Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 24 September 2008, Revised 29 October 2008, Accepted 29 October 2008 Published online 16 December 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1569

Modified PEG-anilinoquinazoline derivatives as potential EGFR PET agents

Samar Dissoki, Renana Eshet, Hana Billauer, and Eyal Mishani*

Inhibition of epidermal growth factor receptor tyrosine kinase (EGFR-TK) has emerged as a major approach for cancer-targeted therapy. Consequently, there has been a great interest in the use of labeled EGFR-TK inhibitors as positron emission tomography (PET) imaging agents. Currently, the developed agents did not yield adequate PET imaging of animal models probably due to poor solubility, rapid washout from blood, and low stability *in vivo*. In order to overcome these hurdles, new derivatives of previously reported inhibitors (ML04, 2) with decreased log *P* and increased solubility were designed and synthesized. These compounds (3–5) exhibited high autophosphorylation inhibitory potency with an IC_{50} of 5–35 nM, decreased log *P*'s (3.1, 3.34, and 3.45, respectively), and significantly increased solubility (630, 300, and 120 μg/mL, respectively) relative to the previously reported parent compound 2 (log *P* = 3.7, solubility = 3.5 μg/mL). The labeling of compound 5 with [^{18}F] and compounds 3 and 4 with [^{11}C] and [^{124}I], respectively, involved a one-step radiosynthesis. Compounds 3–5 were obtained with a total decay-corrected radiochemical yields of 13, 31, and 5%, respectively, and were found to be stable in blood. The positive outcome achieved with compounds 3–5 merits further *in vivo* evaluation as PET bioprobes.

Keywords: PET; EGFR; cancer; fluorine-18; imaging

Introduction

Epidermal growth factor receptor (EGFR) is overexpressed in many epithelial cancers such as head and neck, lung, prostate, and kidney.[1,2] Dysregulation and malfunction of EGFR are associated with several key features of cancer, such as autonomous cell growth, inhibition of apoptosis, increased angiogenic potential, invasion, and metastases.[3,4] Correlation between EGFR overexpression and metastasis formation, therapy resistance, poor prognosis, and short survival has prompted the design and development of various anti-EGFR-targeted therapies.[5–9] Examples of FDA-approved therapies include low-molecular-weight reversible epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors, such as gefitinib (Iressa, ZD1839; AstraZeneca, Wilmington, PA) and erlotinib (Taceva; Genentech, San Francisco, CA).[10,11] Promising results have been obtained using gefitinib and erlotinib in pre-clinical models of EGFR overexpressing cell lines and xenografts.[12,13] Nonetheless, these results failed to reproduce in the clinical setting since both agents appeared to be effective only in the subset of non-small cell lung carcinoma patients.[14,15] Higher response rates to gefitinib and erlotinib therapies have been reported in patients with EGFR expressing tumors containing well-defined activating mutations.[16–20] However, patients who initially responded to these treatments have occasionally developed therapy resistance due to acquired secondary mutation in the EGFR.[21] The chimeric anti-EGFR monoclonal antibody cetuximab (Erbitux; ImClone Systems, Inc.) has yielded positive clinical results against head and neck cancers overexpressing the EGFR and has demonstrated activity in colon cancer regardless of tumor EGFR expression.[22–24]

Additional anti-EGFR-targeted therapies, which are currently undergoing clinical trial, include monoclonal antibodies, such as panitumumab (ABX-EGF),[25–28] and low-molecular-weight compounds, such as the dual EGFR/HER2 inhibitor PKI-166 and the irreversible pan-erbB inhibitor CI-1033.[9,29] The moderate clinical outcome of most approved drugs and the inconsistency between EGFR overexpression and response to EGFR-targeted treatment require further investigation including careful patient selection. The lack of accurate and comprehensive measurements of the phosphorylation status of EGFR in tumors following gefitinib or erlotinib treatment poses difficulties when interpreting clinical data, and it is actually not possible to assess whether the poor response is indeed due to either a lack of specific activating mutations, the absence of a survival function of EGFR, or an insufficient long-term occupancy of the receptor by reversible inhibitors.[15,30,31] Thus, the ability to noninvasively quantitate EGFR content in tumors would aid in selecting patients who are likely to benefit from anti-EGFR-targeted therapy and in monitoring such treatment.[15,30] Therefore, there has been a growing interest in the use of EGFR-TK inhibitors as radiotracers for molecular imaging of EGFR overexpressing tumors via nuclear medicine modalities such as positron emission tomography (PET).[15,30,32–39] Since recent pre-clinical

Cyclotron/Radiochemistry Unit, Nuclear Medicine Department, Hadassah/Hebrew University Hospital, Jerusalem 91120, Israel

**Correspondence to: Eyal Mishani, Cyclotron/Radiochemistry Unit, Nuclear Medicine Department, Hadassah/Hebrew University Hospital, Jerusalem 91120, Israel.*
E-mail: eyalmi@ekmd.huji.ac.il

and clinical publications indicate that irreversible inhibitors of the EGFR-TK appear to circumvent resistance to treatment due to secondary mutations, and may benefit from a larger clinical application,[40] we and others have mainly focused on this category of inhibitors.[15,30,32,41,42]

Results and discussion

Chemistry

The main disadvantage of labeled anilinoquinazoline derivatives developed thus far was their high log *P* and poor solubility, which led to moderate tumor uptake, fast clearance from blood, and high non-specific binding in tumor.[15,30,33] Recently, in order to circumvent these major drawbacks, an fluoro-polyethylenglycol (FPEG) functional group was attached to the quinazoline ring at the 7-position (compounds **1a**–**c** and **2**, Figure 1).[43] While no improvement was obtained with compounds **1a**–**c**, compound **2** (ML04 derivative) exhibited a minor decreased lipophilicity, a significantly improved solubility compared with the parent compound ML04, and an irreversible binding characteristic as opposed to **1a**–**c** derivatives.[43] Since the ML04 derivative (**2**) showed promising results, we have continued the development of this derivative in order to further improve solubility, decrease log *P*, decrease blood clearance, and potentially minimize non-specific binding *in vivo*.[33]

Three different pegylated ML04 derivatives **3**–**5** (Figure 2) were designed, synthesized, and labeling routes were developed with [^{11}C], [^{124}I], and [^{18}F], respectively.

