppe, 0.78	110	46	6
2	146	48	0.39	dppe, 0.78	110	16	5
3	146	48	3.04	dppe, 6.27	110	42	3
4	48	37	0.39	PPh_3, 0.78	110	37	7
5[d]	48	37	0.39	dppe, 0.78	110	86	0
6[e]	48	37	0.39	dppe, 0.78	110	33	0

Standard conditions were 200 μL solution of $[Rh(cod)Cl]_2$, ligand, 4-fluoro-phenylazide **3** and aniline **2** in THF.
[a]Decay-corrected conversion yield of [^{11}C]carbon monoxide to non-volatile products remaining in the reaction mixture after removal of solvent.
[b]Decay-corrected radiochemical yield based on the initial amount of radioactivity at the start of the synthesis and the radioactivity of the isolated product. When the reaction mixture was transferred from the micro-autoclave to an evacuated vial, the radioactivity in the vial was measured. The radioactive residues left in the micro-autoclave were estimated to be less than 1%. Hence, the amount of initial radioactivity of [^{11}C]carbon monoxide could be determined.
[c]Radiochemical purity was >97% in all experiments.
[d]*n*-BuLi 1.1 eq to the aniline added, solvent mixture of DMF (50 μL) and THF (150 μL).
[e]Solvent mixture of DMF (50 μL) and THF (150 μL).



Scheme 3. Rhodium-mediated carbonylative cross-coupling between an azide **4** and 4-fluoroaniline using [^{11}C]carbon monoxide forming an urea.

metal-mediated carbonylation with [^{11}C]carbon monoxide.[25,26] However, the lithium salt of **2** had an even lower solubility in THF than that of **2** itself. Although addition of DMF as a co-solvent effectively dissolved the salt, DMF was found to be incompatible with the reaction (Table 1, entries 5 and 6). The conversion of [^{11}C]carbon monoxide to non-volatile products was significantly higher in the lithium amide reaction, but the radioactivity was mainly composed of highly polar products and the desired urea product **5** could not be obtained.

In the second route, the less sterically hindered, more soluble 4-fluoroaniline acts as a nucleophile and the more sterically hindered 4-(4-azido-2-fluoro-phenoxy)-6,7-dimethoxy-quinoline **4** as an azide (Scheme 3). This approach modestly improved [^{11}C]carbon monoxide conversion and decreased formation of by-products resulting in a higher radiochemical yield of the desired product (Table 2, entry 7). In addition, the higher solubility of 4-fluoroaniline in THF compared with **2** allowed us to increase the concentration of nucleophile, resulting in even higher yields (Table 2, entries 3–6). Lowering the reaction temperature from 140 to 80°C increased the conversion of [^{11}C]carbon monoxide from 84 to 95% and improved the radiochemical yield (Table 2, entries 2, 4 and 9). With regard to the choice of the Rh ligand, triphenylphosphane (PPh_3) was favored over [1,2-bis(diphenylphosphino)ethane] at all temperatures tested (Table 2, entries 1,3 and 8 versus 2, 4 and 9, respectively). When keeping the amine concentration constant at 371 mM and gradually decreasing the rhodium complex and the azide concentrations down to 0.1 and 9.4 mM, respectively, the conversion of [^{11}C]carbon monoxide and radiochemical yield were preserved (Table 2, entries 9 and 10 versus 12 and 13). The

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Table 2. ^{11}C-labelling of a VEGFR-2/PDGFR-β inhibitor using 4-fluoroaniline as nucleophile

Entry	Azide **4** (mM)	Aniline (mM)	$[Rh(cod)Cl]_2$ (mM)	Ligand (mM)	T (°C)	Conversion of [^{11}C]O (%)[a,g]	Analytical rcy (%)[f,g]	Isolated rcy (%)[b,c,g]
1	37	371	0.39	dppe, 0.39	140	71	70	45
2	37	371	0.39	PPh_3, 0.78	140	83 ± 0 (2)	81 ± 3 (2)	62 ± 10 (2)
3	37	371	0.39	dppe, 0.78	110	79	67	49
4	37	371	0.39	PPh_3, 0.78	110	84	71	50
5	19	186	0.19	dppe, 0.38	110	81	69	50
6	37	74	0.39	PPh_3, 0.78	110	74 ± 5 (2)	61 ± 8 (2)	35 ± 7 (2)
7	48	37	0.39	PPh_3, 0.78	110	46 ± 5 (2)	41 ± 9 (2)	27
8	37	371	0.39	dppe, 0.78	80	84	77	44
9	37	371	0.39	PPh_3, 0.78	80	95 ± 1 (2)	91 ± 1 (2)	81
10	19	371	0.19	PPh_3, 0.39	80	95 4 (2)	90 ± 6 (2)	78 ± 1 (2)
11	19	186	0.19	PPh_3, 0.39	80	92	87	72
12	9.4	371	0.10	PPh_3, 0.20	80	90 ± 1 (2)	86 ± 3 (2)	73 ± 0 (2)
13	4.7	371	0.05	PPh_3, 0,10	80	77 ± 10 (2)	71 ± 10 (2)	51
14[h]	9.4	74	0.19	PPh_3, 0.39	80	96	32	

