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Formation of fluorine-18 labeled diaryl ureas—labeled VEGFR-2/PDGFR dual inhibitors as molecular imaging agents for angiogenesis

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Abstract—Urea subunits are common components of various pharmaceuticals' core structure. Since in most cases the design and development of PET biomarkers is based on approved or potential drugs, there is a growing need for a general labeling methodology of urea-containing pharmacophores. As a part of research in the field of molecular imaging of angiogenic processes, we synthesized several highly potent VEGFR-2/PDGFR dual inhibitors as potential PET biomarkers. The structure of these inhibitors is based on the *N*-phenyl-*N*′-{4-(4-quinolyloxy)phenyl}urea skeleton. A representative inhibitor was successfully labeled with fluorine-18 by a three-step process. Initially, a two-step radiosynthesis of 4-[^{18}F]fluoro-aniline from 1,4-dinitrobenzene (60 min, EOB decay corrected yield: 63%) was performed. At the third and final step, the 4-[^{18}F]fluoro-aniline synthon reacted for 30 min at room temperature with 4-(2-fluoro-4-isocyanato-phenoxy)-6,7-dimethoxy-quinoline to give complete conversion of the labeled synthon to 1-[4-(6,7-dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-[^{18}F]fluoro-phenyl)-urea.
The desired labeled product was obtained after total radiosynthesis time of 3 h including HPLC purification with 46 ± 1% EOB decay corrected radiochemical yield, 99% radiochemical purity, 99% chemical purity, and a specific activity of 400 ± 37 GBq/mmol (n = 5).

1. Introduction

Urea subunits are common components of lead pharmaceuticals. Recently, urea moieties have been used as part of colchicine-binding site antagonists[1] and polycyclic imides with antimicrobial activity,[2] while diaryl ureas specifically have emerged in multitarget tyrosine kinase inhibitors,[3] p38 MAPK inhibitors,[4] insulin-like growth factor I receptor (IGF-1R) inhibitors[5] and as combined vascular endothelial growth factor receptor 2 and platelet-derived growth factor receptor (VEGFR-2/PDGFR) inhibitors.[6] Most of the above-mentioned pharmacophores were designed as highly selective targeted therapies. In order to evaluate and monitor efficiency of targeted therapies, a sensitive and quantitative molecular imaging modality is needed.[7,8] This may be achieved by means of Positron Emission Tomography (PET) coupled with selective labeled biomarkers. In the last decade, the field of PET imaging has witnessed great progress, fueled by specific radiolabeled biomarkers. With this approach, careful patient selection combined with extensive therapy monitoring provides an optimal platform for in vivo drug efficacy evaluation. Moreover, pharmacodynamic interactions, pharmacokinetics and target quantity and occupancy can be measured in humans by PET. Development of a general synthetic route for the labeling of aryl-ureas moieties with positron emitting isotopes will broaden the use of PET and specifically, accelerate the development of potential drugs containing this moiety, allowing for more effective therapeutic monitoring.

Cyclic ureas have been labeled with ^{11}C by the production of [^{11}C]phosgene, which further reacts with free amines to give the desired urea product. There are two major drawbacks of using [^{11}C]phosgene as a labeling agent. The first is the multi-step process of [^{11}C]phosgene production which takes about one half-life of ^{11}C and predominantly involves the use of the toxic chlorine gas. The second drawback is the highly reactive nature of phosgene which makes the synthesis of asymmetric diaryl ureas extremely difficult, thus limiting this

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approach to cyclization reactions of diamino or hydroxyamino precursor molecules.[9–12] Another approach for [^{11}C]urea production is the rhodium(I)-promoted carbonylation reaction using phenyl azide and [^{11}C]carbon monoxide developed by Doi et al.[13] This approach allows the formation of a wide spectrum of diaryl ureas since an azide and an amine are used rather than two amines. Since the half-life of ^{11}C is relatively short (20.4 min), PET studies are limited to a short time window following injection of the radiolabeled biomarker. In order to counter this time restraint, labeling with longer half-life isotopes such as ^{18}F and ^{124}I (109.8 min and 4.2 days, respectively) is required.

To the best of our knowledge, there is no report on fluorine-18 labeling of a diaryl urea and only one report has surfaced on the labeling of an urea-containing molecule with fluorine-18.[14] In this particular report, the dialkyl urea was formed in a 5 h, four-step radiosynthesis route using diphosgene to yield the desired product with a radiochemical yield of 12% (decay corrected).

One approach to label diaryl ureas is first to produce ^{18}F labeled anilines.[15,16] This radiolabeled synthon can further react with a precursor carrying a reactive functional group to yield the labeled diaryl urea. In order to evaluate the feasibility of this approach, we focused on the VEGFR-2/PDGFR dual inhibitors which contain a diaryl urea moiety. Three potential precursors with different reactive functional groups were chosen: isocyanate, isopropenyl carbamate, and carbamoyl imidazoles (Scheme 1).

2. Results and discussion

2.1. Chemistry

The synthesis of two new (**1** and **2**) and one published (**3**) derivatives of VEGFR-2/PDGFR dual inhibitors (Scheme 2) was performed based on the chemical course described by Kubo et al.[6] with several modifications. The first step was the cyclization of 2-amino-4,5-dimethoxyacetophenone in dimethoxyethane at room temperature to give 6,7-dimethoxy-4-quinolone (**4**, yield: 95%). This quinolone was first chlorinated using oxalyl chloride in DCM at reflux (**5**) rather than phosphoryl chloride and then reacted with (3-fluoro-4-hydroxy-phenyl)-carbamic acid *tert*-butyl ester and DMAP in DMF at 145 °C to give the protected phenoxyquinoline (**6**, 50%) which was de-protected with HCl in dioxane to give the free amine (**7**, 66%). It should be noted that both reaction of **5** with nitrophenol followed by nitro reduction and a direct reaction of **5** with an aminophenol in the presence of NaH either did not produce **7** or furnished the desired molecule accompanied with a large quantity of side products. In the last step, reaction of **7** with triphosgene followed by the addition of the corresponding fluoroaniline in dry DCM yielded the title compounds, **1**–**3** (50–65%) (Scheme 3).

The isocyanate precursor (**8**) for radiosynthesis was prepared by reacting **7** with triphosgene in DCM at room temperature. The reaction was monitored using IR via absorption at 2285 cm^{-1} which is associated with isocyanate formation. Upon completion of the reaction, all volatiles were evaporated to give a yellow solid which was used as such for radiosynthesis.

