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Research Article

Synthesis of 6-acrylamido-4-(2-[^{18}F]fluoroanilino)quinazoline: a prospective irreversible EGFR binding probe

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Summary

Acrylamido-quinazolines substituted at the 6-position bind irreversibly to the intracellular ATP binding domain of the epidermal growth factor receptor (EGFR). A general route was developed for preparing 6-substituted-4-anilinoquinazolines from [^{18}F]fluoroanilines for evaluation as EGFR targeting agents with PET. By a cyclization reaction, 2-[^{18}F]fluoroaniline was reacted with *N*′-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylimidoformamide to produce 6-nitro-4-(2-[^{18}F]fluoroanilino)quinazoline in 27.5% decay-corrected radiochemical yield. Acid mediated tin chloride reduction of the nitro group was achieved in 5 min (80% conversion) and subsequent acylation with acrylic acid gave 6-acrylamido-4-(2-[^{18}F]fluoroanilino)quinazoline in 8.5% decay-corrected radiochemical yield, from starting fluoride, in less than 2 h.

Key Words: EGFR; fluorine-18; PET; quinazoline

Introduction

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases (tk) and is often overexpressed in a variety of tumors. Inhibition of the EGFR-tk is a focal point in therapeutic development and considered to be an important target for cancer therapy.[1] Recently,

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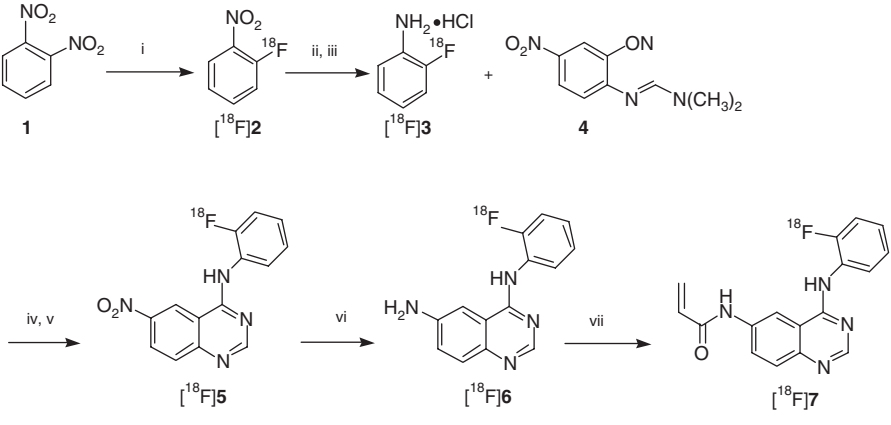
6-acrylamido-4-anilino-quinazolines have been developed as a class of therapeutic agents that bind irreversibly to the intracellular ATP binding domain of the EGFR-tk and inhibit downstream cell signaling events.[2,3] Radiolabeled EGFR-tk probes may be instrumental in understanding tumor biology or as tools for drug development.

Mishani and coworkers[4–7] have synthesized and evaluated 6-substituted-4-haloanilinoquinazolines labeled with carbon-11 (half-life = 20.4 min) as novel irreversible positron emission tomography (PET) probes for EGFR-tk. These tracers have been explored to overcome the ATP competitive binding that may have interfered with the retention of the carbon-11 labeled reversible probes.[4,7] As a longer-lived alternative to the carbon-11 probes, we report herein the synthesis of 6-acrylamido-4-(2-[^{18}F]fluoroanilino)quinazoline ([^{18}F]**7**) as a potential probe for EGFR-tk. The increased tracer lifetime (fluorine-18 half-life = 109.7 min) may provide a better match to the pharmacokinetics and EGFR turnover.

Our synthetic strategy involves the following steps: (1) preparation of a radiolabeled aniline; (2) ring closure of *N*′-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylimidoformamide, **4**, with the labeled aniline to form 6-nitro-4-anilinoquinazoline;[8] (3) reduction of the nitro group to form 6-amino-4-anilinoquinazoline, a key intermediate for several labeled analogs; and (4) acylation of the amine with acrylic acid.[8,9] This strategy may be extended to provide a series of labeled 6-substituted-4-anilinoquinazolines.