Standard conditions were 200 μL solution of $[Rh(cod)Cl]_2$, ligand, azide **4** and 4-fluoroaniline in THF. For explanations see Table 1. [f]Decay-corrected analytical radiochemical yield was determined from the conversion yield multiplied by the decay-corrected radiochemical purity assessed with analytical HPLC. [g]The numbers in brackets indicate the number of experiments. [h]*n*-BuLi 0.9 eq to aniline.

lithium salt of 4-fluoroaniline was highly soluble in THF and gave good conversion of [^{11}C]carbon monoxide, however, to a complex mixture of labelled compounds. The desired product was co-eluted with several labelled by-products and could not be isolated from the crude mixture (Table 2, entry 14). The most optimal conditions for obtaining maximal radiochemical yield combined with minimal concentration of starting materials are indicated in Table 2, entry 10.

In traditional radiochemistry, the amount of transition metal complex exceeds both the amount of [^{11}C]carbon monoxide and the obtained labelled product by a factor of approximately 50.[27] The amount of the rhodium complex in entry 12 is approximately half of the amount of the product formed suggesting that the reaction is catalytic in nature.

The use of [^{11}C]carbon monoxide enables the production of a broad spectrum of substituted ^{11}C-labelled ureas in a fast and systematic manner. Using the most optimal reaction conditions (Table 2, entry 10), a small library of eight urea derivatives was synthesized with decay-corrected radiochemical yields (dc rcy) in the range of 38–78% (Table 3). Tracer libraries may accelerate the research of new radiopharmaceutical structure–activity relationships by enabling the synthesis of a multitude of tracers using the same chemical system with only minor modifications.

Characterization

The radiolabelled products (Table 3, entries 1–3) were characterized using HPLC by co-eluting with an authentic sample[16] and also by analyzing them with ESI+MS/MS. References as well as labelled compounds all showed similar fragmentation patterns. The molecular ion [M+H] and two primary fragmentation ions that arose and differed by $m/z = 26$ were observed. This type of fragmentation is characteristic of 1,3-diphenyl-urea derivatives where the corresponding aniline ($m/z = 315$) and isocyanate ($m/z = 341$) are formed.[28–30] The other compounds (Table 3, entries 4–8) were characterized by only using ESI+MS/MS and all gave the expected molecular weight and fragmentations.

Specific activity

Carbon monoxide is present at low levels in the atmosphere (0.5–5 ppm), while carbon dioxide is present at much higher concentrations (380 ppm). Thus, with the use of [^{11}C]carbon monoxide, the isotopic dilution can be kept at a minimal level resulting in higher specific activity.[17] Further, throughout this investigation, the amount of product formed was determined after each synthesis and ranged from 15 to 175 nmol with a median of approximately 40 nmol and was unrelated to the amount of radioactivity produced in the cyclotron target. The most important factor for isotopic dilution was found to be the conditioning of the zinc-oven used for reduction of [^{11}C]O_2 to [^{11}C]O. After a service where the zinc-oven was loaded with fresh zinc granules, the oven needed to be flushed for 3 h with a flow of helium (20 mL min^{-1}) at 400°C prior to synthesis for low isotopic dilution. When higher quantities of activity are produced, higher specific activity may be obtained. A 10 μA h bombardment gave 10.7 ± 0.5 GBq of [^{11}C]carbon monoxide and, after 45 min, 23 ± 1 nmol of product **5** was isolated as determined by HPLC-UV using a calibration curve. This 23 nmol of product corresponds to 2.1 GBq giving a specific radioactivity of 92 ± 4 GBq μmol^{-1} at 45 min from EOB.