The isopropenyl carbamate precursor (**9**) was prepared by reaction of **7** with isopropenyl chloroformate in THF as described before.[17] This precursor proved to be stable enough for column chromatography purification and characterization.

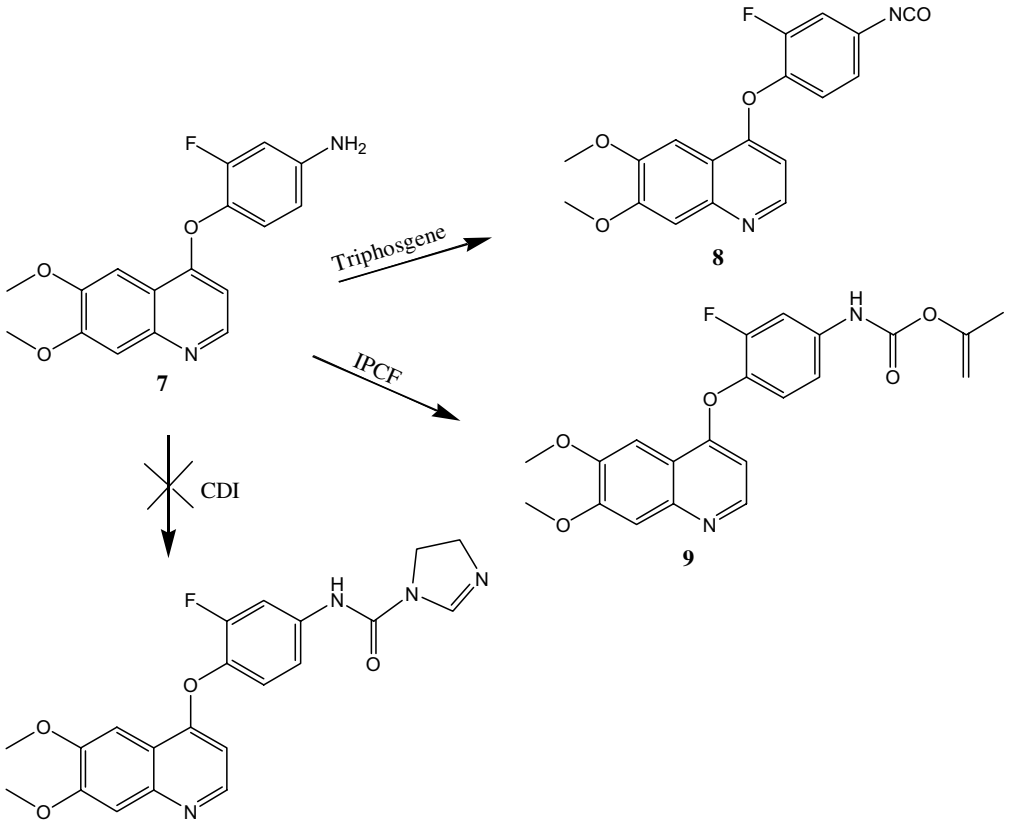
Reaction of **7** with *N*,*N*′-carbonyldiimidazole (CDI) was attempted under different conditions,[18–22] but unfortunately the carbamoyl imidazole intermediate could not be isolated. When 4-fluoroaniline was added to the above-mentioned reaction mixtures, formation of **1** was evident by mass spectrometry analysis. At the outset, our primary reason of choosing the carbamoyl imidazole intermediate over the isocyanate precursor was to afford precursor purification and isolation; however, this procedure turned out to be unfeasible, and thus this approach was no longer explored in radiosynthesis.

2.2. Radiochemistry

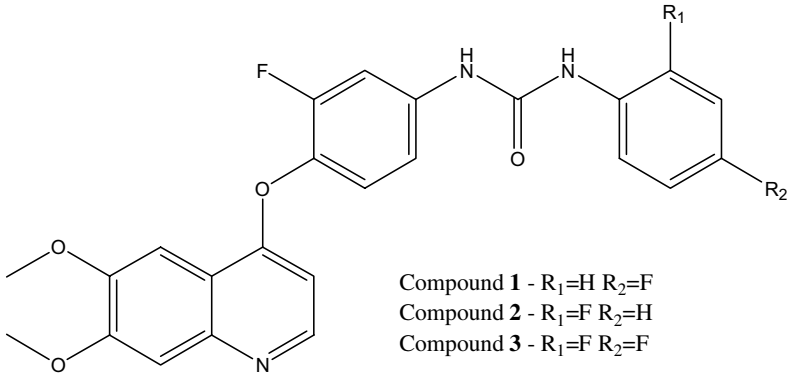
2.2.1. 4-[^{18}F]fluoroaniline ([^{18}F]-11). The labeling of the 4-[^{18}F]fluoro-aniline was described earlier.[23] Briefly, 1,4-dinitrobenzene was first ^{18}F-fluorinated using a [^{18}F]KF/Kryptofix complex in DMF and then the resulting 4-[^{18}F]fluoro-nitrobenzene ([^{18}F]-**10**) was reduced by means of raney-nickel and hydrazine hydrate (Scheme 4). This labeled intermediate was purified by an Oasis cartridge and eluted from the cartridge with various solvents such as DCM, DCE, and toluene in order to afford several conditions for the next step. The synthesis to furnish the desired labeled product lasted approximately 60 min. This labeled intermediate was meant to assess the feasibility of using the different precursor molecules, namely the isocyanate and isopropenyl carbamate, to furnish [^{18}F]-**1**.

2.2.2. Carbamoyl imidazoles. The first approach for labeling one of the title compounds involved the CDI-mediated urea synthesis. CDI is a phosgene equivalent which is commercially available and readily handled as a crystalline solid. Usually, the carbamoyl imidazole intermediate is formed in situ and further reacted without purification. In some cases, this intermediate can be isolated[18] and this could be an advantage over the highly reactive isocyanates which are extremely hard to purify. As mentioned above, precursor isolation was not possible and this route was no longer explored in radiosynthesis.

2.2.3. Isopropenyl carbamate. The second method chosen for the radiosynthesis of unsymmetrical ureas was via the isopropenyl carbamate intermediate. Although carbamates have the advantage of being sufficiently stable for purification and analysis, most carbamates form urea bonds via lengthy equilibrium reactions. Since PET radiochemistry often deals with short-lived isotopes, the use of carbamates, which require long reac-



Scheme 1. Candidate precursors for labeling diaryl urea.

