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Combinatorial synthesis of labelled drugs and PET tracers: synthesis of a focused library of ^{11}C-*carbonyl*-labelled acrylamides as potential biomarkers of EGFR expression

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Combinatorial synthesis is extensively used in drug development and lead optimisation. However, this approach has rarely been used for positron emission tomography because of limitations in available technologies. [^{11}C]Carbon monoxide is amenable to combinatorial synthesis in transition-metal-catalysed reactions because it can react with a wide variety of electrophiles and nucleophiles, which opens up the possibilities for combinatorial radiochemistry. Herein, we exemplify the combinatorial approach by ^{11}C-labelling a library of epidermal growth factor receptor inhibitors. The selection of candidates was guided by molecular docking. Epidermal growth factor receptor is overexpressed in a variety of tumours, and it has become an important drug target. The ^{11}C-labelling reactions were performed using four substituted vinyl iodides and three different 4-anilino-6-aminoquinazolines using a palladium-mediated reaction with [^{11}C]carbon monoxide using a single set of reaction conditions. In total, 12 labelled acrylamide derivatives were radiolabelled and obtained in 24–61% decay-corrected radiochemical yield (from [^{11}C]carbon monoxide). Starting from 5.6 GBq [^{11}C]carbon monoxide, 0.85 GBq of formulated *N*-[4-(3-bromo-phenylamino)-quinazolin-6-yl]-acryl[^{11}C]amide [^{11}C]12da was obtained within 47 min from end of bombardment (specific activity of 60 GBq μmol^{-1}). This strategy is an example of how [^{11}C]carbon monoxide can be utilised in the labelling of libraries of drug candidates and positron emission tomography tracers for *in vitro* and *in vivo* testing.

Keywords: combinatorial chemistry; carbonylation; palladium; PET chemistry; radiolabelling

Introduction

Positron emission tomography (PET) is a non-invasive imaging technology that is based on the spatial detection of a positron-emitting radionuclide incorporated into a biologically relevant molecule, that is, a radiotracer. The use of high specific activity ^{11}C ($t_{1/2}$ = 20.4 min) allows a labelled molecule to be administered in very small quantities (usually in the order of μg) and followed over time *in vivo* using a PET scanner. The small mass administered means that the regulatory burden is significantly lower when compared with the administration of pharmacologically active doses.[1] Because of this reason, PET is increasingly used in drug development for evaluation of new drug candidates and first test in man following the micro-dosing concept.[1] These phase 0 studies usually include biodistribution and dose occupancy studies and often lead to a more rapid decision-making process on new candidate drugs and save time and resources. Despite these benefits, today, only the optimised lead in a typical drug development programme is evaluated using this powerful technology. This is to an extent associated with limitations in what type of chemical reactions that can be performed with the most commonly used labelled precursors, that is, [^{11}C]methyl iodide/triflate and [^{18}F] fluoride. Far from all drug candidates contain a methyl group or a fluorine suitable for labelling. ^{18}F-labelling often involves harsh reaction conditions, thus lengthy multi-step reactions and prosthetic groups are needed to overcome this issue. Another shortcoming of methylation and fluorination is that only one chemical bond is formed to the radionuclide, thus the chemical variation possible in a series of analogues is limited. The carbonyl group, on the other hand, is commonly found in biologically active molecules, and the carbon binds two other atoms except for the doubly bound oxygen atom. Incorporation of a carbonyl group can be achieved via carbonylation. A unique and attractive feature of carbon monoxide is that two bonds are formed in a typical cross-coupling reaction, thus the possibility of using carbonylations for combinatorial chemistry.[2]

Several chemical and technological methods to overcome the low solubility and reactivity of [^{11}C]carbon monoxide have been evaluated including recirculation techniques,[3] chemical complexation with THF·BH_3,[3] or copper(I)scorpionates,[4] high pressure reactors,[5] micro-fluidic reactors[6] and xenon-based atmospheric

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pressure reactors.[7] These efforts have the potential to make [^{11}C] carbon monoxide available for any lab with an on-site cyclotron. [^{11}C]Carbon monoxide can be used to synthesise a plethora of functional groups commonly found in drugs via transition-metal-mediated carbonylative cross-coupling reactions. These include but are not limited to amides, ureas, sulfonylureas, ketones, carboxylic acids, carbonates, esters, thioesters, carbamates and thiocarbamates.[8]

Radiolabelling and screening of libraries is a powerful optimisation tool and has the potential to bring valuable knowledge about structure activity relationship for both tracer and drug candidates as exemplified by ^{11}C-labelled analogues of WAY-100635,[9] ^{11}C-labelled inhibitors of the vascular endothelial growth factor receptor 2,[10] ^{18}F-labelled peptides for the integrin $\alpha_v\beta_6$,[11] ^{11}C-labelled cyclooxygenase inhibitors[12] and ^{11}C-labelled DAA1106 analogues.[13]

In this manuscript, we wish to emphasise the usefulness of [^{11}C]carbon monoxide for synthesis of focused radiolabelled compound libraries, here exemplified with a series of epidermal growth factor receptor (EGFR) inhibitors labelled in the carbonyl position. The development of a PET tracer has similarities to drug development and radiolabelled drugs, but there are also differences. Occasionally, radiolabelled drugs can serve as tracers as exemplified with the EGFR tyrosine kinase inhibitor (TKI) [^{11}C]erlotinib.[14] On the other hand, the EGFR TKI [^{18}F]gefitinib was inefficient for *in vivo* tumour imaging because of high non-specific binding and non-saturable cellular uptake.[15] From the two examples mentioned, it is clear that even small molecular differences will have a profound effect on a molecule's chance to work as a tracer. Our library approach enables important molecular properties such as pK_a, lipophilicity and reactivity to be systematically varied.