Experimental

General

^{11}C was prepared by the ^{14}N(p,α)^{11}C nuclear reaction using 17 MeV proton beam produced by a Scanditronix MC-17 Cyclotron at Uppsala Imanet, GE Healthcare, and obtained as [^{11}C]carbon dioxide. The target gas used was nitrogen (AGA Nitrogen 6.0) containing 0.05% oxygen (AGA Oxygen 4.8). The carbonylation reactions were carried out in a 200 μL Teflon-coated micro-autoclave according to a previously described method.[27,31]

THF was freshly distilled over sodium and benzophenone under a nitrogen atmosphere. All chemicals were purchased

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Table 3. ^{11}C-labelling of a small library of VEGFR-2/PDGFR-β inhibitors using substituted anilines, azide **4**, Rh–PPh_3 complex, in THF, 80°C, 5 min

Compound	Aniline substituent(s)	Conversion of [^{11}C]O [%][a,g]	Analytical rcy (%)[f,g]	Isolated rcy (%)[b,c,g]
5	4-F	95 4 (2)	90 ± 6 (2)	78 ± 1 (2)
6	2-F	65 ± 7 (2)	53 ± 3 (2)	31 ± 10 (2)
7	2,4-diF	79	59	48
8	4-Cl	86	86	70
9	2-OMe	92	91	77
10	4-OMe	63	58	50
11	2-Me	90	90	74
12	4-Me	93	89	76

For explanations see Tables 1 and 2.

from Aldrich/Fluka and used without further purification. The identities of the ^{11}C-labelled compounds (Table 3, entries 1–3) were determined by analytical HPLC using authentic samples as references. Analytical HPLC was performed on a Beckman system, equipped with a Beckman 126 pump, a Beckman 166 UV detector in series with a Bioscan β^+-flow count detector and a Beckman Ultrasphere ODS dp 5 μm column (250 × 4.6 mm). Mobile phase: (A) 50 mM ammonium formate pH 3.5, (B) acetonitrile; 50% B for 10 min then 95% B for 15 min. A Gilson 231 XL autoinjector was used. Further characterization of the purified labelled products was made using a Waters Quattro Premier triple quadrupole mass spectrometer with electrospray ionization operated in positive mode. Purification with semi-preparative HPLC was performed on a similar Beckman system equipped with a Genesis C18 120 4 μm (250 × 10 mm). Mobile phase: (A) 50 mM ammonium formate pH 3.5, (B) acetonitrile.

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400 or 500 MHz spectrometer and chemical shifts are given in ppm (δ) using $CHCl_3$ as the internal standard. GC-MS analyses were performed on a Finnigan MAT Thermoquest GCQ mass spectrometer operated in EI+mode, equipped with a non-polar column SE-54, using a temperature gradient of 70–250°C over 10 min. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT/IR spectrometer as neat compounds. Melting point was recorded on a Bibby Stuart melting point apparatus and is uncorrected. Elemental analysis was preformed by Mikro kemi AB (Uppsala, Sweden).

Synthesis of precursors

*4-Fluoro-phenylazide (**3**)*[32]

4-Fluoroaniline (1.00 g, 9.00 mmol) was dissolved in 2 M HCl (10 mL) and cooled in an ice bath. To the cold solution, a solution of sodium nitrite (2.29 g, 33.3 mmol) in water (10 mL) was added dropwise while keeping the temperature below 3°C. The mixture was stirred for another 15 min and a saturated solution of sodium azide (7.60 g, 117 mmol) in water (18 mL) was added dropwise while keeping the solution temperature below 3°C. The solution was stirred for an additional 15 min. After neutralization with saturated $NaHCO_3$, the reaction mixture was extracted three times with diethyl ether and the combined organic extracts were washed with brine and dried over $MgSO_4$ and purified on SiO_2 using flash chromatography eluting with pentane to give the product as a pale yellow oil (0.8 g, 66%). The product was kept in darkness and under nitrogen at 4°C. ^{1}H-NMR (500 MHz, $CDCl_3$, 25°C): δ = 7.04 (m, 2H), 6.98 (m, 2H) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$, 25°C): 160.1 (d, J = 248 Hz), 135.9 (d, J = 2.9 Hz), 120.4 (d, J = 8.3 Hz), 116.7 (d, J = 24 Hz) ppm. IR λ: 2100 (azide), 1496, 1301, 1223, 825 cm^{-1}. GC-MS for $C_6H_4FN_3$ R_t = 4.8 min, *m/z* 137 (90), 123 (31), 109 (100).