Compounds **3** and **4** contain a free hydroxyl group at the end of the polyethylenglycol (PEG) chain attached at the 7-position of the quinazoline ring, rather than fluorinated PEG as in compound **2**. The major disadvantage with compound **3** was that it could not be easily labeled with [^{18}F] as opposed to compound **2** and was required to be labeled with carbon-11 on the dimethylamine moiety. Since carbon-11 has a short half-life (20 min), biological studies such as blood stability, biodistribution, or PET are limited to a relatively narrow time window post-injection of the tracer. In order to afford prolonged *in vitro* and *in vivo* measurements, we synthesized compound **4**, which has a similar structure as **3**, but contains an iodine atom attached at the aniline ring, to afford labeling with the longer-lived isotope [^{124}I] (4.2 days). In order to decrease the lipophilicity while enabling labeling with fluorine-18 at the end of the PEG chain in one radiochemical step, compound **5** was synthesized (Figure 2). Compound **3** was prepared according to the synthesis shown in Scheme 1. The synthesis of compounds **6**–**10** has already been described.[36,44] Compound **10** was dissolved in EtOH/H_2O, hydrazine monohydrate and Raney® nickel were added to yield the reduced compound **11** (75%). Compound **11** was reacted with bromo/chlorocrotonylchloride (**12**) at 0°C to yield **13** (52%), and the latter was reacted with dimethylamine in the presence of *N,N*-diisopropylethylamine to yield **3** (28%). Compound **13** was dissolved in DMSO and reacted with monomethylamine at 0°C for 15 min to obtain **14** (Scheme 1), a precursor for the [^{11}C] radiolabeling synthesis. The crude product **14** was kept at −20°C without any further purification.[42] The synthesis of compound **4** is shown in Scheme 2. Compound **4** was obtained after five synthetic steps with 12% total yield.

The synthesis of compound **25**, a precursor for the [^{124}I] radiosynthesis, is outlined in Scheme 3. Compound **8** was reacted with 3-bromoaniline at room temperature to give **20** (54%).[36] Compound **20** was reacted with **9** in the presence of potassium trimethylsilanolate at room temperature to yield **21** (48%). The latter was reduced to amine at 40–50°C to give **22** (57%). Bromo/chlorocrotonylchloride (**12**) was reacted with **22** at 0°C for 1 h to yield **23** (23%). Compound **23** was reacted with dimethylamine in THF in the presence of *N,N*-diisopropylethylamine at 0–60°C for 2 h to yield **24** (25%). Tetrakis(triphenylphosphine) palladium(0) and *bis*(tributyltin) were added to a solution of **24** dissolved in dry toluene and refluxed for 2 h to yield **25** (28%).

The synthesis of compound **5** is outlined in Scheme 4. The preparation of compounds **26**–**28** has been described previously.[43] Compound **28** in THF was reacted with diethanolamine and DMF at 0–60°C for 2 h to obtain **5** (14%). The synthesis of compound **30**, a precursor for the [^{18}F] labeling of **5** in one radiochemical step, is shown in Scheme 5. Compound **13** in dichloromethane was reacted with mesyl chloride (MsCl) in the presence of triethylamine at 0°C to obtain **29** (76%). Compound **29** dissolved in THF was added to a solution of diethanolamine

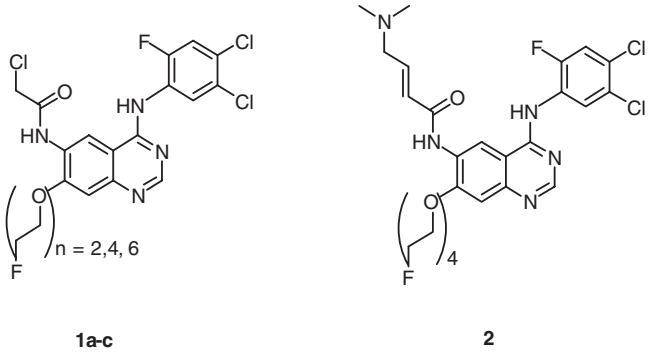


Figure 1. Chemical structures of compounds **1a**–**c** and **2**.