Experimental

General

Reagent grade THF was freshly distilled from sodium and benzophenone in a nitrogen atmosphere. All chemicals were purchased from Aldrich/Fluka (Stockholm, Sweden) or Fluorochem (Derbyshire, UK) and used as received. The identities of the purified ^{11}C-labelled compounds [^{11}C]**12bb**, [^{11}C]**12da**, [^{11}C]**12db** and [^{11}C]**12dc** were determined with liquid chromatography–mass spectrometry (LC-MS) and analytical HPLC equipped with a ultraviolet (UV) and radioactivity detector using authentic samples as references. For the remaining compounds, LC-MS or LC-MS equipped with a radioactivity detector was used. LC-MS analyses on ^{11}C-labelled compounds (decayed and/or radioactive) were performed on a Gilson (Middleton, WI, USA) reverse-phase HPLC equipped with a Waters (Milford, MA, USA) Quattro Premier triple quadrupole mass spectrometer with ESI, operating in selective positive ion mode. Analytical HPLC was performed on a Beckman (Bromma, Sweden) 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-μ column (250 × 4.6 mm). A Gilson 231 XL was used as the auto-injector. Purification with semi-preparative HPLC was performed on a similar Beckman system equipped with an Alltech Alltima HP C18 5-μ guard cartridge (7.5 × 4.6 mm) and a Grace Genesis C18 4-μ column (250 × 10 mm). Flow 4 mL min^{-1}. Method A: 30–70% acetonitrile over 10 min in 50-mM ammonium formate pH 3.5. Method B: 45–50% acetonitrile over 10 min in 10-mM KH_2PO_4. Method C: 30–80% acetonitrile over 10 min in 50-mM ammonium formate pH 3.5. ^{11}C was prepared by the ^{14}N(p,α)^{11}C nuclear reaction using 17-MeV protons 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). [^{11}C]Carbon dioxide was transferred in a stainless steel tubing concentrated on silica in a cold trap (−196 °C). [^{11}C]Carbon dioxide was reduced to [^{11}C]carbon monoxide over Zn granules at 400 °C and concentrated silica in a cold trap (−196 °C). The carbonylation reactions were carried out in a 200-μL stainless steel micro-autoclave using a previously described technique.[5a]

Thin-layer chromatography was performed on pre-coated Merck silica gel plates (60 F_{254}) and visualised with UV light. ^{1}H and ^{13}C NMR spectra were recorded on a Varian (Palo Alto, CA, USA) 400 or 500-MHz spectrometer, and chemicals shifts are given in ppm (δ) and referenced to residual solvent. *J* values are given in Hz. LC-MS analyses of reference compounds and precursors were performed on a Gilson reverse-phase HPLC equipped with a Finnigan ESI mass spectrometer (MeCN/H_2O and 0.1% formic acid). Intermediates, precursors and references were synthesised using known methods, and their spectroscopic characteristics corresponded well with those reported; 3-chloro-4-fluoroaniline,[16] 3*H*-quinazoline-4-one (**1**), 6-nitro-3*H*-quinazolin-4-one (**2**), 4-chloro-6-nitro-quinazoline (**3**),[17] (3-bromo-phenyl)-(6-nitro-quinazolin-4-yl)-amine (**4a**),[18] (3-chloro-4-fluoro-phenyl)-(6-nitro-quinazolin-4-yl)-amine (**4b**),[19] [3-chloro-4-(3-fluoro-benzyloxy)-phenyl]-(6-nitro-quinazolin-4-yl)-amine (**4c**), 2-chloro-1-(3-fluoro-benzyloxy)-4-nitro-benzene (**6**), 3-chloro-4-(3-fluoro-benzyloxy)-phenylamine (**7**),[20] (*E*)-3-(tributylstannyl)-2-propen-1-ol (**8**),[21] (*E*)-3-iodo-2-propen-1-ol (**9**),[22] (*E*)-3-bromo-1-iodo-propene (**10**),[23] (*E*)-4-morpholin-4-yl-but-2-enoic acid [4-(3-chloro-4-fluoro-phenylamino)-quinazolin-6-yl]-amide (**12bb**),[24] *N*-[4-(3-bromo-phenylamino)-quinazolin-6-yl]-acrylamide (**12da**),[19] *N*-[4-(3-chloro-4-fluoro-phenylamino)-quinazolin-6-yl]-acrylamide (**12db**)[19] and *N*-{4-[3-chloro-4-(3-fluoro-benzyloxy)-phenylamino]-quinazolin-6-yl}-acrylamide (**12dc**).[25]

*N^4-(3-Bromo-phenyl)-quinazoline-4,6-diamine (**5a**)*[26]

To a slurry of **4a** (0.538 g, 1.56 mmol) in ethanol (20 mL) and water (10 mL) was added iron (0.190 g, 3.41 mmol) and acetic acid (1 mL). The reaction mixture was stirred under heating (95 °C heating bath) for 90 min. After neutralisation with 2 M NaOH, the mixture was filtered. The filtrate was concentrated to approximately half volume and extracted with ethyl acetate, and the combined organic phases were washed with brine. The solvent was removed under reduced pressure to give the product as a yellow solid (0.197 g, 25%). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) δ = 9.49 (s, 1H), 8.36 (s, 1H), 8.24 (s, 1H), 7.88 (m, 1H), 7.52 (m, 1H), 7.37 (s, 1H), 7.32–7.20 (m, 2H), 5.62 (br s, 2H, NH_2) ppm. LC-MS, ESI + for $C_{14}H_{11}BrN_4$ *m/z* [M + H]: 315/317.