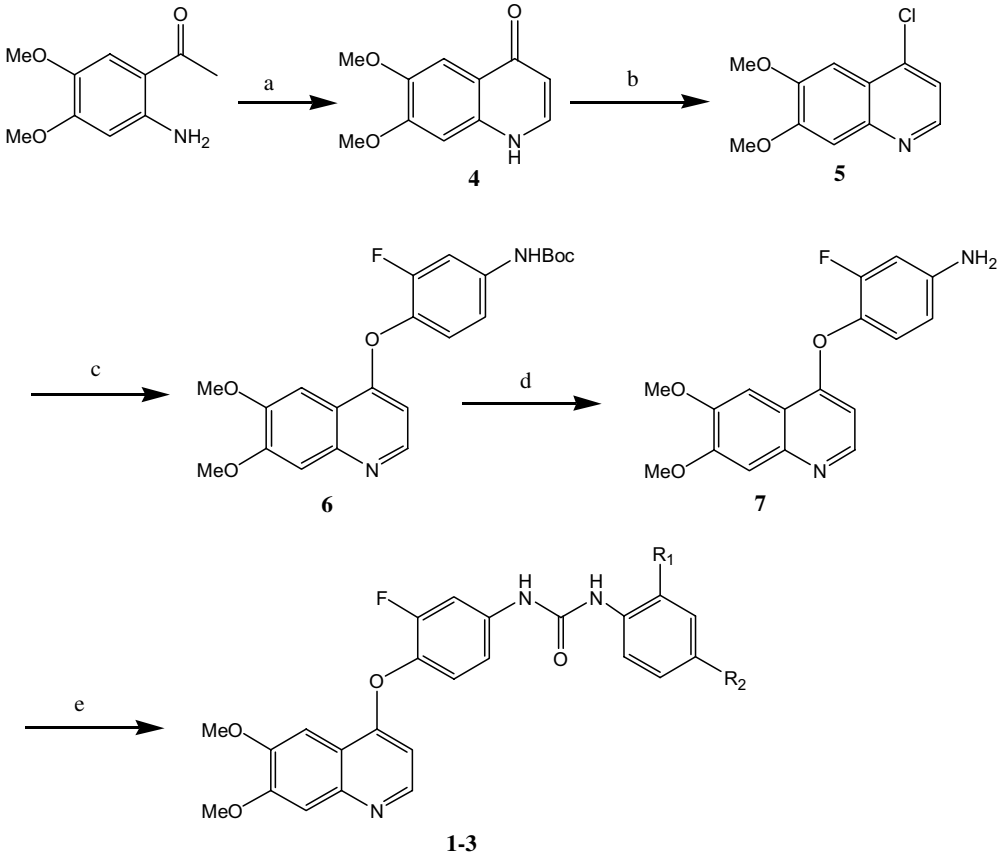


Scheme 2. VEGFR-2/PDGFR dual inhibitors.

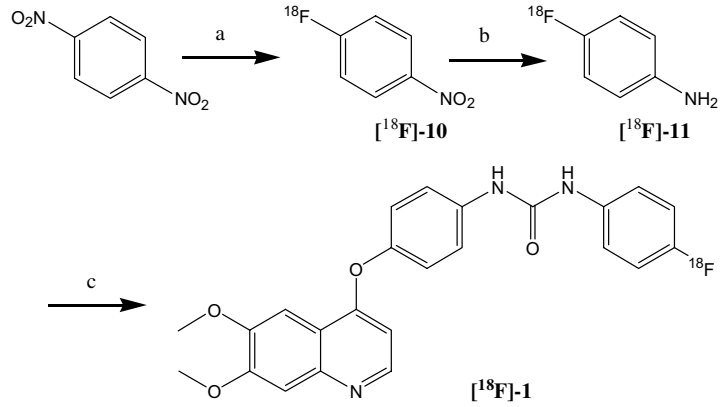
tion times, may be inapplicable. However, isopropenyl carbamates, formed by reaction with isopropenyl chloroformate, are unique in this family since they react with amines rapidly and irreversibly to give unsymmetrical ureas in high yield and purity.[17]

The synthesis and purification of the carbamate precursor **(9)** were performed as described by Gallou et al.[17] According to this report, different isopropenyl carbamates may require entirely different conditions for urea formation. In our case, in non-radioactive experiments, compound **1** formation was obtained at reaction temperatures above 145 °C. Under these temperatures, some precursor degradation was also evident in mass spectroscopy analysis. When these conditions were applied at the tracer level, extensive degradation was observed while conversion of [^{18}**F**]-**11** to [^{18}**F**]-**1** reached a maximum EOB decay corrected radiochemical yield of 10% (n = 3).

2.2.4. Isocyanate. The final method is the commonly used isocyanate-mediated urea formation. While isocyanates react with amines rapidly, under mild conditions, their formation requires the use of phosgene or one of its analogs (diphosgene or triphosgene). A major drawback in this approach is the use of phosgene which is a toxic gas and while both analogs are less toxic, they spontaneously release phosgene.[24–26] Furthermore, the most common way of purifying the reactive isocyanates is via distillation, which is a challenging undertaking when dealing with high molecular weight derivatives requiring distillation at high temperatures.



Scheme 3. Synthetic routes for the production of VEGFR-2/PDGFR dual inhibitors. Reagents: (a) NaOMe, ethyl formate; (b) oxalyl chloride, DCM; (c) (3-fluoro-4-hydroxy-phenyl)-carbamic acid *tert*-butyl ester, DMF, DMAP; (d) HCl/dioxane; (e) triphosgene followed by a reaction with a corresponding aniline.



Scheme 4. Radiosynthesis of [^{18}F]-**1**. Reagents and conditions: (a) [^{18}F]KF/Kryptofix complex, DMF, 120 °C; (b) raney-Ni/hydrazine hydrate, EtOH:H_2O, 57 °C; (c) isocyanate precursor, DCM, rt, 30 min.

Although triphosgene was successfully used for the synthesis of our non-labeled title compounds, the isocyanates were used without any purification and therefore several hurdles might evolve at the tracer level transformation. At the tracer level, since the concentration of the impurities is, in most cases, higher than that of the radioactive intermediate, it may lead to the formation of numerous side products.