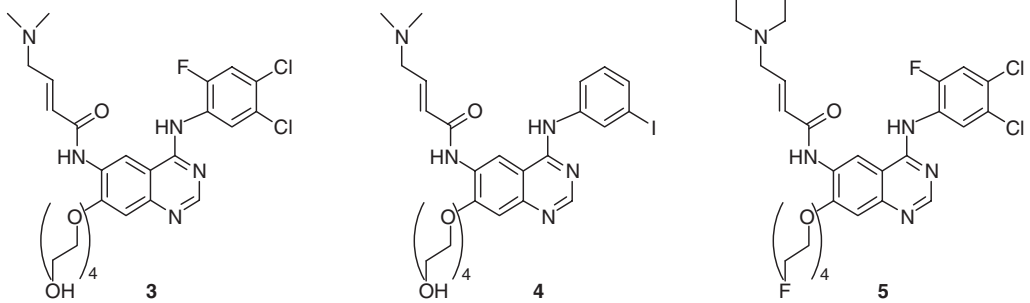
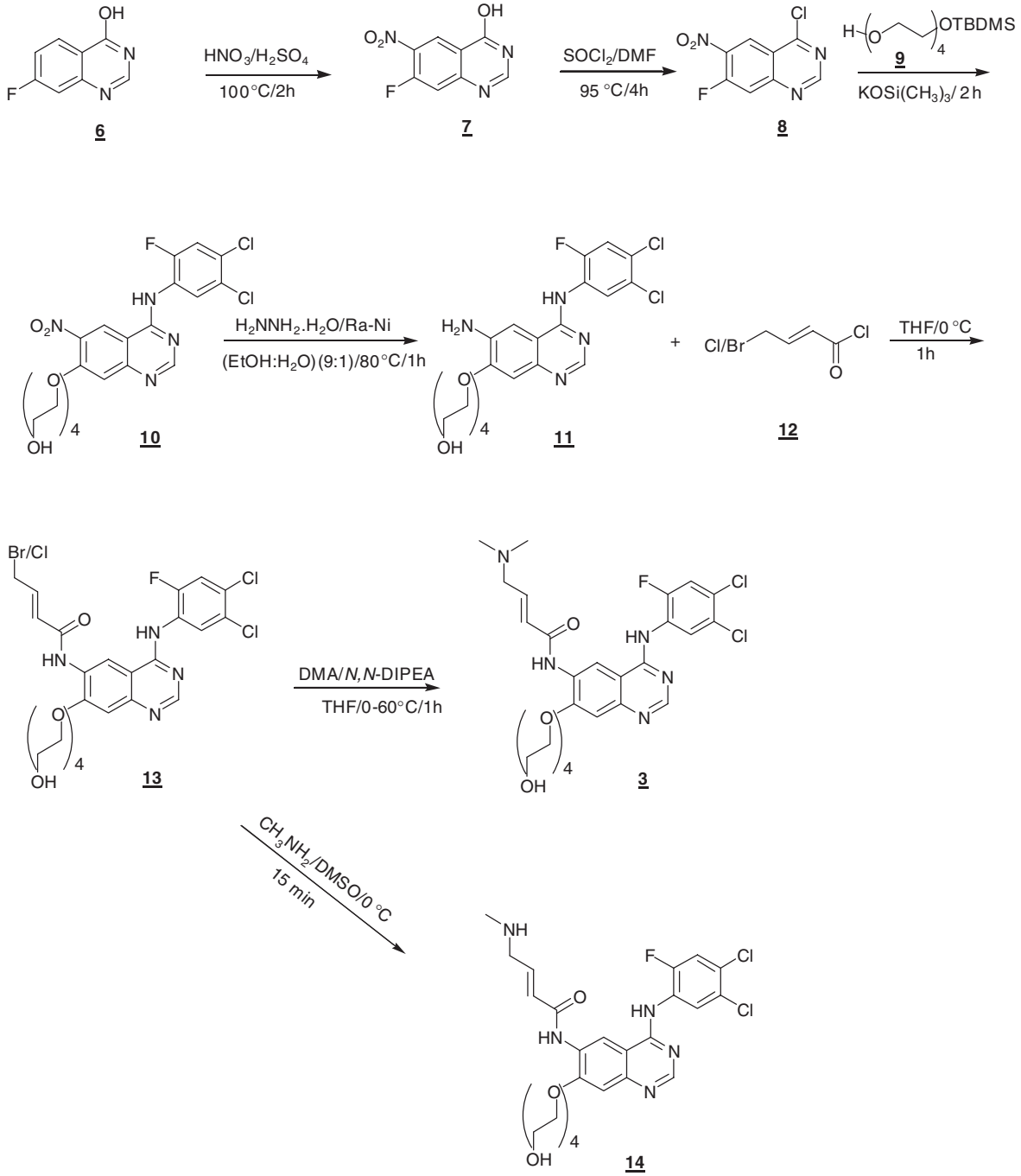


Figure 2. Chemical structure of ML04 derivatives **3**, **4**, and **5**.