*N^4-(3-Chloro-4-fluoro-phenyl)-quinazoline-4,6-diamine (**5b**)*[27]

Synthesised according to the procedure for **5a**. Yield 78% as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ = 9.47 (br s, 1H, NH), 8.36 (s, 1H, H-2), 8.20 (dd, ^4J 2.6, $^4J_{H,F}$ 6.9, 1H, H-2′), 7.82 (ddd, $^3J_{H,H}$ 9.0, $^4J_{H,H}$ 2.6, $^4J_{H,F}$ 4.4, 1H, H-6′), 7.54 (d, $^3J_{H,H}$ 8.9, 1H, H-8), 7.40 (dd, $^3J_{H,H}$ 9.0, $^3J_{H,F}$ 9.0, 1H, H-5′), 7.31 (d, $^4J_{H,H}$ 2.4, 1H, H-5), 7.24 (dd, $^3J_{H,H}$ 8.9, $^4J_{H,H}$ 2.4, 1H, H-7), 5.62 (br s, 2H, NH_2) ppm. LC-MS ESI + for $C_{14}H_{10}ClFN_4$ *m/z* [M + H]: 289/291.

*N^4-[3-Chloro-4-(3-fluoro-benzyloxy)-phenyl]-quinazoline-4,6-diamine (**5c**)*[28]

Synthesised according to the procedure for **5a**. Yield 70% as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ = 9.33 (br s, 1H, NH), 8.31 (s, 1H, H-2), 8.03 (d, $^4J_{H,H}$ 2.6, 1H, H-5), 7.72 (dd, $^4J_{H,H}$ 2.6, $^3J_{H,H}$ 9.0, 1H, H-7), 7.51 (d, $^3J_{H,H}$ 8.8, 1H, H-5′), 7.46 (m, 1H), 7.34–3.30 (m, 3H), 7.23 (dd, $^4J_{H,H}$ 2.5, $^3J_{H,H}$ 8.8, 1H, H-6′), 7.22 (d, $^3J_{H,H}$ 9.0, 1H, H-8), 7.17 (m, 1H), 5.72 (br s, 2H, NH_2), 5.23 (s, 2H, CH_2) ppm. LC-MS, ESI + for $C_{21}H_{16}ClFN_4O$ *m/z* [M + H]: 395/397.

*4-((E)-3-Iodo-allyl)-morpholine (**11b**)*

To an ice-cold stirred slurry of morpholine (0.117, 1.35 mmol) and K_2CO_3 (0.186 g, 1.35 mmol) in MeCN (5 mL) was added dropwise (*E*)-3-bromo-1-iodo-propene **10** (0.302 g, 1.22 mmol) as a solution in MeCN (5 mL) over 10 min. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The solids were filtered off, and the filtrate was concentrated under reduced pressure to give an oil, which was purified using flash chromatography eluting with 0–50% EtOAc in pentane and a few drops of triethylamine to give the product as an oil (0.146 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ = 6.53 (dt, *J* 7.0, 14.4, 1H), 6.25 (dt, *J* 1.4, 14.4, 1H),

3.68 (m, 2H), 2.93 (dd, *J* 1.4, 7.0, 1H), 2.41 (m, 2H) ppm. δ_C (100 MHz, $CDCl_3$, 25 °C) 142.5, 79.0, 67.0, 63.1, 53.4 ppm.

*1-((E)-3-Iodo-allyl)-piperidine (**11a**)*

Synthesised according to the procedure for **11b**. Yield 37% as an oil. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ = 6.58 (dt, *J* 7.0, 14.3, 1H), 6.19 (dt, *J* 1.3, 14.3, 1H), 2.91 (dd, *J* 1.3, 7.0, 2H), 2.35 (m, 4H), 1.57 (m, 4H), 1.42 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ = 143.4, 78.2, 63.6, 54.4, 26.0, 24.3 ppm.

*4-((E)-3-Iodo-allyl)-thiomorpholine (**11c**)*

Synthesised according to the procedure for **11b**. Yield 40.4 mg, 58% as an oil. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ = 6.54 (dt, *J* 6.7, 14.5, 1H), 6.26 (dt, *J* 1.4, 14.5, 1H), 2.96 (dd, *J* 1.4, 6.7, 1H), 2.68 (m, 8H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ = 142.9, 78.4, 63.6, 54.8, 28.2 ppm.