With the triphosgene, there are two possible approaches. Either the [^{18}F]-**11** or compound **7** can be transformed into isocyanates and then reacted with the counter amine to give the desired [^{18}F]-**1**. Although both options were attempted, only one succeeded. When [^{18}F]-**11** was reacted with either a commercially available solution of phosgene in toluene or triphosgene in DCM, HPLC revealed that a multiple number of radioactive moieties had formed after 10 min at room temperature. None of these moieties reacted with **7** ($n = 2$). We suspect that since the 4-[^{18}F]fluoro-aniline synthon ([^{18}F]-**11**) was eluted from the Oasis cartridge with a significant amount of 1,4-

diaminobenzene, there was already an urea forming reaction before the addition of **7**.

In order to overcome this problem, triphosgene was added to a solution of [^{18}**F**]-**11** and **7** in DCM, but the formation of [^{18}**F**]-**1** was not detected by analytical HPLC. Although it is possible to separate [^{18}**F**]-**11** from all the other non-radioactive species using HPLC purification prior to transformation into labeled isocyanates, this procedure is not customary practice in the

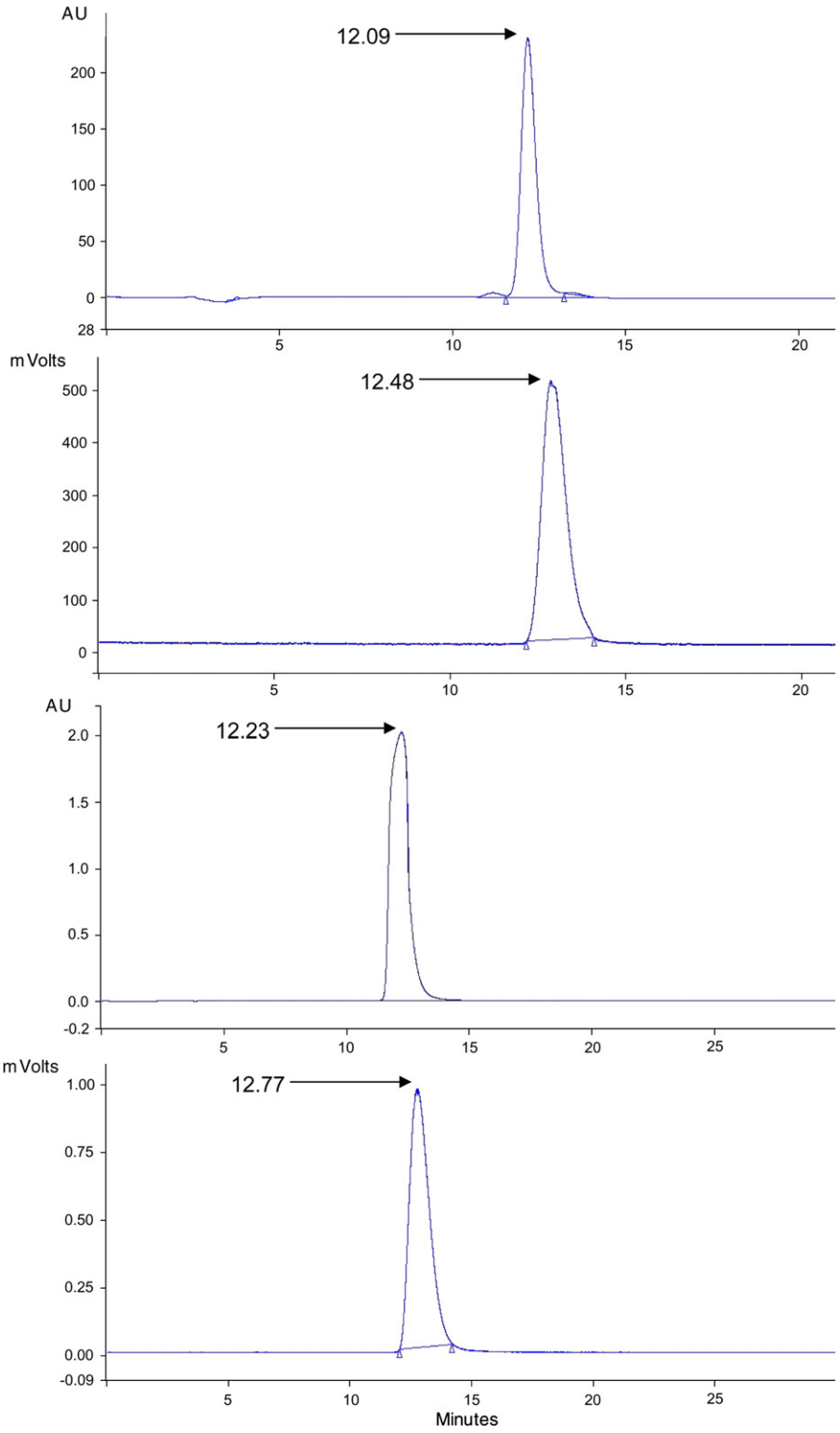


Figure 1. HPLC chromatograms of analytical injections of **1**, [^{18}**F**]-**1** and a co-injection of the two. From top to bottom: UV chromatogram of **1**, Radio chromatogram of [^{18}**F**]-**1**, A co-injection of **1** and [^{18}**F**]-**1** showing both UV and radio chromatograms. A difference of ~0.5 min exists between the UV and radio peaks since the solvent passes through UV detector and only then through the radioactivity detector.

realm of radiochemical transformation with short-lived isotopes, and thus one last alternative was attempted.

The non-labeled isocyanate precursor **8** formed as described in Section 2.1 was reacted with [^{18}**F**]-**11** in dry DCM to give complete conversion of the latter to [^{18}**F**]-**1** after 30 min at room temperature (Fig. 1). The reaction proceeded efficiently and no other radioactive moieties were detected by HPLC (Scheme 4). [^{18}**F**]-**1** was purified using semipreparative HPLC giving an overall 46% radiochemical yield decay corrected to EOB, a specific activity of 400 ± 37 GBq/mmol and a 99% radiochemical purity (n = 5).

2.3. Biology

Several inhibitors of both vascular endothelial growth factor receptor 2 (VEGFR-2) and platelet-derived growth factor receptor (PDGFR) were synthesized, based on the *N*-phenyl-*N*′-{4-(4-quinolyloxy)phenyl}urea chemical core structure. This family of molecules was selected due to its high specificity toward these receptors, known to be upregulated in angiogenic processes.[27] The three derivatives (**1**–**3**) were synthesized with varying fluorine positioning on the aniline ring for structure–activity relationship evaluation of receptor phosphorylation inhibition (Scheme 2).