*4-(4-Azido-2-fluoro-phenoxy)-6,7-dimethoxy-quinoline (**4**)*

4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenylamine (**2**) (0.500 g, 1.59 mmol) was dissolved in 2 M HCl (40 mL) under gentle warming. The solution was cooled to 0°C and a solution of $NaNO_2$ (0.346 g, 5.30 mmol) in water (15 mL) was added while keeping the temperature under 1°C. The reaction mixture was stirred at 0°C for 30 min, and a saturated solution of NaN_3 (5 mL) was added slowly while keeping the temperature under 1°C with stirring continued for another 30 min during which a white precipitate was formed. The mixture was diluted with water and extracted with dichloromethane. The combined organic extracts were dried over $MgSO_4$ and evaporated to give an orange solid. The solid was purified using flash chromatography with EtOAc as eluent to give the product of 0.37 g (68%) as an orange oil, which crystallized upon standing cold overnight. This azide proved to be stable when kept in the dark at room temperature under normal atmosphere for several months. Melting point 131–133°C. ^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): 8.51 (s, 1H), 7.57 (s, 1H), 7.47 (s, 1H), 7.28 (m, 1H), 6.93 (m, 2H), 6.40 (m, 1H), 4.07 (s, 3H), 4.06 (s, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$, 25°C): 160.0 (d, J = 1.2 Hz), 156.5, 154.0, 153.3, 150.0, 148.9, 147.3, 139.0 (d, J = 8.4 Hz), 138.6 (d, J = 12.5 Hz), 124.7 (d, J = 1.9 Hz), 115.6 (d, J = 3.7 Hz), 108.8 (d, J = 22.1 Hz), 108.3, 102.5, 99.6, 56.3, 56.2 ppm. IR λ: 2984, 2120 (azide), 1737, 1372, 1231, 1043 cm^{-1}. ^{19}F-NMR (376 MHz, $CDCl_3$, 25°C): 125.9 (unresolved dd, $J_{F,H}$ = 9.0, 10.0 Hz) ppm. Direct inlet EI+ for $C_{17}H_{13}FN_4O_3$ *m/z*: 340 (83), 312 (100), 277 (75). Elementary analysis for $C_{17}H_{13}FN_4O_3$, expected C 60.0%, H 3.85%, N 16.46%, found C 59.8%, H 4.0%, N 16.2%.

Labelling chemistry

*1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-fluoro-phenyl)-[^{11}C]-urea (**5**)*

In a typical experiment, chloro(1,5-cyclooctadiene)rhodium(I) dimer ([Rh(cod)Cl]$_2$) (0.038 mg, 0.076 μmol) and triphenylphosphane (0.041 mg, 0.16 μmol) was added as a solution in 100 μL THF to a capped 1-mL vial under argon. To this solution, 4-(4-azido-2-fluoro-phenoxy)-6,7-dimethoxy-quinoline (2.55 mg, 7.50 μmol) dissolved in 100 μL THF and 4-fluoro-phenylaniline (16.5 mg, 148 μmol) dissolved in 200 μL THF were added. The resulting 400 μL solution was degassed with argon and 200 μL of the solution was loaded onto an injection loop. THF was

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pumped through the loop and the reagents were transferred into a 200 μL Teflon-coated stainless steel micro-autoclave containing [^{11}C]carbon monoxide and helium immersed in an oil bath. The reaction mixture was kept at 80°C for 5 min and then transferred into an evacuated capped 3-mL vial and the radioactivity was measured (2.4 GBq at 20.0 min from EOB). The vial was purged with a stream of nitrogen for 1 min at 80°C and the radioactivity was measured (1.9 GBq, 25 min and 30 s from EOB) giving the conversion yield of [^{11}C]carbon monoxide 92%. The crude product was transferred to a 0.8 mL vial and the 3 mL vial was rinsed with acetonitrile, 300 μL, and then water, 200 μL, and in this manner 97% of the radioactivity was transferred. A small sample of the solution was withdrawn and analyzed by reversed-phase HPLC for determination of the radiochemical purity of the crude product. The crude product was purified on a reversed-phase semi-preparative HPLC, eluent 60% B, flow: 4 mL min^{-1}, R_t = 7.6 min. The radioactivity of the purified product was measured (0.79 GBq at 45 min and 30 s from EOB) to determine the dc rcy (78%) calculated from [^{11}C]carbon monoxide. Analytical HPLC was used to assess the identity of the labelled compound as compared with the reference compound (R_t = 6.6 min). In addition, ESI+MS/MS was used for identification of the purified product, cone voltage 45 V, source temperature 120°C, desolvation temperature 350°C showing peaks for $C_{24}H_{19}F_2N_3O_4$ at *m/z* [M+H]: 452 and fragments at 341 and 315.