General procedure for synthesis of [*carbonyl*-^{11}C]acrylamides [^{11}C]12aa–dc

$Pd_2(dba)_3$ (0.70 mg, 1.2 μmol) and PPh_3 (2.70 mg, 10 μmol) were placed in a 0.8-mL vial equipped with a rubber septum. THF (600 μL) was added, and the resulting solution was degassed with argon. Vinyl halide **11a–c** (6.0 μmol) was added, whereupon the colour of the solution changed from yellow to pale yellow. Quinazoline precursor **5a–c** (12.5 μmol) was added, and the mixture was loaded through a filter (13-mm Teflon, 0.45-μm pore size) into a 200-μL injection loop. THF was pumped through the loop using an HPLC pump (30–40 MPa), and the reagents were transferred into a pre-heated 200-μL, stainless steel micro-autoclave containing [^{11}C]carbon monoxide (10–100 nmol, 0.12–1 Pa) in helium (40 mmol, 500 kPa). The reagents were kept in the micro-autoclave for 5 min at 110 °C. The reaction mixture was then transferred to a 1-mL, septum-equipped evacuated glass vial, and the radioactivity was measured. The vial was heated at 80 °C for 1–2 min during the removal of volatiles under a stream of nitrogen gas, and radioactivity was measured again. The crude product was redissolved in acetonitrile (400 μL) and then water (400 μL) and purified with semi-preparative HPLC. The radioactivity remaining in the vial was less than 2%. The radioactivity of the purified product was measured, and analytical HPLC equipped with a UV and radioactivity detector and LC-MS was used to assess its identity. The radiochemical purity was >98% for all products. Formulation of the purified product was performed by diluting the purified fraction (3–8 mL) with 15-mL phosphate buffer (10 mM, pH 7.4) and concentrated on a solid phase extraction (SPE) column (Phenomenex (Torrance, CA, USA) Strata-X, previously activated with EtOH and washed with H_2O). The solid phase extraction (SPE) column was washed with H_2O (3 mL) before the radioactive product was eluted using EtOH (0.5 mL) followed by a solution of cyclodextrin (100 μL, 300 mg/mL) diluted in phosphate buffer (950 μL, pH 7.4).

*(E)-4-Piperidin-1-yl-but-2-enoic [carbonyl-^{11}C]acid [4-(3-bromo-phenylamino)-quinazolin-6-yl]-amide (**12aa**)*

HPLC method B. Product t_R = 7.2–8.9 min, aniline precursor t_R = 10.5–16.0 min. LC-MS ESI + for $C_{23}H_{24}BrN_5O$ *m/z*: 466 $(M+H)^+$.

*(E)-4-Piperidin-1-yl-but-2-enoic [carbonyl-^{11}C]acid [4-(3-chloro-4-fluoro-phenylamino)-quinazolin-6-yl]-amide (**12ab**)*

HPLC method B. Product t_R = 8.5–10.0 min, aniline precursor t_R = 10.9–13.9 min. LC-MS ESI + for $C_{23}H_{23}ClFN_5O$ *m/z*: 440 $(M+H)^+$.

*(E)-4-Piperidin-1-yl-but-2-enoic [carbonyl -^{11}C]acid {4-[3-chloro-4-(3-fluoro-benzyloxy)-phenylamino]-quinazolin-6-yl}-amide (**12ac**)*

HPLC method B. Product t_R = 13.0–14.2 min, aniline precursor t_R = 11.8–14.5 min. LC-MS ESI + for $C_{30}H_{29}ClFN_5O_2$ *m/z*: 273.5 $(M+2H)^{2+}$.

*(E)-4-Morpholin-4-yl-but-2-enoic [carbonyl-^{11}C]acid [4-(3-bromo-phenylamino)-quinazolin-6-yl]-amide (**12ba**)*

HPLC method B. Product t_R = 10.0–11.8 min, aniline precursor t_R = 11.8–16 min. LC-MS ESI + for $C_{22}H_{21}ClFN_5O_2$ *m/z*: 468 and 470 $(M+H)^+$.

*(E)-4-Morpholin-4-yl-but-2-enoic [carbonyl-^{11}C]acid [4-(3-chloro-4-fluoro-phenylamino)-quinazolin-6-yl]-amide (**12bb**)*

HPLC method B. Product t_R = 9.9–11.7 min, aniline precursor t_R = 11.8–14 min. LC-MS ESI + for $C_{22}H_{21}ClFN_5O_2$ *m/z*: 221.5 $(M+2H)^{2+}$.

*(E)-4-Morpholin-4-yl-but-2-enoic [carbonyl-^{11}C]acid {4-[3-chloro-4-(3-fluoro-benzyloxy)-phenylamino]-quinazolin-6-yl}-amide (**12bc**)*

HPLC method C. Product t_R = 10.1–10.5 min, aniline precursor t_R = 10.1–13.9 min. The product mass peak could not be detected because of ion suppression by the precursor.

*(E)-4-Thiomorpholin-4-yl-but-2-enoic [carbonyl-^{11}C]acid [4-(3-bromo-phenylamino)-quinazolin-6-yl]-amide (**12ca**)*

HPLC method C. Product t_R = 7.2–8.5 min, aniline precursor t_R = 7.1–9.5 min. The product mass peak could not be detected because of ion suppression by the precursor.

*(E)-4-Thiomorpholin-4-yl-but-2-enoic [carbonyl-^{11}C]acid [4-(3-chloro-4-fluoro-phenylamino)-quinazolin-6-yl]-amide (**12cb**)*

HPLC method B. Product t_R = 13.9 min, aniline precursor t_R = 10.2–13.8 min. The product mass peak could not be detected because of ion suppression by the precursor.