Evaluation of median inhibitory concentration (IC_{50}) values for inhibition of receptor phosphorylation was carried out using porcine aortic endothelial (PAE/KDR) and NIH/PDGFR cells, expressing VEGFR-2 and $PDGFR_{\beta}$, respectively.[28,29] Inhibitory selectivity with respect to the tyrosine kinase receptors EGFR and HER2 was determined using DHER14 and CSH12 cells, which express EGFR and a HER1-HER2 chimera, respectively.[30–32] Selectivity toward the IGF-1R was assessed by ELISA.[33] Although published results reported varying VERGFR-2/PDGFR selectivity as a result of fluorine positioning on the different phenyl rings,[6] we observed minor influence of fluorine positioning on VERGFR-2/PDGFR selectivity in whole cell assays. All three compounds exhibited high autophosphorylation inhibition with IC_{50} values of around 5–15 nm for both VEGFR-2 and $PDGFR_{\beta}$, in addition to having very high selectivity in comparison to other members of the receptor tyrosine kinase (RTK) superfamily (Table 1).

Table 1. IC_{50} values (nM) obtained in whole cell assays for the three tested compounds

Compound	PDGFR[b]	VEGFR[b]	EGFR[c]	Her-2[c]	IGF-1R[a,b]
1	2–5	8–20	>500	>500	>2000
2	2–5	4–10	>500	>500	>2000
3	2–5	n.d.	>500	>500	>2000

n.d., not determined.
[a] IGF-1R values were obtained in cell-free assays.
[b] n = 3.
[c] n = 2.

Table 2. Extracted fraction and extracted intact [^{18}**F**]-**1** from ex vivo blood stability experiments

Incubation (min)	Extracted fraction (%)	Intact tracer (HPLC, TLC) (%)
0	60–68	99
30	74–77	99
60	70–72	99
120	79–80	99

2.4. Ex vivo blood stability

[^{18}**F**]-**1** was evaluated ex vivo for its stability in human blood as described in the past[32] with several modifications. After incubation in blood samples, the tracer was extracted at different time points (0, 30, 60, and 120 min). The extracted radioactivity fraction was measured, and formation of metabolites was evaluated using both HPLC (Table 2 and Fig. 2) and TLC [data not shown]. The extracted fraction was in the range of 68–79% (n = 2, in duplicates) of the total radioactivity. Both HPLC and TLC revealed that 99% of the extracted radioactivity corresponded to the intact tracer at all time points.

3. Conclusion

In conclusion, out of the three approaches, only one proved to be valuable in our attempt to develop a general method for labeling diaryl ureas. The isocyanate group reacted rapidly with a n.c.a fluorine-18 labeled aniline to give high yields of diaryl urea moiety under mild conditions and short reaction times. This successful approach was used to label the high affinity and selective VEGFR-2/PDGFR inhibitor [^{18}**F**]-**1** for the potential of being used as PET imaging agents in angiogenesis. [^{18}**F**]-**1** was found to be highly stable in ex vivo blood tests.

4. Experimental

4.1. General methods

All operations with air- and moisture-sensitive compounds were performed by the Schlenk techniques under argon atmosphere. All solvents were of analytical grade or better. THF was distilled over sodium/benzophenone; other solvents were purchased as anhydrous. ^{1}H and ^{19}F NMR spectra were recorded on 300 MHz spectrometers in DMSO-d_6. ^{1}H signals are reported in ppm. ^{1}H NMR signals are referenced to the residual proton (2.50 ppm for DMSO-d_6) of a deuterated solvent and for ^{19}F NMR spectra; the signal of $CFCl_3$ (0 ppm) was used as an internal reference. Mass spectra were obtained on a spectrometer equipped with CI, EI, and FAB probes and on a spectrometer equipped with ESI probe. HRMS results were obtained on MALDI-TOF and ESI mass spectrometers. IR spectra were recorded on a FTIR spectrometer. Reaction progress was monitored by TLC (SiO_2) and visualized using UV light. Flash chromatography was carried out on SiO_2 (0.04–