*1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(2-fluoro-phenyl)-[^{11}C]-urea (**6**)*

Similar procedure as for **5**. 2-Fluoroaniline (16.5 mg, 148 μmol). Dc rcy 38%. Analytical R_t = 8.3 min. ESI+MS/MS $C_{24}H_{19}F2N_3O_4$ *m/z*: 452, 341, 315.

*1-(2,4-Difluoro-phenyl)-3-[4-(6,7-dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-[^{11}C]-urea (**7**)*

Similar procedure as for **5**. 2,4-Difluoroaniline (19.1 mg, 148 μmol). Dc rcy 48%. Analytical R_t = 8.3 min. ESI+MS/MS $C_{24}H_{18}F_3N_3O_4$ *m/z*: 470, 341, 315.

*1-(4-Chloro-phenyl)-3-[4-(6,7-dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-[^{11}C]-urea (**8**)*

Similar procedure as for **5**. 4-Chloroaniline (18.9 mg, 148 μmol). Dc rcy 70%. Analytical R_t = 9.4 min. ESI+MS/MS $C_{24}H_{19}ClFN_3O_4$ *m/z*: 468, 341, 315.

*1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(2-methoxy-phenyl)-[^{11}C]-urea (**9**)*

Similar procedure as for **5**. 2-Methoxyaniline (18.3 mg, 148 μmol). Dc rcy 77%. Analytical R_t = 8.0 min. ESI+MS/MS $C_{25}H_{22}FN_3O_5$ *m/z*: 464, 341, 315.

*1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-methoxy-phenyl)-[^{11}C]-urea (**10**)*

Similar procedure as for **5**. 4-Methoxyaniline (18.3 mg, 148 μmol). Dc rcy 50%. Analytical R_t = 5.8 min. ESI+MS/MS $C_{25}H_{22}FN_3O_5$ *m/z*: 464, 341, 315.

*1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-o-tolyl-[^{11}C]-urea (**11**)*

Similar procedure as for **5**. *o*-Toluidine (15.9 mg, 148 μmol). Dc rcy 74%. Analytical R_t = 6.6 min. ESI+MS/MS $C_{25}H_{22}FN_3O_4$ *m/z*: 448, 341, 315.

*1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-p-tolyl-[^{11}C]-urea (**12**)*

Similar procedure as for **5**. *p*-Toluidine (15.9 mg, 148 μmol). Dc rcy 76%. Analytical R_t = 7.8 min. ESI+MS/MS $C_{25}H_{22}FN_3O_4$ *m/z* [M+H]: 448, 341, 315.

Determination of specific activity

The synthesis of 1-[4-(6,7-dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-fluoro-phenyl)-[^{11}C]-urea (**5**) was performed as described above. A cyclotron bombardment of 10 μA h gave 10.7 ± 0.5 GBq of [^{11}C]carbon monoxide at 18–20 min after EOB. After 45 min, 2.1 GBq of the product was isolated. The product fraction had a volume of 6.2 mL as measured with a syringe. A calibration curve was made from a series of three calibration standards, three injections each (1, 5 and 15 μM), which were analyzed using analytical reversed-phase HPLC coupled to a UV detector (λ = 254 nm), inj. volume was 50 μL. A sample from the isolated product was analyzed in a similar manner. The concentration of the analyte was 3.67 μM corresponding to an amount of 23 nmol of product, giving a specific activity of 92 ± 4 GBq μmol^{-1} at 45 min after EOB.

Conclusions

^{11}C-labelling of a series of asymmetric phenyl-ureas has been performed via rhodium-mediated carbonylation reaction with an azide **4**, [^{11}C]carbon monoxide and various anilines.

The reaction conditions were explored by altering reagent concentrations, temperature and Rh ligands. High concentrations of the amine nucleophile were found to be the key factor for obtaining high radiochemical yields. After a 10 μA h bombardment, 2.08 GBq of the product **5** was isolated at 45 min from EOB with a specific activity of 92 ± 4 GBq μmol^{-1}. The ^{11}C-labelled VEGFR-2/PDGFR-β dual inhibitors are now being further explored as potential PET angiogenic probes.

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