*(E)-4-Thiomorpholin-4-yl-but-2-enoic [carbonyl-^{11}C]acid {4-[3-chloro-4-(3-fluoro-benzyloxy)-phenylamino]-quinazolin-6-yl}-amide (**12cc**)*

HPLC method B. Product t_R = 14.2–16.3 min, aniline precursor t_R = 12.1–15.0 min. LC-MS ESI + for $C_{29}H_{27}ClFN_5O_2S$ *m/z*: 564 $(M+H)^+$.

*N-[4-(3-Bromo-phenylamino)-quinazolin-6-yl]-[carbonyl-^{11}C]acrylamide (**12da**)*[29]

HPLC method A. Product t_R = 11.1–12.0 min, aniline precursor t_R = 7.5–10 min. LC-MS ESI + for $C_{17}H_{13}BrN_4O$ *m/z*: 369 $(M+H)^+$.

*N-[4-(3-Chloro-4-fluoro-phenylamino)-quinazolin-6-yl]-[carbonyl-^{11}C]acrylamide (**12db**)*

HPLC method A. Product t_R = 10.2–11.4 min, aniline precursor t_R = 7.5–10.1 min. LC-MS ESI + for $C_{17}H_{12}ClFN_4O$ *m/z*: 343 $(M+H)^+$.

N-{4-[3-Chloro-4-(3-fluoro-benzyloxy)-phenylamino]-quinazolin-6-yl}-[carbonyl-^{11}C]acrylamide (**12dc**)

HPLC method B. Product t_R = 13.1–14.0 min, aniline precursor t_R = 10–12.3 min. LC-MS ESI + for $C_{24}H_{18}ClFN_4O_2$ *m/z*: 449 $(M+H)^+$.

Specific activity

The synthesis of [^{11}C]**12da** was performed as described earlier, starting with 5.6 GBq of [^{11}C]carbon monoxide. After 47 min (from end of bombardment (EOB)), the product was isolated with a radioactivity of 0.85 GBq. The volume of the product fraction was 3.1 mL. The concentration was determined to be 4.50 μM using an HPLC UV (λ = 254 nm) calibration curve, corresponding to 14 nmol of product and a specific radioactivity of 60 GBq μmol^{-1}.

Computational docking studies

The following X-ray structures representing the inactive conformation of EGFR were downloaded from http://www.rcsb.org and prepared for comparisons; 1XKK, 2RGB, 3GT8, 2JIV, 2RFD and 2GS7. The docking calculations for the inactive conformation were performed on 1XKK.[30] The missing amino acid side chains on the following residues K713, R748, E749, K754, K757, E758, N808, E866, K867, V876, M945, R962, E985, R986, S995, R999, E1004, V1010, V1011, E1015, L1017 and T1018 were modelled. None of these residues was found within 11 Å from the ligand. The atom types of the co-crystallised ligand GW-502016 were adjusted manually, and all water molecules except three in the close proximity of the ligand were deleted as well as two phosphate ions, using the Schrödinger Maestro Interface. Orientation of hydroxyls in Ser, Thr, and Tyr and sulfhydryls in Cys chain in

the protein structure was made using the Protein Preparation script in Schrodinger Glide. The same script was used for setting formal charges and protonation of the protein.[31] The X-ray structure of the inhibitor GW-502016 bound to the active site of the kinase domain of EGFR (PDB entry 1XKK) was used as template to construct the three-dimensional models of test compounds as well as **12ac**, **12bc**, **12cc** and **12dc**. All the structures were minimised in the absence of the protein using Macro Model[32] and Merck molecular force field (MMFFs)[33] force field, with an implicit solvent model of water. The Polak-Ribiere Conjugate Gradient (PRCG) method was used with a gradient threshold of 0.05, and minimisation was continued until convergence (usually <3000 steps). Ionisable ligands (at physiological pH) were modelled both in their charged and neutral forms. The protein active site was defined by a 10 × 10 × 10 Å box around the ligand, and no constraints were used. The docking model was tested by docking a set of 58 known potent compounds (1–1900 nM),[34] and the lowest energy poses were inspected visually. The same method was used for the active conformer 1M17 and the remaining ligands. Docking and scoring of **12aa–dc** were performed using standard precision mode in the software Glide from Schrodinger, and the distance between the sulfur atom of Cys^{797} and the β-carbon of the acrylamide was measured. A covalent bond was manually added between the β-carbon of the acrylamide to the sulfur atom of Cys^{797}. Atoms more than 6 Å away from the ligand were frozen, whereas the atoms within 6 Å from the ligand were constrained with a force constant of 100. The ligand, the side chain of Cys^{797} and the hydrogens of the water molecule were allowed to move freely during the minimisation using Macro Model and OPLS 2005 force field with an implicit solvent model of water. PCRG was used with a gradient threshold of 0.05 kcal/mol and continued until converged.