Results and discussion

Scheme 1 shows the synthetic route to [^{18}F]**7**. Fluorine-18 labeled 2-fluoroaniline hydrochloride ([^{18}F]**3**) was selected for coupling to **4** because it



Scheme 1. ***Reagents*****: (i) $K^{18}F/K_{222}$, DMSO, 130°C, 5 min; (ii) $NaBH_4$/Pd-C, rt, 5 min; (iii) HCl; (iv) HOAc, 100°C, 20 min; (v) NaOH; (vi) $SnCl_2 \cdot 2H_2O$, HCl, 100°C, 5 min; (vii) acrylic acid, EDCI · HCl, pyridine, DMF, rt, 5 min**

is efficiently prepared[10] and potentially possesses suitable metabolic properties when coupled to quinazolines.[11,12] Cyclization provides 6-nitro-4-(2-[^{18}F]fluoroanilino)quinazoline ([^{18}F]**5**), incorporating the labeled 4-anilino moiety in a single step. Compound [^{18}F]**5** was obtained (after HPLC purification) in 27.5% decay-corrected radiochemical yield, relative to the initial ^{18}F-fluoride activity. Acid mediated nitro group reduction of [^{18}F]**5** to [^{18}F]**6** with tin chloride was accomplished in 5 min with 80% conversion. Subsequent acylation with acrylic acid followed by HPLC purification provided chemically and radiochemically pure [^{18}F]**7** ($>95\%$). The overall radiochemical yield was 8.5% from starting fluoride in less than 120 minutes. The measured specific activity at the end of synthesis was 12.1 GBq/mmol. The corresponding fluorine-19 analogs, compounds **5**–**7**, were prepared by the same route (Scheme 1) and were characterized by ^{1}H NMR spectroscopy and electron impact mass spectrometry. The identities of [^{18}F]**5** - [^{18}F]**7** were confirmed by comparison of the HPLC retention times and co-injection with unlabeled **5**–**7**.

We have recently described the optimized synthesis of 2-, 3- and 4-[^{18}F]fluoroanilines.[10] As the [^{18}F]fluoroaniline isomers are similarly prepared, one could easily substitute the 3- and 4-[^{18}F]fluoroanilines in the current approach. Likewise, one could envision extending the series with radiobromo- or radioiodo-anilines. Additionally, elaboration in the 6 position such as the acetamido analogs reported by Mishani and coworkers[9] expands the series of potential EGFR-tk probes. Thus, the chosen synthetic approach permits the preparation of a variety of labeled quinazolines compounds for assessment as biologic probes.

Experimental section

Materials

Absolute EtOH USP (Aaper), $NaBH_4$ (Spectrum), HPLC grade MeOH (Burdick and Jackson), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI · HCl; Pierce), stannous chloride dihydrate (Mallinckrodt) and [^{18}O]H_2O (Rotem Industries Ltd., Israel, >94.1 atom%) were used without further purification. All other reagents were purchased from Aldrich and were used without further purification, with the exception of 1,2-dinitrobenzene (Aldrich, $>99\%$), which was recrystallized from EtOAc prior to use.

Standard techniques

Preparative HPLC was performed at room temperature using a Beckman Ultrasphere C-18 5-μm column (250 mm × 10 mm) with 65 : 35 (v/v) CH_3OH : H_2O (1% Et_3N in H_2O adjusted to pH = 7.4 with H_3PO_4) as the mobile phase. A flow rate of 6 ml min^{-1} was used (pump: Waters model 590) and the eluent

was monitored for UV absorbance at 254 nm (detector: Linear UV-106) and radioactivity (detector: Carroll and Ramsey Associates Model 105S). Analytical HPLC was performed with the same solvent system on a Phenomenex Hypersil C18 (ODS) 5-μm column (250 mm × 4.6 mm) with a flow rate of 1 ml min^{-1} and the eluent was monitored for UV absorbance at 254 nm (detector: Linear UVIS 200). The ^1H NMR spectra were recorded in CD_3OD on a Bruker AV-300 (300 MHz) spectrometer at 30°C. The mass spectrometry analyses were conducted using a VG ProSpec spectrometer equipped with an electron impact (EI) source operated at 70 eV.