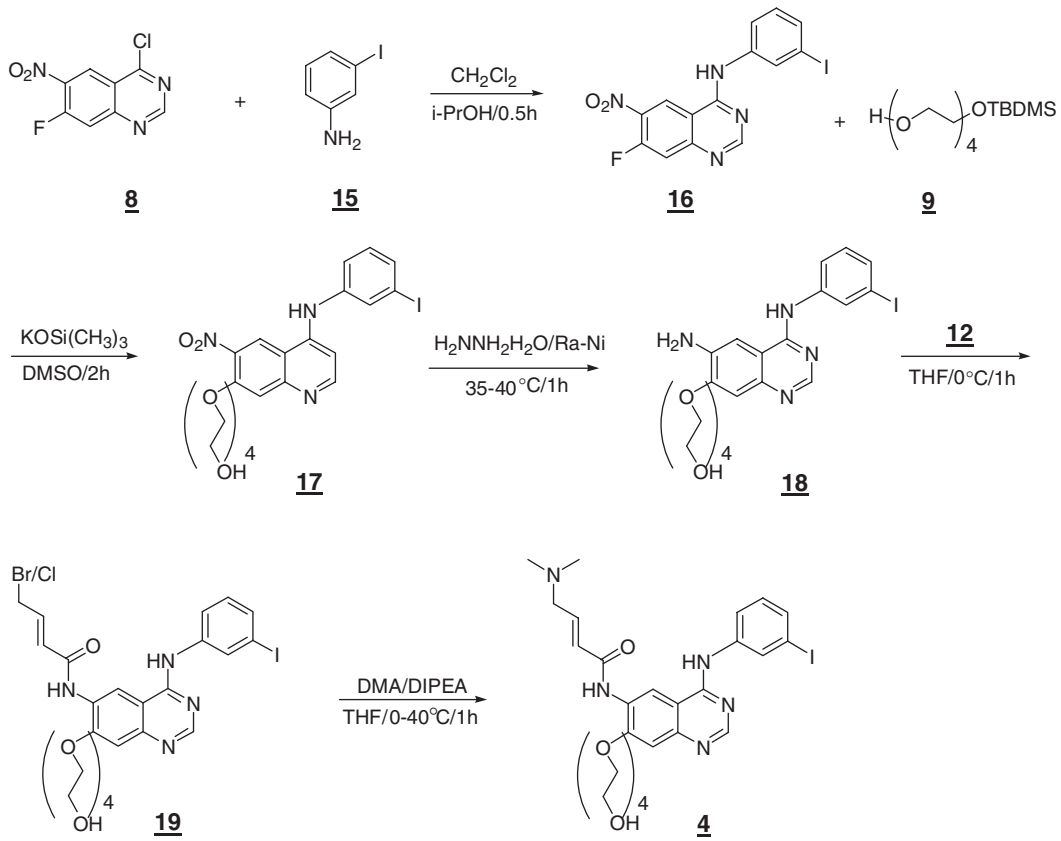


Scheme 1.

in THF followed by the addition of DMF and stirred for 1 h at 0°C to obtain **30** (28%).

Radiochemistry

The carbon-11 radiolabeling reaction to obtain compound **[^{11}C]-3** is outlined in Scheme 6. The strategic radiolabeling approach is based on the [^{11}C] methylation reaction of the monomethylamine group of the amide attached at the 6-position of the quinazoline ring. The [^{11}C] methyliodide labeling reagent was prepared according to a previously described procedure[45] and then was distilled to a second reactor that contained precursor **14** dissolved in a mixture of THF, DMSO, and CH_3CN (with this solvent composition the higher yield was obtained) at −15°C.[42] The [^{11}C] methylation reaction was carried out at 80°C for 5 min, and the crude mixture was automatically injected onto the HPLC preparative column to yield **[^{11}C]-3** (radiochemical purity = 98%, decay-corrected radiochemical yield of 13%, specific activity of 2300 Ci/mmol ($n = 6$)) in a total radiosynthesis time of 1 h including purification and formulation. The iodine-124 radiosynthetic route is described in Scheme 7, **[^{124}I]-4** was obtained with radiochemical yield of 31% ($n = 3$), 97% purity, and specific activity over 6000 Ci/mmol (detection limit) in a total radiosynthesis time of 2.5 h including purification and formulation.



Scheme 2.

Scheme 8 shows the one-step [^{18}F] labeling of compound **5**. **[^{18}F]-5** was obtained with a decay-corrected radiochemical yield of 5%, specific activity of 2200 Ci/mmol ($n = 3$), and radiochemical purity of 97%. The achieved low radiochemical yield was probably due to the reactive double bond of the butenamide residue attached at the 6-position of the quinazoline ring. It should be noted that the identity of all labeled compounds was confirmed by analytical HPLC co-injection with standards.