Results and discussion

During the last decade, several research groups have reported radiolabelling and imaging evaluation of small molecule TKIs, and their efforts have recently been reviewed.[35] For EGFR TKIs, the scaffold is the 4-(phenylamino)quinazoline or 4-(phenylamino)-3-cyanoquinoline. It has been shown that PET tracers with a reactive acrylamide moiety that irreversibly binds to Cys^{797} at the entrance of the adenosine triphosphate (ATP) binding pocket were superior to reversible ones. This is likely due to fast washout of reversible inhibitors because of ATP present in mM concentration competing for the binding site.[36] So far, the success of irreversible binding EGFR TKI PET-tracers has been limited because of slow tumour uptake, fast metabolism and high non-specific binding. One cause of fast metabolism and unspecific binding may be due to high reactivity of the acrylamide moiety towards other endogenous nucleophiles such as glutathione, a feature that possibly can be altered by altering the substituents of the β-carbon of the acrylamide (R′ in **12aa–dc**). Another cause of non-specific binding could be low selectivity over other kinases because the ATP binding pocket is highly conserved in the over 500 kinases known in the human genome.[37] It has been postulated that targeting the inactive conformation of kinases may be a way to achieve higher selectivity because the inactive conformation does need to fulfil the requirements to fit ATP, which could allow for more specific ligand-receptor interactions.[38] For the EGFR/HER-2 inhibitor lapatinib, it has been shown that by adding an aromatic moiety in the para position of the aniline, the inhibitor reaches a hydrophobic pocked deep within the binding site and locks the kinase in its inactive form, which resulted in a remarkable selectivity profile that could be beneficial for imaging purposes.[38,39]

During the design of the focused library, we performed docking studies on **12aa–12dc** (Table 1). The quinazoline core and the aromatic moieties at the 4-position were docked deep in the hydrophobic binding pocket and were oriented in a similar way as erlotinib (active) or lapatinib (inactive) in their X-ray structures. The distance between the electrophilic β-carbon of the acrylamide and the sulfur of Cys^{797} was measured (3.8–5.4 Å) to confirm that irreversible binding would in theory be possible. The acrylamide moiety of **12aa–12dc** pointing towards the solvent interface

Table 1. Products obtained from aminocarbonylation of vinyl iodides with 6-aminoquinazoline derivatives

Vinyl iodide	Nucleophile	Product	cLogD$_{7.4}$[a]	Calculated pK_a[a]	Conversion of [^{11}C]O (%)[b]	Isolated RCY[c]
11a	**5a**	[^{11}C]**12aa**	3.7	8.6	92 ± 1 (3)	36 ± 2 (3)
	5b	[^{11}C]**12ab**	3.7	8.6	86 ± 4 (3)	31 ± 6 (3)
	5c	[^{11}C]**12ac**	5.2	8.6	91 ± 4 (2)	24 ± 9 (3)
11b	**5a**	[^{11}C]**12ba**	3.8	6.3	89 ± 3 (2)	39 ± 0 (2)
	5b	[^{11}C]**12bb**	3.8	6.3	94 ± 1 (3)	35 ± 1 (3)
	5c	[^{11}C]**12bc**	5.3	6.3	92 ± 0 (2)	36 ± 2 (2)
11c	**5a**	[^{11}C]**12ca**	4.0	7.6	90	31
	5b	[^{11}C]**12cb**	4.0	7.6	90	38
	5c	[^{11}C]**12cc**	5.6	7.6	90	32
11d	**5a**	[^{11}C]**12da**	4.2	n.a.	84 ± 11(4)	47 ± 15 (4)
	5b	[^{11}C]**12db**	4.2	n.a.	89 ± 7 (6)	48 ± 9 (6)
	5c	[^{11}C]**12dc**	5.8	n.a.	95 ± 5 (4)	61 ± 13 (4)

n.a.. not applicable.
[a]pK_a values of the aliphatic amine calculated using Chemaxon Marwin online software.
[b]Decay-corrected conversion yield of [^{11}C]carbon monoxide to non-volatile products remaining in the reaction mixture after removal of solvent.
[c]The number in parentheses is the number of experiments.
[d]Decay-corrected radiochemical yield based on the amount of radioactivity at the start of the synthesis and the radioactivity of the isolated product. After the reaction mixture was transferred from the micro-autoclave to an evacuated 1-mL 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 could be determined.
[e]Radiochemical purity was >97% in all experiments.