No-carrier-added ^{18}F-fluoride was produced by 10 MeV proton irradiation of 30% enriched $[^{18}O]H_2O$ at low pressure in a silver target by the $^{18}O(p,n)^{18}F$ nuclear reaction on the Biomedical Isotope Facility CTI RDS 111-DV01 cyclotron. The $[^{18}O]H_2O/^{18}F^-$ (100–450 μl) was transferred into a conical glass vial containing Kryptofix® 222 (5 mg, 13.2 μmol), K_2CO_3 (0.5 mg, 3.62 μmol) in 500 μl of CH_3CN. The water was removed by azeotropic distillation with anhydrous CH_3CN (1 ml) at 100°C in vacuo, under a stream of nitrogen. The azeotropic drying process was repeated.

Radiochemistry

*6-nitro-4-(2-[^{18}F]fluoroanilino)quinazoline ([^{18}F]**5**).* Fluorine-18 labeled 2-fluoroaniline hydrochloride ([^{18}F]**3**) was prepared as previously described[10] and subsequently reacted with 400 μl of a saturated solution of *N*′-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylimidoformamide (1 ml of glacial AcOH added to 50 mg of **4**) at 100°C for 20 min to provide 6-nitro-4-(2-[^{18}F]fluoroanilino)-quinazoline ([^{18}F]**5**). The reaction mixture was made basic with NaOH (1 ml, 1 M) and diluted to 4 ml with H_2O. The mixture was passed sequentially through a cartridge loaded with Celite® 521 filtering agent (Aldrich), an activated C18 solid phase extraction cartridge (Sep-Pak®, Waters) and a 45 μm nylon disc-filter (Acrodisc Premium, Pall), connected in series, and rinsed with 4 ml H_2O. [^{18}F]**5** was eluted with MeOH (3 ml) into a separate vial, diluted with 1 ml of H_2O and injected onto the semi-preparative HPLC system. [^{18}F]**5** ($t_R = 6.5$ min) was well separated from excess **4** ($t_R = 4.0$ min). [^{18}F]**5** was identified by the semi-preparative ($t_R = 6.5$ min) and analytical ($t_R = 6.9$ min) HPLC retention times and co-injection with authentic compound **5**. The HPLC peak containing [^{18}F]**5** was collected, diluted to 20 ml with H_2O, concentrated on a C18 cartridge and eluted with 2.5 ml EtOH. Isolated [^{18}F]**5** was obtained in 27.5 ± 1.5% ($n = 3$) decay-corrected radiochemical yield, relative to the initial ^{18}F-fluoride activity.

*6-amino-4-(2-[^{18}F]fluoroanilino)quinazoline ([^{18}F]**6**).* The ethanolic solution of [^{18}F]**5** was added to a vial containing $SnCl_2 \cdot 2H_2O$ (8 mg, 35 μmol), followed by the addition of 20 μl conc. HCl. The reaction vessel was sealed

with a Teflon lined screw cap and heated at 100°C for 5 min to yield 6-amino-4-(2-[^{18}F]fluoroanilino)quinazoline ([^{18}F]**6**), which was used without further purification. In separate experiments, semi-preparative HPLC was used to determine the reduction efficiency. The conversion from [^{18}F]**5** to [^{18}F]**6** was found to be 80%. [^{18}F]**6** was identified by the semi-preparative ($t_R = 3.2$ min) and analytical ($t_R = 4.1$ min) HPLC retention times and co-injection with authentic compound **6**.

*6-acrylamido-4-(2-[^{18}F]fluoroanilino)quinazoline ([^{18}F]**7**).* The ethanolic reaction mixture containing [^{18}F]**6** was evaporated to dryness at 100°C in vacuo. To the dry residue, a DMF solution (300 µl) containing EDCI · HCl (6 mg, 31 µmol), followed by pyridine (50 µl) and acrylic acid (10 µl of a 20% acrylic acid solution in DMF, 0.03 µmol) were added. The reaction vessel was sealed with a Teflon lined screw cap and the acylation proceeded at room temperature for 5 min. The reaction mixture was diluted to 5 ml with H_2O and loaded onto a C18 cartridge, then rinsed with an additional 2 ml H_2O. [^{18}F]**7** was eluted with 3 ml MeOH into a separate vial, diluted with 1 ml H_2O and injected onto the semi-preparative HPLC system. The collected fraction containing compound [^{18}F]**7** was diluted to 20 ml with H_2O, concentrated onto a C-18 cartridge and extracted into a test tube with methanol. The methanol was evaporated at 100°C in vacuo and [^{18}F]**7** was obtained in 8.5 ± 2% decay-corrected radiochemical yield ($n = 3$) in less than 2 h with greater than 95% chemical and radiochemical purity as measured by analytical HPLC. [^{18}F]**7** was identified by the semi-preparative ($t_R = 5.5$ min) and analytical ($t_R = 6.0$ min) HPLC retention times and co-injection with authentic compound **7**. [^{18}F]**7** is resolubilized by heating with 20 µl of EtOH at 100°C for 10 s, followed by the addition of saline or aqueous buffer (e.g. Krebs–Henseleit buffer) to achieve the desired final concentration. The specific activity was determined by analytical HPLC.