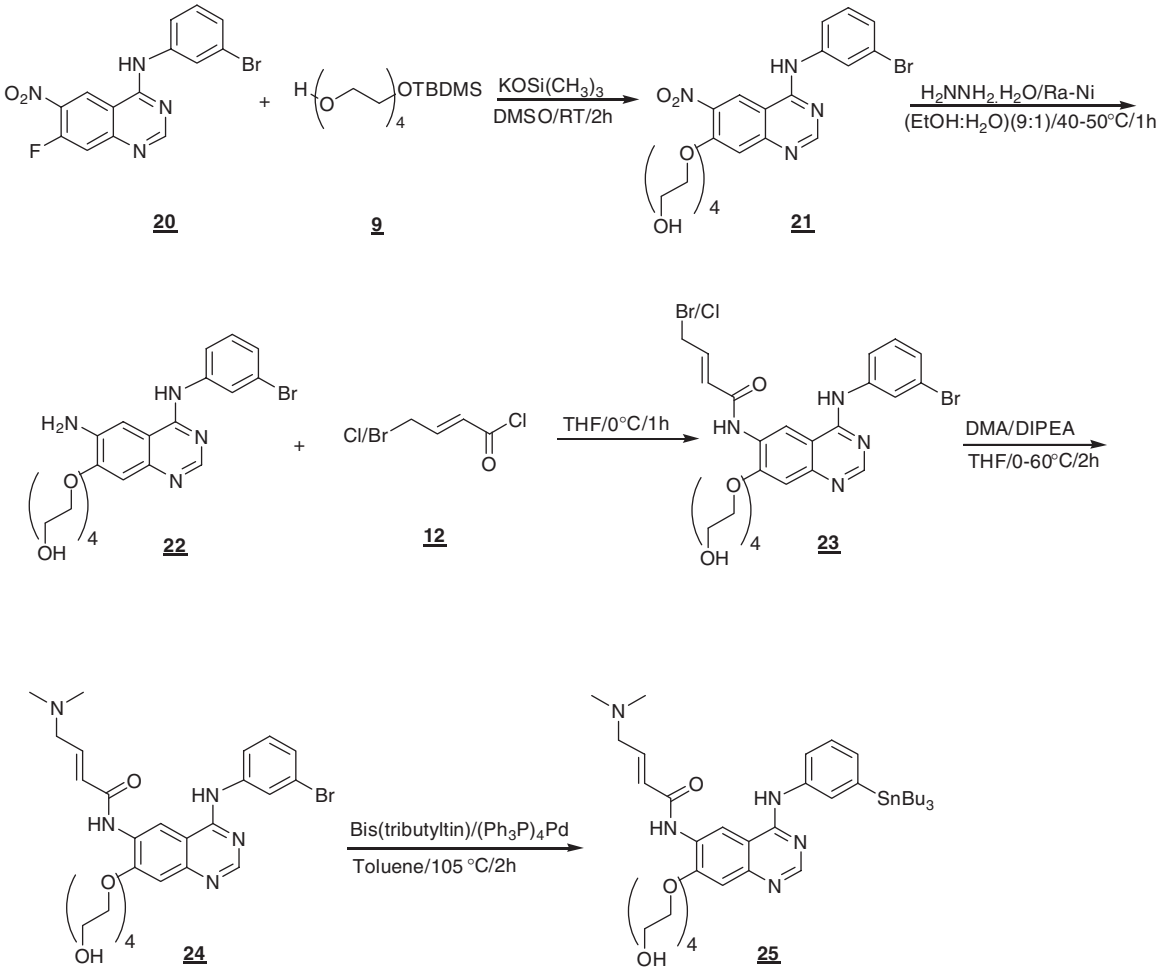
Log *P* and solubility

As far as passive diffusion into tissues and cells is involved, the lipophilicity of the molecule (generally denoted by log *P*) should be sufficiently high to allow penetration through the cell membrane. However, high log *P*'s usually lead to fast clearance from blood, accumulation in metabolic tissue, and non-specific binding in tumor. Therefore, since these EGFR inhibitors target the internal TK binding domain, a log *P* value between 1.5 and 3 would commonly be desired. The lipophilicity of compound **3** was decreased significantly (log $P = 3.1$), and the solubility was increased (solubility = 640 μg/mL) compared with the parent compound **2** (log $P = 3.7$, solubility = 3.5 μg/mL)[43] (Table 1). Therefore, it is evident that the free hydroxyl polyethylenglycol (PEG) group led to a significant decrease in lipophilicity and increase in solubility as opposed to the minor effect of the fluorinated polyethylenglycol (PEG) chain witnessed in compound **2**.[43] The log *P* and the solubility of compound **4** were also decreased to 3.3 and increased to 300 μg/mL, respectively (Table 1), although to a lesser extent than in compound **3**. It appears that the iodine atom, which is more hydrophobic and polarizable than the fluorine and the chlorine atoms, led to a higher lipophilicity and a lower solubility. The log *P* value of compound **5** was decreased to 3.45 and the solubility was increased to 120 μg/mL (Table 1). All of the above log *P* values were significantly better than those of ML04 (log $P = 3.9$, solubility = 0.14 μg/mL) and compound **2**.