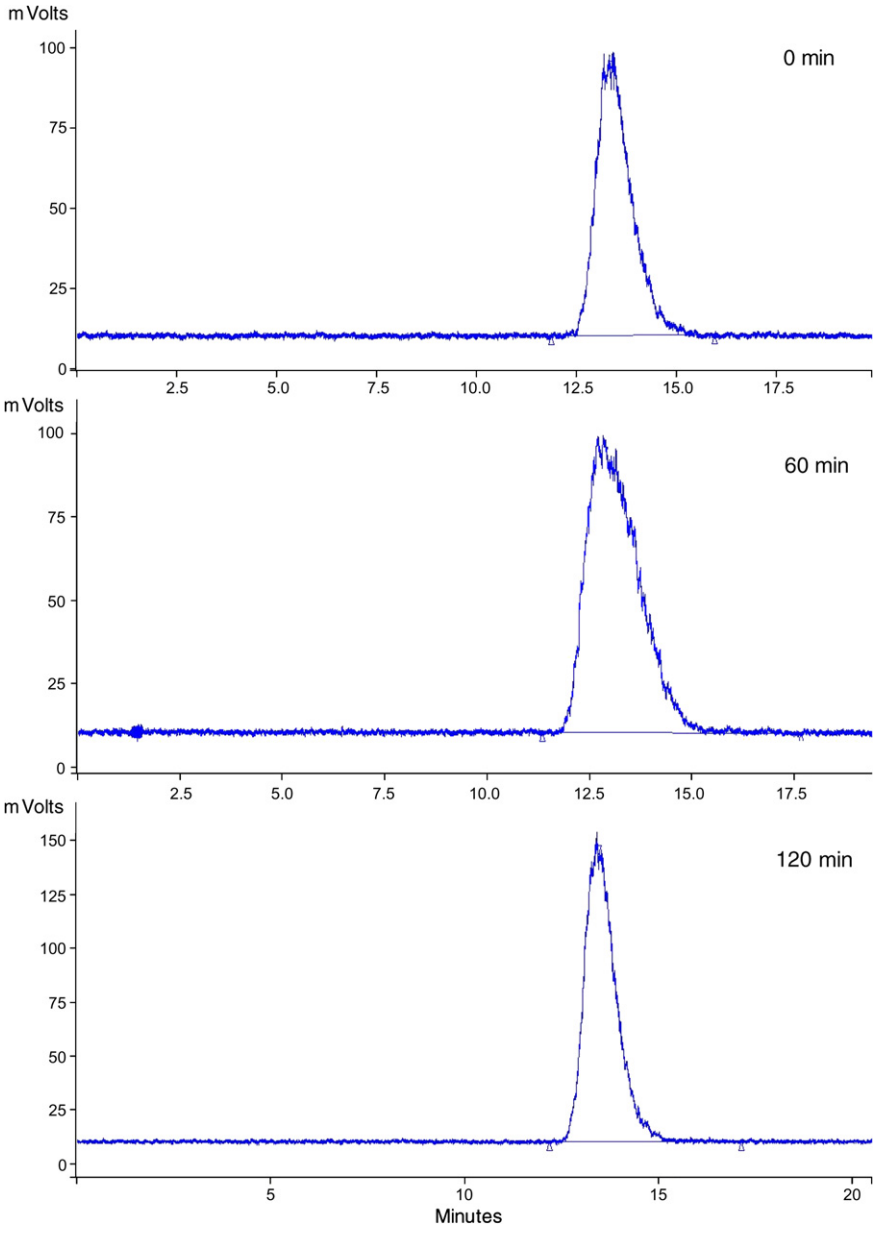


Figure 2. HPLC chromatograms of the radioactivity extracted from blood sample at 0, 60, and 120 min.

0.063 mm). HPLC was performed on a system with a variable wavelength detector operating at 254 nm and a radioactivity detector with a NaI crystal.

Thin layer chromatography (TLC) for blood stability experiments was preformed using normal phase TLC plates (LK6DF, Whatman) and 1:9 methanol/dichloromethane as eluent. TLC plates were dried and exposed for 1.5 h to phosphor imager plates (BAS-IP MS 2040 Fuji Photo Film Co., LTD) for visualization of radioactive bands. The plates were scanned with a BAS reader 3.1-version scanner, and analyzed with TINA 2.10 g software. Two HPLC systems were used: (A) a reversed-phase system employing a Bishoff 100 C-18 column (7 μm, 250 × 10 mm) and 45% CH_3CN/55% acetate buffer 0.1 M/pH 3.8 as eluent, at a flow rate of 4.8 mL/min. Fractions of 4.0 mL were collected. (B) Analysis of formulated radiotracer was performed on a reversed-phase system using a Waters μBondapak C-18 column (10 μm, 125A, 300 × 3.9 mm) and 55% CH_3CN/45% acetate buffer 0.1 M/pH 3.8 as solvents at a flow rate of 1 mL/min. Fluorine-18 was produced on an IBA 18/9 cyclotron by irradiation of 2 mL water target (97%-enriched [^{18}O]water) by the [^{18}O(p,n)^{18}F] nuclear reaction and was transferred to the appropriate hot cell.

4.2. Chemistry

4.2.1. 6,7-Dimethoxy-4-quinolone (4).[6] To a solution of 2-amino-4,5-dimethoxyacetophenone (10.0 g, 51 mmol) in dimethoxyethane (250 mL), NaOMe (8.3 g, 154 mmol) was added and the mixture stirred at room temperature for 70 min. Ethyl formate (21 mL, 261 mmol) was then added and stirring continued for 2 h. Reaction was stopped by adding water (10 mL).

The solution was neutralized with 10% aq HCl solution forming a white precipitate. The resulting solid was collected by filtration and washed with water, dioxane, and AcOEt. The solid was dried under vacuum to obtain 10.0 g (95%) of **4**. ^{1}H NMR (DMSO-d_6): δ 7.75 (d, J = 7.5 Hz, 1H), 7.41 (s, 1H), 6.95 (s, 1H), 5.91 (d, J = 7.5 Hz, 1H), 3.83 (s, 3H), 3.8 (s, 3H). MS (ESI, *m*/*z*): 206 (M+1).

4.2.2. 4-Chloro-6,7-dimethoxy-quinoline[34] (5). 6,7-Dimethoxy-4-quinolone (4, 2 g, 10 mmol) and oxalyl chloride (4.4 mL, 50 mmol) were dissolved in DCM (40 mL) and DMF (0.2 mL) was added. The mixture was refluxed overnight, the volatiles were distilled out, and the purity of the product, 4-chloro-6,7-dimethoxy-quinoline, was determined using a reversed-phase C-18 analytical HPLC column (94% purity). The compound was kept at 0 °C, and used without any further purification for the next step. Mp 130–131 °C. ^{1}H NMR (DMSO-d_6): δ 8.68 (d, J = 5.1 Hz, 1H), 7.66 (d, J = 4.8 Hz, 1H), 7.49 (s, 1H), 7.4 (s, 1H), 3.99 (s, 3H), 3.98 (s. 3H). MS (ESI, *m*/*z*): 224 (M+1).

4.2.3. [4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-carbamic acid *tert*-butyl ester (6). Compound **5** (4.33 g, 19.4 mmol) and (3-fluoro-4-hydroxy-phenyl)-carbamic acid *tert*-butyl ester (7.22 g, 31.7 mmol) and DMAP (4 g, 31.7 mmol) were dissolved in DMF (150 mL) and stirred at 145 °C for 5 h. The DMF was evaporated under reduced pressure and replaced with DCM, washed with brine, dried over Na_2SO_4, and evaporated to give a brown oil. Purification was achieved using flash column chromatography with 40% EtOAc:Hexane as eluent to give 4.13 g (51%) of yellow oil. ^{1}H NMR (DMSO-d_6): δ 9.73 (s, 1H), 8.44 (d, J = 5.1 Hz, 1H), 8.432 (s, 1H), 7.93 (s, 1H), 7.64 (d, J = 12.3 Hz, 1H), 7.5 (s, 1H), 7.28-7.4 (m, 2H), 3.92 (s, 6H), 1.48 (s, 9H). MS (ESI, *m*/*z*): 415 (M+1).