Syntheses of non-radioactive standard compounds

*6-nitro-4-(2-fluoroanilino)quinazoline (**5**).* *N′*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylimidoformamide,[8] **4**, (2.4 g, 11.0 mmol) and 2-fluoroaniline (1.41 g, 13.0 mol) were stirred in AcOH (100 ml) at reflux for 2 h. The resulting solid was filtered hot and washed with ether, followed by drying at 50°C in vacuo to give 1.86 g (60%) of **5** as a yellow solid. ^{1}H NMR spectrum, δ (ppm, relative to TMS):9.58 (bs, 1H), 8.61 (s, 1H), 8.57 (dd, $J = 2.5$ Hz, $J = 9.2$ Hz, 1H), 7.94 (d, $J = 9.2$ Hz, 1H), 7.54 (t, $J = 7.5$ Hz, 1H), 7.25–7.37 (m, 3H); MS (EI) m/z 284.

*6-amino-4-(2-fluoroanilino)quinazoline (**6**).* A mixture of 6-nitro-4-(2-fluoroanilino)quinazoline, **5**, (1.2 g, 4.33 mmol) was stirred in EtOH (7.5 ml) with

Fe (1.2 g, 21.5 mmol) and AcOH (2 ml). Upon heating to reflux, additional EtOH (2 ml) and AcOH (0.5 ml) were added and heating was continued for 3 h. After cooling the mixture to room temperature, the solution was filtered and the residue washed with H_2O (20 ml), then MeOH (3×20 ml). The combined filtrates were neutralized with NH_4OH and extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4), decolorized with charcoal, and the solvent was removed in vacuo. The residue was purified by chromatography (10% MeOH–$CHCl_3$, silica gel) to provide 0.5 g (45%) of **6**, a yellow solid. ^{1}H NMR spectrum, δ (ppm, relative to TMS):9.25 (bs, 1H), 8.19 (bs, 1H), 7.56 (t, $J = 7.19$ Hz, 1H), 7.51 (d, $J = 8.7$ Hz, 1H), 7.20–7.28 (m, 4H); MS (EI) m/z 254.

*6-acrylamido-4-(2-fluoroanilino)quinazoline (**7**).* Acrylic acid (91 µl, 1.1 mmol) was added dropwise into an ice-cold solution of 6-amino-4-(2-fluoroanilino)-quinazoline, **6**, (0.127 g, 0.5 mmol) in pyridine (0.5 ml) and DMF (4 ml). After a few minutes EDCI · HCl (0.239 g, 1.25 mmol) was added. The reaction was warmed to room temperature and stirred for 1 h. The solvent was removed in vacuo and the residue was purified by chromatography (5% MeOH–$CHCl_3$, silica gel) to provide 0.2 g (8.5%) of **7**. ^{1}H NMR spectrum, δ (ppm, relative to TMS): 8.75 (d, $J = 2.2$ Hz, 1H), 8.39 (s, 1H), 7.84 (dd, $J = 2.2$ Hz, $J = 8.9$ Hz, 1H), 7.75 (d, $J = 8.9$ Hz, 1H), 7.67 (dt, $J = 1.8$ Hz, $J = 7.8$ Hz), 7.35 – 7.20 (m, 3H), 6.47 (d, $J = 3.8$ Hz, 1H), 6.46 (d, $J = 8.1$ Hz, 1H), 5.83 (dd, $J = 3.8$ Hz, $J = 8.1$ Hz, 3H); MS (EI) m/z 308.

Conclusions

The first prospective fluorine-18 labeled irreversibly-binding EGFR-tk tracer, 6-acrylamido-4-([^{18}F]2-fluoroanilino)quinazolines, ([^{18}F]**7**), has been prepared. The synthetic strategy reported herein may be applied to develop a series of labeled compounds through acylation of the amine at position 6 and/or coupling with substituted anilines for assessment as EGFR-tk probes.

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