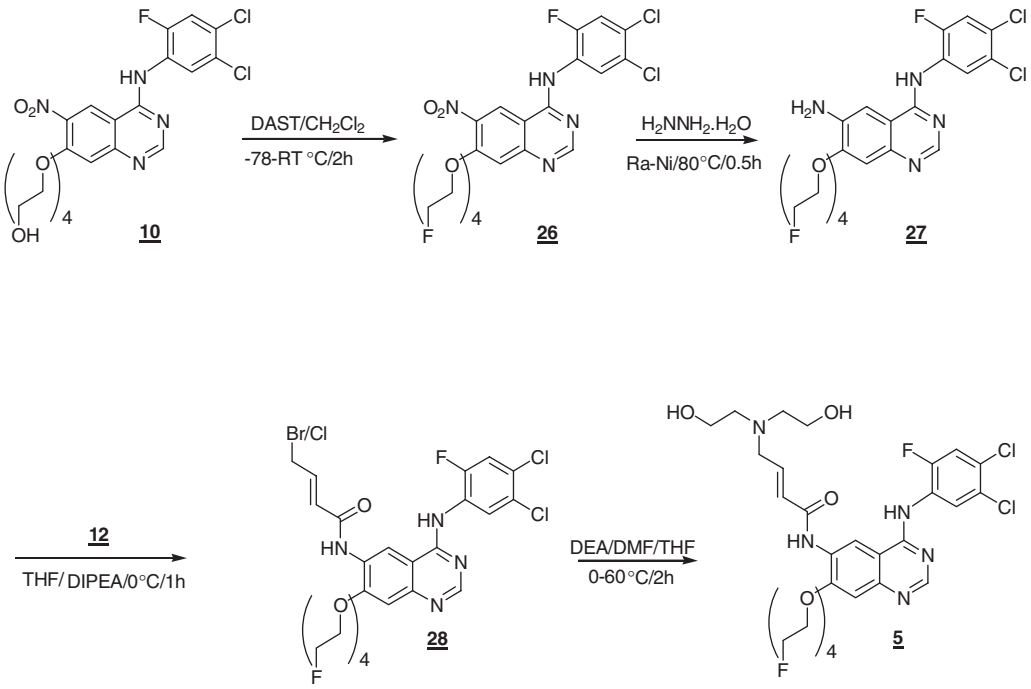
Biological evaluations

Inhibitory potency test protocol

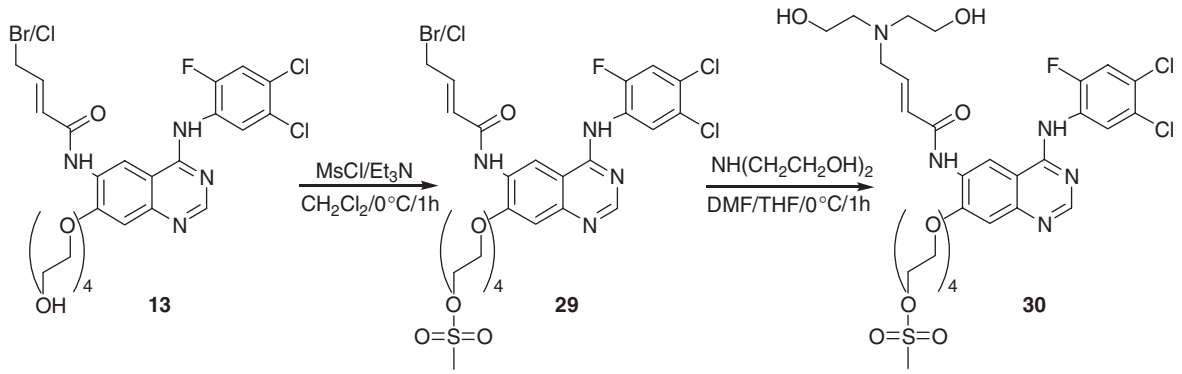
The inhibitory potency and the extent of irreversible inhibition, which compounds **3**–**5** exerted on the autophosphorylation of the EGFR, were evaluated in A431 vulval carcinoma intact cells. The inhibitors were incubated with intact A431 cells for 3 h, and the degree of EGFR phosphorylation was measured either immediately after or 8-h post-removal of the inhibitor from the medium. As previously described,[33] an 80% inhibition of phosphorylation (or more), 8 h after removal of the inhibitor compared with the 3-h samples, suggested that the compound was irreversible, while a 20–80% inhibition classified the compound as partially irreversible. The IC_{50}'s of compounds **3**–**5** were 5–35 nM, a slightly decreased potency as compared with compound **2** (Table 1). This variance could also be due to the hydrophilic characteristic of these compounds, which might slow their penetration into the cell. Indeed, when either a shorter or a longer incubation time was carried out, a decreased or an increased (only up to 3 h) affinity, respectively, was obtained. It should be noted that their irreversible inhibitory characteristic was successfully preserved.



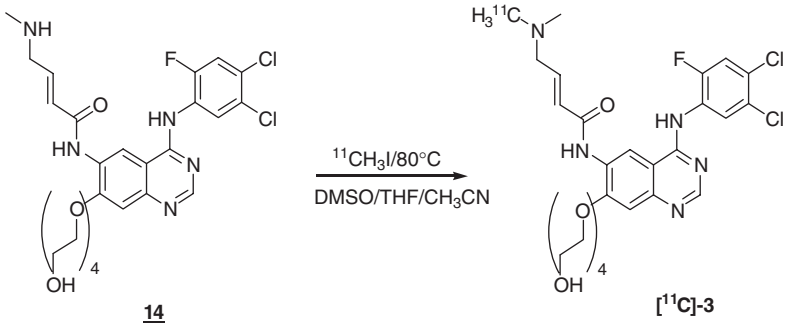
Scheme 3.



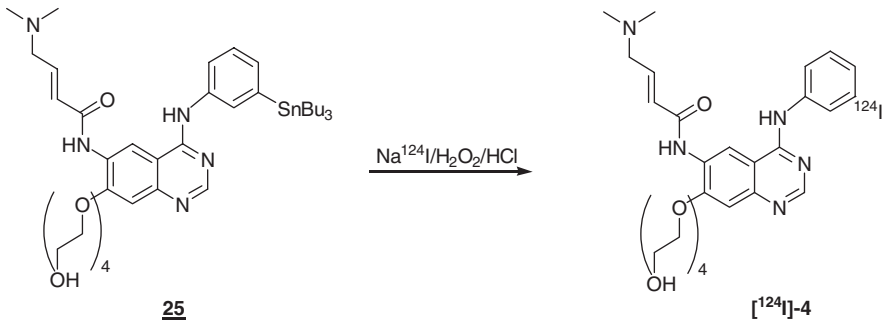
Scheme 4.



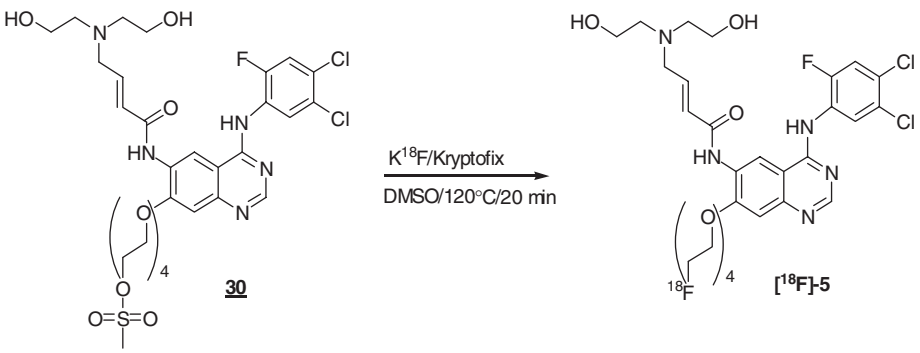
Scheme 5.



Scheme 6.



Scheme 7.



Scheme 8.

Table 1. IC_{50} values (nM) after 3-h incubation or 8-h post-incubation with A431 intact cells, log *P* and solubility values of ML04 and its derivatives

Compound	IC_{50} (nM)/3-h after incubation ($n=4$)	IC_{50} (nM)/8-h post-incubation ($n=4$)	Log *P* ($n=3$)	Solubility (μg/mL) ($n=3$)
ML04	5±3	25±5	3.9±0.4	0.14±0.56
2	3±2	15±7	3.7±0.2	3.5±0.12
3	8±2.5	20±5	3.1±0.88	630±0.78
4	15±1	79±14	3.34±0.96	300±0.33
5	35±6.5	95±10	3.45±0.8	120±0.48

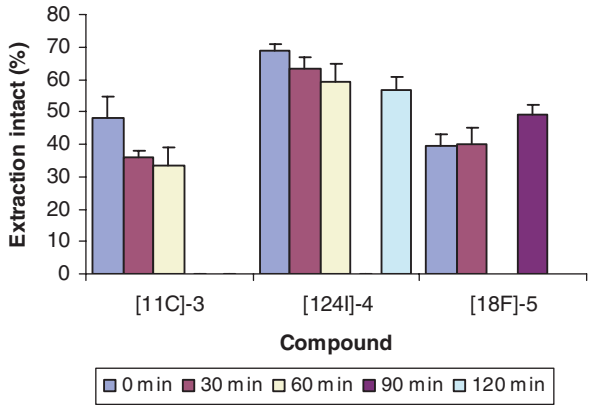


Figure 3. Extraction intact values of compounds **[^{11}C]-3**, **[^{124}I]-4**, and **[^{18}F]-5** in blood stability assay at different time periods ($n=3$).