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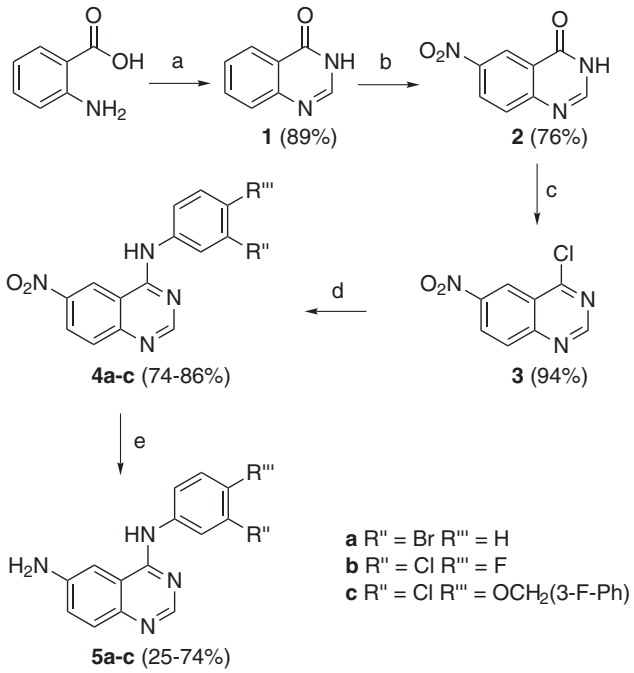
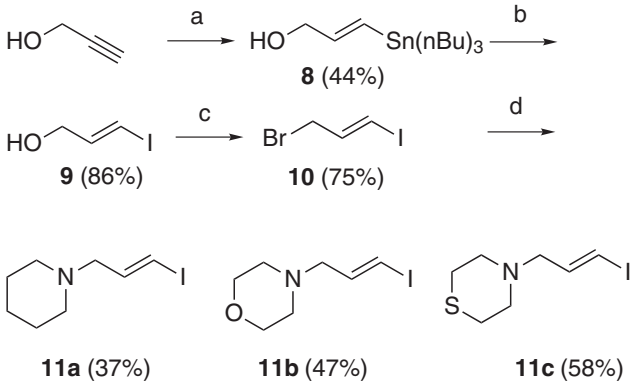
appeared to have many low energy conformations and high rotational freedom, which is in agreement with the less defined electron densities reported for the flexible substituents at the 6-position and 7-position of the quinazoline cores of lapatinib and erlotinib. Ligands **12ac**, **12bc**, **12cc** and **12dc** with the extra phenyl–ether moiety targeting the inactive conformation were not able to dock into the active conformation because of steric clash. We also confirmed that β-substitution of the acrylamide (piperidine, morpholine or thiomorpholine) did not affect the binding mode of the quinazoline core, which promoted us to go ahead with the synthesis of precursors and reference compounds. The final binding modes exemplified with **12bb** (active) and **12ac** (inactive) (Figure 1) were obtained by manual formation of the covalent carbon–sulfur bond followed by a constrained minimisation. The non-covalent ligand–protein interactions include a hydrogen bond between *N*1 and the backbone N–H of Met[793] in addition to the water-mediated bond via the *N*3 of the inhibitor and the hydroxyl of Thr[790] (active) or hydroxyl Thr[854] (inactive). In the deep hydrophobic pocket that becomes accessible in the inactive conformation, we observed a favourable π-interaction between the 4-fluoro-benzyloxy substituent in an edge-to-face manner to Phe[856]. The tertiary amine substituent on the acrylamide interacts with Asp[800] via hydrogen bonding. The docking results are in good agreement with recently published X-ray structures of structurally related irreversibly bound EGFR inhibitors.[40]



Scheme 1. (a) Formamide, 130 °C, 7 h. (b) HNO_3/H_2SO_4, 95 °C, 40 min. (c) $SOCl_2$, reflux, 3 h. (d) Ar–NH_2, *i*-PrOH, reflux, 2 h. (e) Fe, HOAc, EtOH, H_2O, reflux, 90 min.

Synthesis of precursors

The syntheses of the precursors were performed by previously described procedures with minor modifications. Briefly, the 4-anilino-6-amino-quinazolines **5a–c** were synthesised starting from anthranilic acid and formamide to give the quinazolinone **1** (Scheme 1), which was nitrated in the 6-position to form **2**.[41] Deoxychlorination was accomplished using thionyl chloride, and different anilines were reacted with **3** to give the 4-anilino-6-nitro-quinazolines **4a–c**. Finally, the nitro group was reduced using iron and acetic acid to yield the nucleophile precursors 4-anilino-6-amino-quinazolines **5a–c** (Scheme 1). The β-position substituent of the acrylic amide is pointing towards the solvent interface of the binding site (Figure 1). These substituents may influence the reactivity of the acrylamide as well as the overall solubility and lipophilicity of the molecule (Scheme 3).[17] Vinyliodide precursors **11a–c** with different substituents were synthesised according to



Scheme 2. (a) Bu_3SnH, AIBN, 80 °C, 2 h. (b) I_2, dichloromethane, room temperature, 2 h. (c) Ph_3P, CBr_4, dichloromethane, 0 °C, 10 min. (d) K_2CO_3, MeCN, a secondary amine, 0 °C to room temperature, 18 h.

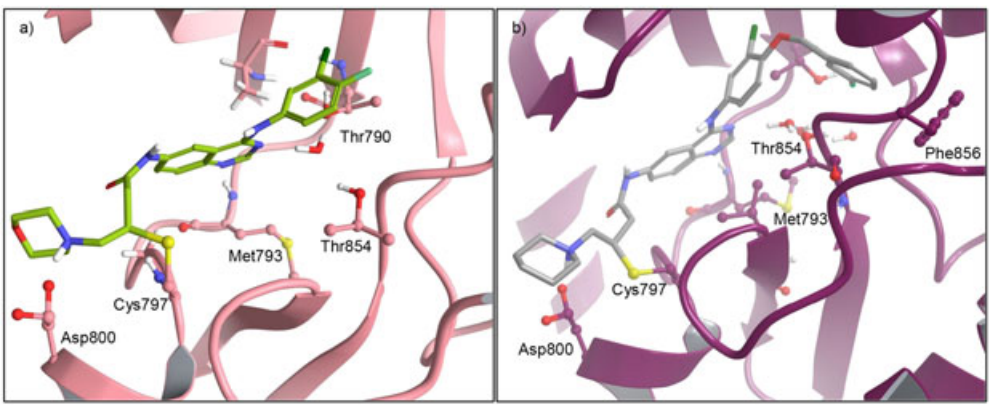
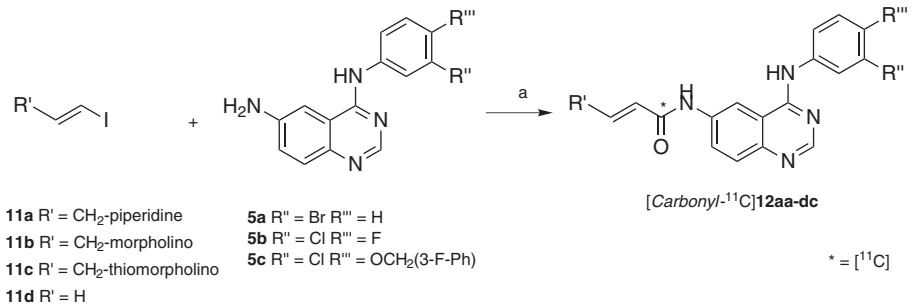