4.2.4. 4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenylamine[6] (7). Compound **6** (4.13 g, 9.98 mmol) was dissolved in HCl in dioxane (2 M, 300 mL) and stirred at room temperature for 5 h. A white sediment formed. The solution was diluted with ether and filtered to give the HCl salt as a white paste. This paste was first dissolved in water and washed with DCM and then by increasing the pH, the free base sediments were collected by filtration. To ascertain product purity, the solid was cleaned using a short silica plug with EtOAc as eluent to give 2.07 g (66%) of the title compound. Mp 196–197 °C. ^{1}H NMR (DMSO-d_6): δ 8.43 (d, J = 6.3 Hz, 1H), 7.48 (s, 1H), 7.35 (s, 1H), 7.05 (t, J = 8.7 Hz, 1H), 6.35–6.55 (m, 3H), 5.48 (s, 2H), 3.91 (s, 6H). MS (ESI, *m*/*z*): 315 (M+1).

4.2.5. 1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(2-fluoro-phenyl)-urea (2). A solution of triphosgene (190 mg, 0.65 mmol) in dry DCM (10 mL) was cooled to 0 °C and a solution of compound **7** (200 mg, 0.64 mmol) and Et_3N (180 μL, 1.3 mmol) in dry DCM (10 mL) were added slowly over a period of 1 h under nitrogen. The solution was then stirred at room temperature for 2 h and evaporated to dryness. The solid obtained was redissolved in dry DCM (10 mL) and a solution of 2-fluoroaniline (0.12 mL, 1.3 mmol) and Et_3N (0.18 mL, 1.3 mmol) in dry DCM (10 mL) were added in one portion. After stirring at room temperature overnight, the solvent was evaporated and the product purified using flash column chromatography with 50% EtOAc:Hexane to give 185 mg of the title compound as a white solid (65%). Mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 9.38 (s, 1H), 8.64 (s, 1H), 8.47 (d, J = 5.1 Hz, 1H), 8.11 (t, J = 8 Hz, 1H), 7.75 (d, J = 14 Hz, 1H) 7.52 (s, 2H), 7.39 (t, J = 9.5 Hz, 1H) 7–7.29 (m, 5H), 6.46 (d, J = 5.1 Hz, 1H), 3.93 (s, 6H). ^{19}F NMR (DMSO-d_6): δ −128.89 (dd, J_1 = 13.5 Hz, J_2 = 9 Hz, 1F), −130.45 (m, 1F). HRMS 452.1404 (M+1).

4.2.6. 1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-fluoro-phenyl)-urea (1). A similar procedure as for **2** was used with 4-fluoroaniline to give a white solid which was purified using flash column chromatography with 100% EtOAc to give 140 mg of the title compound as a white solid (50%). Mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 9.08 (s, 1H), 8.88 (s, 1H), 8.46 (dd, J_1 = 6.6 Hz, J_2 = 1.5 Hz, 1H), 7.73 (d, J = 13.5 Hz, 1H), 7.34–7.51 (m, 5H), 7.24 (d, J = 8.4 Hz, 1H) 7.12 (t, J = 7.8 Hz, 2H), 6.43 (d, J = 5.4 Hz, 1H), 3.93 (s, 6H). ^{19}F NMR (DMSO-d_6): δ −129.13 (dd, J_1 = 14.1 Hz, J_2 = 8.7 Hz, 1F), −121.92 (m, 1F). HRMS 452.1439 (M+1).

4.2.7. 1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(2,4-difluoro-phenyl)-urea[6] (3). A similar procedure as for **2** was used with 2,4-difluoroaniline to give a white solid which was purified using flash column chromatography with 100% EtOAc to give 152 mg of the title compound as a white solid (50%). Mp 205–206 °C. ^{1}H NMR (DMSO-d_6): δ 9.34 (s, 1H), 8.6 (s, 1H), 8.46 (d, J = 5.1 Hz, 1H), 8.03 (m, 1H), 7.73 (dd, J_1 = 10.8 Hz, J_2 = 2.4 Hz, 1H) 7.51 (s, 1H), 7.28–7.42 (m, 3H), 7.22 (d, J = 9.3 Hz, 1H), 7.305 (t, J = 9.3 Hz, 1H) 6.43 (d, J = 5.4 Hz, 1H) 3.93 (s, 6H). ^{19}F NMR (DMSO-d_6): δ −128.92 (dd, J_1 = 12.9 Hz, J_2 = 9.9 Hz, 1F), −125.21 (m, 1F), −118.39 (m, 1F). HRMS 470.1350 (M+1).

4.2.8. 4-(2-Fluoro-4-isocyanato-phenoxy)-6,7-dimethoxy-quinoline (8). A solution of triphosgene (12 mg, 41 μmol) in dry DCM (2 mL) was cooled to 0 °C and a solution of compound **7** (20 mg, 64 μmol) and Et_3N (17 μL, 12 mg, 122 μmol) in dry DCM (4 mL) was added slowly over a period of 1 h. The solution was then stirred at room temperature for 3 h and a sample taken under nitrogen for IR evaluation. Volatiles were evaporated under nitrogen to dryness to give a yellow solid which was kept at 4 °C under nitrogen until further use. IR (NaCl) 2285 cm^{-1}.

4.2.9. [4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-carbamic acid isopropenyl ester (9). Compound 7 (100 mg, 0.32 mmol) and *N*-methylmorpholine (40 μL, 0.38 mmol) were dissolved in THF (8 mL) and the solution was cooled in an ice bath. Isopropenyl chlorofor-

mate (40 μL, 0.38 mmol) was added dropwise over 30 min and the reaction stirred at room temperature for 4 h. Reaction was quenched by adding water, extracted with EtOAc and the organic fractions washed with brine, dried over Na_2SO_4 and evaporated to give a yellow solid. Purification was performed by flash column chromatography with 60% EtOAc:Hexane as eluent to give 94 mg of the title compound as a white solid (74%) ^{1}H NMR (DMSO-d_6): δ 10.31 (s, 1H), 8.45 (d, J = 5.4 Hz, 1H), 7.61 (dd, J_1 = 10.5 Hz, J_2 = 2.4 Hz, 1H), 7.5 (s, 1H), 7.31–7.45 (m, 3H), 6.41 (d, J = 4.2 Hz, 1H), 4.77 (d, J = 8.1 Hz, 2H), 3.92 (s, 6H), 1.94 (s, 3H). MS (ESI, m/z): 399 (M+1).