In vitro stability in blood

In vitro blood stability was determined by incubating the radioligands, **[^{11}C]-3**, **[^{124}I]-4**, and **[^{18}F]-5**, in human blood and plasma at 37°C for various time periods. Blood samples were extracted with THF/CH_3CN (30:70) (v/v) at different time points (Figure 3). The extracted radioactivity was measured and loaded onto reversed-phase TLC plates and radioactive bands were visualized by a phosphor image plate. No radioactive degradation products were observed in blood and plasma by a phosphor image plate during these different time points.

Experimental

General

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific, Merck, or J. T. Baker. THF was refluxed over sodium/benzophenone and dichloromethane was refluxed over phosphorous pentoxide (P_2O_5). Other solvents were purchased as anhydrous. ^{1}H-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 either the residual proton (2.50 ppm for DMSO-d_6; 7.25 ppm for $CDCl_3$) of a deuterated solvent or TMS as the internal standard. Mass spectra were obtained on a spectrometer equipped with CI, EI, and FAB probes and on a spectrometer equipped with an ESI probe. HRMS results were obtained on MALDI-TOF and ESI mass spectrometers. Elemental analysis was performed on a Perkin-Elmer 2400 series II analyzer at the Hebrew University Microanalysis Laboratory. Reversed-phase HPLC: analytical, semi-preparative, and preparative columns C-18 μBondapak® Waters using the eluents 53:47 (v/v) ammonium formate 0.1 M:acetonitrile for the semi-preparative column, 50:48:2 (v/v/v) ammonium formate 0.1 M:acetonitrile:THF for the preparative column, 55:45 (v/v) acetate buffer:acetonitrile, and 53:47 (v/v) ammonium formate 0.1 M:acetonitrile for the analytical C-18 columns. A Varian 9012Q pump, a Varian 9050 variable wavelength detector operating at 254 nm, and a Bioscan Flow-Count radioactivity detector with a NaI crystal (GE) were used. Specific radioactivities were determined by HPLC, using cold mass calibration curves. Radiosyntheses, using fluorine-18 and carbon-11, were carried out on [^{18}F] and [^{11}C] GE modules. 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. [^{11}C]CO_2 was produced by the [^{14}N(p,α)^{11}C] nuclear reaction on nitrogen containing 0.5% oxygen. [^{124}I]-NaI was purchased as a 0.02 M solution from Ritverc GmbH, Germany. A431 human epidermoid vulval carcinoma cells were grown in Dulbeccco's modified Eagle medium (Biological Industries, Beit Haemek, Israel) supplemented with 10% fetal calf serum and antibiotics (penicillin 10^5 units/L, streptomycin 100 mg/L) at 37°C, 5% CO_2.

Chemistry

Compounds **6**–**10**, **20**, and **26**–**28** were prepared according to the literature procedures.[36,43]

*2-[2-(2-2-[6-Amino-4-(4,5-dichloro-2-fluoro-phenylamino)-quinazolin-7-yloxy]-ethoxy-ethoxy)-ethoxy]-ethanol (**11**)*

Hydrazine monohydrate (0.71 mmol, 34.5 μL) and Raney® nickel solution (0.8 mL) were added to a solution of compound **10** (0.183 g, 0.35 mmol) dissolved in EtOH/H_2O (9:1) (25 mL). The reaction mixture was stirred at 80°C for 30 min. The solution was cooled and filtered over Celite®; the filtrate was evaporated under reduced pressure, and the residue was extracted with dichloromethane (×3), washed with brine (×1), dried with sodium sulfate, and evaporated to afford **11** (0.139 g, 75%). ^{1}H-NMR (DMSO-d_6): δ 9.27 (s, 1H), 8.26 (s, 1H), 7.94–7.97 (m, 1H), 7.73–7.76 (m, 1H), 7.27 (s, 1H), 7.1 (s, 1H), 5.39 (s, 2H), 4.58 (m, 1H), 4.28 (m, 2H), 3.86 (m, 2H), 3.25–3.62 (m, 11H). MS (*m/z*) 515.67 (MH+). HRMS (EI): calcd. for $C_{22}H_{25}Cl_2FN_4O_5$: 515.1293, found: 515.1264. M.p. = 115–116°C.

*4-Bromo/chloro-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenylamino)- 7 - (2-2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**13**)*

Compound **11** (0.118 g, 0.22 mmol) was dissolved in dry THF (3 mL), and added dropwise to a solution of

4-bromo/chloro-but-2-enoyl chloride (**12**)[36] (0.151 g, 0.91 mmol) in THF (1.5 mL) at 0°C and stirred for 1 h. The solvent was evaporated and the residue was purified by silica gel chromatography and eluted with 5% MeOH in CH_2Cl_2 to obtain **13** (0.079 g, 52%). ^{1}H-NMR ($CDCl_3$): δ 9.19 (s-br, 1H), 9.14 (s, 1H), 8.8 (d, J = 9 Hz, 1H), 8.71 (s, 1H), 7.32 (s, 1H), 7.29 (s, 1H), 7.08–7.15 (m, 1H), 6.47–6.52 (m, 1H), 4.39 (m, 3H), 3.99–4.03 (m, 3H), 3.61–3.75 (m, 11H). MS (*m/z*) 617.6/663.27 (MH+). HRMS (EI): calcd. for $C_{26}H_{28}Cl_3FN_4O_6$: 617.1158/661.0627, found: 617.1137/661.0632.