Figure 1. (a) Plausible binding modes obtained using computational docking for **12bb** bound to the adenosine triphosphate site of the active epidermal growth factor receptor (EGFR) conformation followed by manually adding the covalent bond to Cys[797] and minimisation of (b) **12ac** docked and covalently bound to the inactive conformation of EGFR.



Scheme 3. [^{11}C]O in a helium carrier, $Pd_2(dba)_3$, PPh_3, THF, 110 °C, 5 min.

Scheme 2. Addition of an excess of tributyltin hydride to propargyl alcohol gave mainly the syn addition product **8**.[21] Iodination and subsequent bromination gave the intermediate **10**, which was combined with secondary amines to obtain vinyl iodides **11a–c** used as substrates in the labelling reactions.[23]

Labelling chemistry

Compound libraries and combinatorial chemistry have since the early nineties been extensively used for structure activity relationship (SAR) analyses and lead optimisation in drug discovery.[42] On a laboratory scale, carbon monoxide has been used to synthesise libraries using microwave techniques and *in situ* produced carbon monoxide.[43] In the current work, we use on-line produced [^{11}C]carbon monoxide to radiolabel a focused library of EGFR inhibitors in a combinatorial fashion.

An active palladium complex was formed by mixing tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba_3)$] and triphenylphosphane in THF under argon to form a yellow solution. To the *in situ* formed complex was added vinyl iodide **11a–d**, whereupon the colour of the solution changed to pale yellow. The amine nucleophile **5a–c** was then introduced, and the mixture was injected into a micro-autoclave pre-charged with no-carrier-added [^{11}C]carbon monoxide in helium obtained on-line via reduction of [^{11}C]carbon dioxide over zinc granules at 400 °C. To ensure a high radiochemical yield, the reaction time must be kept short because of the short half-life of ^{11}C, and thus, the rate of the reaction must be high. To meet these criteria, all reagents were used in large excess compared with [^{11}C]carbon monoxide, and around 90% of the [^{11}C]carbon monoxide could be converted to non-volatile products within 5 min. The stoichiometric relationship between the reagents Pd/PPh_3/vinyl iodide/amine nucleophile in the reaction mixture was 1:4.2:2.5:5.2, whereas the amount of [^{11}C]carbon monoxide was much lower (0.004–0.04 equivalents). Unsubstituted acrylamides have been ^{11}C-labelled previously via a Grignard reaction followed by chlorination and finally by reaction with an amine nucleophile.[29] However, it is a clear advantage to use a single-step reaction, especially in view of the short half-life of ^{11}C as well as the physical handling of the radioactivity. Another advantage using [^{11}C]carbon monoxide is the low abundance of carbon monoxide in the atmosphere (0.05–0.2 ppm) compared with carbon dioxide (380 ppm), which will decrease the risk of isotopic dilution while handling the reagents. Isotopic dilution leads to low specific activity that can lead to undesired pharmacological effects and saturation of binding sites in biological systems.

The crude products were purified using semi-preparative HPLC to give the labelled products [^{11}C]**12aa–dc** (Scheme 3, Table 1). The yields were in the range 24–61% (Table 1). As a comparison, [^{11}C]**12da**, a known irreversible inhibitor with a reported IC_{50} value of 2 nM in an enzyme assay,[24] was obtained in higher decay-corrected radiochemical yield and specific activity (47%, 60 GBq μmol^{-1}) than was reported previously (12%, 10 GBq μmol^{-1}).[29] In general, there was only one radio peak with a retention time of more than 5 min. The hydrophilic by-products formed were not analysed, but we believe these could be the corresponding hydroxy carbonylation product from a reaction where water acts as the nucleophile, as well as labelled carbonates as seen in previous studies.[44]

Conclusion

The abundance of the carbonyl group in many drug candidate libraries and other biologically active molecules and the generality and functional group tolerance of the carbonylation labelling chemistry make [^{11}C]carbon monoxide versatile in labelling synthesis. A combinatorial approach to synthesise a library of ^{11}C-labelled potential irreversible EGFR inhibitors based on the 4-anilino-6-acrylamido-quinazoline core has successfully been explored. The labelling was performed using various vinyl iodides as substrates in palladium-mediated carbonylations with [^{11}C]carbon monoxide and 4-anilino-6-amino-quinazolines as the nucleophiles using the same conditions. In total, 12 analogues were ^{11}C-labelled at the carbonyl position and were obtained in biologically useful radiochemical yields and low isotopic dilution. Biological evaluation of several of these tracers will follow.

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Conflict of Interest

The authors did not report any conflict of interest.

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