4.2.10. 4-[^{18}F]fluoro-nitrobenzene[23] ([^{18}F]-10). An [^{18}O]H_2O/[$^{18}F^-$] (26 GBq) mixture was trapped and transferred to a reactor through an ion exchange column (preactivated with 0.8 mL of EtOH and 3 mL of HPLC water) by elution with 0.5 mL of potassium carbonate (2.76 mg/mL). Following the addition of Kryptofix 2.2.2 solution (6 mg, 16 μmol in 1 mL CH_3CN), azeotropic removal of water and acetonitrile was achieved by heating the reactor to 100 °C under a stream of nitrogen for 3 min and under vacuum for another 1 min. 1,4-Dinitrobenzene (2.2 mg, 13 μmol) dissolved in DMF (600 μL) was added, the reactor temperature was increased to 120 °C and the reaction mixture was stirred for 20 min. After cooling the mixture to 30 °C, 7 mL of water was added to the reactor, and the mixture was loaded on a C-18 cartridge (Waters Sep-Pak; preactivated with 5 mL of EtOH and 10 mL of sterile water). The product, 4-[^{18}F]fluoro-nitrobenzene, was washed with another 10 mL of water and eluted with 2 mL of EtOH into a collection vial after a total radiosynthesis time of 40 min and was obtained with an 82% radiochemical yield. The product was analyzed by HPLC using system A (rt = 7.4 min) and compared to a standard material.

4.2.11. 4-[^{18}F]fluoro-aniline[23] ([^{18}F]-11). Hydrazine monohydrate (150 μL) was added to a reactor containing 200 μL of EtOH/H_2O (4:1) and raney (Ra)-Ni (400 μL) at 57 °C, and then [^{18}**F]-10** in EtOH/H_2O (2.5 mL, 4:1) was added dropwise to the reactor. Synthesis continued at 57 °C for 7 min, another portion of hydrazine monohydrate (150 μL) was added to the reactor and synthesis continued for an additional 5 min. The product was diluted with 10 mL of water, passed through an Oasis HLB plus cartridge (Waters; preactivated with 5 mL of MeOH and 10 mL of sterile water) and dried under argon for 20 min to yield 4-[^{18}F]fluoro-aniline with a 76% decay corrected radiochemical yield. The product was eluted with our solvent of choice (2 mL), dried over Na_2SO_4, filtered via 0.45 μm filter (Whatman, Puradisc), and analyzed by HPLC using system A (rt = 5.8 min) and compared to a standard material.

4.2.12. 1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-3-fluoro-phenyl]-3-(4-[^{18}F]fluoro-phenyl)-urea ([^{18}]F-1). To the isocyanate precursor described above dry (sodium distilled) Et_3N (30 μL, 0.2 mmol) and [^{18}**F]-11** in dry DCM (2 mL) were added. Reaction was stirred at room temperature under nitrogen for 30 min and then the DCM was evaporated and replaced with CH_3CN/H_2O (1:1) (1 mL). The product was purified using HPLC system B, generating [^{18}]F-U8 at rt = 24 min. The fractions collected were diluted with water, loaded onto a C-18 Sep-Pak, dried and eluted with EtOH (1 mL). A 50 μL sample was injected into an analytical HPLC using system A for purity and specific activity analysis. The labeled compound was obtained with a 74% radiochemical yield, a specific activity of 400 ± 37 GBq/mmol and a 99% radiochemical purity (n = 5). Co-injection of [^{18}]**F-1** and **1** standard (system A) followed to ensure of the compound's identity (Fig. 1).

4.3. Biology

4.3.1. Inhibition of VEGFR-2 phosphorylation in PAE/KDR cells. PAE/KDR cells (1×10^5 cells/well) were grown in 6-well plates for 24 h, reaching 80–90% confluence. Cells were incubated with inhibitors **1**–**3** at increasing concentrations (0.05% DMSO, 0.1% EtOH) for 1 h. Following incubation of the tested compound, cells were supplemented with Na_3VO_4 (100 μM, 5 min) (Sigma) to inhibit endogenous phosphatase activity, and stimulated for 5 min at 37 °C with 50 ng/mL VEGF-A_{165} (CytoLab Ltd, Israel). Following a wash with ice-cold PBS containing 100 μM Na_3VO_4, cell lysates were prepared in boiling Laemmli buffer. The extent of VEGFR-2 phosphorylation was evaluated by Western blot analysis as described in the past.[32]

4.3.2. Selectivity assay. CSH12, DHER14, and NIH/PDGFR cells, expressing either the HER1-HER2 chimera, EGFR or $PDGFR_\beta$, respectively, were used for the determination of inhibitory selectivity. Cells (7.5×10^4) were grown in 6-well plates (Nalge Nunc Int.) for 24 h and then incubated in 0.25% FCS-containing medium for an additional 24 h to ~90% confluence. Duplicate sets of cells were treated with the tested compound at varying concentrations for 1 h. The final concentration of the vehicle in the medium was 0.05% DMSO, 0.1% EtOH. After removal of the inhibitor from the medium, PBS wash (×2) and addition of 0.25% serum-containing medium to the wells, the cells were stimulated with either 20 ng/mL hEGF (Sigma) for 5 min (CSH12 and DHER14 cells) or 50 ng/mL human $PDGF_{\beta\beta}$ (Sigma) for 10 min (NIH/PDGFR cells) at 37 °C. Following stimulation with the growth factor, the cells were washed with cold PBS. Cell lysate preparation and Western blot analysis were described in the past.[32]

4.3.3. Cell-free assay. An evaluation of the IGF-1R autophosphorylation IC_{50} values was obtained by means of an ELISA screen, performed as depicted earlier.[35] For each compound, at least three independent assays that gave similar results were considered. Each assay was carried out using duplicate samples.

4.3.4. In vitro blood stability. Fresh human blood samples were collected in a Vacutainer™ containing EDTA (K3, BD, USA), divided into 1 mL aliquots in glass-vials and incubated for 5 min at 37 °C with gentle shaking.

Approximately 300 μCi (~200 μL, 10% ethanol in saline) of [^{18}]**F-1** was added to blood samples for pre-determined incubation time periods, after which the samples were removed from the incubator and centrifuged at 3500 rpm for 5 min. The plasma was separated from the precipitant and radioactivities were measured for both fractions in a dose calibrator (CAL/RAD Mark IV, Nuclear Associated). To the volume of plasma extracted, a double volume of cold 30% THF/ACN solution was added. The solution was vortexed for 30 s and then centrifuged again for 5 min at 10,600 rcf (g) and pellet and plasma were separated and measured for radioactivity. The protein free plasma was then filtered through a 0.45 μm filter (Puradisc, Whatman) and samples were both injected into HPLC using system B and loaded on a TLC plate as described in the general methods paragraph.

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