*4-Methylamino-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenylamino)- 7 - (2-2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**14**)*

Methylamine (2 mol/L in THF, 1 mL) was added to a solution of compound **13** (0.01 g, 0.015 mmol) in DMSO (70 μL) at 0°C. After a 15-min reaction, NaOH (0.015 mol/L, 10 mL) was added and the solution was vortexed for 3 min. The solution was passed through 2 × C-18 cartridges (Waters Sep-Pak Plus), preactivated with 10 mL EtOH and 20 mL of sterile water, and dried under a stream of nitrogen. The Sep-Pak columns were eluted with THF (5 mL) and the eluent was dried with Na_2SO_4 and filtered with a 0.45-μm filter. THF evaporation under nitrogen stream yielded the crude product **14** (>70% purity). MS (*m/z*) 612.47 (MH+).

*4-Dimethylamino-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenylamino) - 7 - (2 -2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**3**)*

Compound **13** (0.128 g, 0.193 mmol) dissolved in dry THF (2 mL) was added dropwise to a cooled solution of dimethylamine (2 mL) in THF (2 M), then diisopropylethylamine (0.38 mmol, 67.3 μL) was added. The reaction mixture was stirred at 0°C for 30 min, and heated to 60°C for an additional 30 min. The solvent was evaporated, extracted with ethylacetate (× 3), washed with a $NaHCO_3$ solution (4%) (× 1), brine (× 1), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel chromatography and eluted with 15% MeOH in CH_2Cl_2 to obtain **3** (0.034 g, 28%). ^{1}H-NMR ($CDCl_3$): δ 9.26 (s-br, 1H), 9.16 (s, 1H), 8.8 (d, J = 6 Hz, 1H), 8.71 (s, 1H), 7.63 (s-br, 1H), 7.32 (s, 1H), 7.02–7.07 (m, 1H), 6.41–6.46 (m, 1H), 4.37–4.39 (m, 2H), 3.98–4 (m, 2H), 3.59–3.76 (m, 12H), 3.2 (m, 2H), 2.33 (s, 6H). MS (*m/z*) 626.73 (MH+). HRMS (EI): calcd. for $C_{28}H_{34}Cl_2FN_5O_6$: 626.1929, found: 626.1948. M.p. = 120–121°C.

*(7-Fluoro-6-nitro-quinazolin-4-yl)-(3-iodo-phenyl)-amine (**16**)*

3-Iodo-aniline (**15**) (0.639 g, 2.9 mmol) dissolved in isopropanol (13 mL) was added dropwise to a solution of compound **8** (0.6 g, 2.65 mmol) in CH_2Cl_2 (5 mL), and stirred at room temperature for 30 min.[36] The precipitation was completed by the addition of hexane; the crystals were filtered, dissolved with methanol, and neutralized with triethylamine. The orange solution was diluted with water to yield a yellow precipitate, which was filtered and washed with water. The yellow crystals were extracted with ethylacetate and washed with $NaHCO_3$ solution (4%) (× 3) and brine (× 1). The organic phase was dried over sodium sulfate, filtered, and evaporated under reduced pressure to yield **16** (0.496 g, 45%). ^{1}H-NMR ($CDCl_3$): δ 8.85 (s, 1H), 8.79 (d, J = 7.5 Hz, 1H), 8.21 (m, 1H), 7.76 (s, 1H), 7.68–7.72 (m, 1H), 7.55–7.61 (m, 2H), 7.19 (t, J = 7.8 Hz, 1H). MS (*m/z*) 411.13 (MH+). HRMS (EI): calcd. for $C_{14}H_8FIN_4O_2$: 410.9709, found: 410.9754. M.p. = 160–161°C.

*2-[2-(2-2-[4-(3-Iodo-phenylamino)-6-nitro-quinazolin-7-yloxy]-ethoxy-ethoxy)-ethoxy]-ethanol (**17**)*

Compound **16** (0.496 g, 1.21 mmol), 2-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethoxy]-ethanol (**9**) (0.561 g, 1.81 mmol), and potassium trimethylsilanolate (0.466 g, 3.63 mmol) were dissolved in dry DMSO (25 mL) and stirred for 1.5 h at room temperature.[43] The crude product was extracted with ethylacetate (× 4) and water, washed with $NaHCO_3$ (4%) and brine (× 1), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified on silica gel chromatography, and eluted with 3% MeOH in CH_2Cl_2 to obtain **17** (0.608 g, 48%). ^{1}H-NMR ($CDCl_3$): δ 8.76 (s, 1H), 8.53 (s, 1H), 8.22 (s-br, 1H), 7.71–7.73 (m, 1H), 7.64 (s-br, 1H), 7.53–7.56 (m, 1H), 7.37 (s, 1H), 7.16 (t, J = 7.8 Hz, 1H), 4.34–4.37 (m, 2H), 3.95–3.98 (m, 2H), 3.56–3.8 (m, 12H). MS (*m/z*) 585.2 (MH+). HRMS (EI): calcd. for $C_{22}H_{25}IN_4O_7$: 585.0820, found: 585.0846.

*2-[2-(2-2-[6-Amino-4-(3-iodo-phenylamino)-quinazolin-7-yloxy]-ethoxy-ethoxy)-ethoxy]-ethanol (**18**)*

Compound **18** was prepared from **17** (0.453 g, 0.77 mmol) similarly to **11** (0.173 g, 23%). ^{1}H-NMR ($CDCl_3$): δ 8.58 (s, 1H), 8.15 (s, 1H), 7.71–7.73 (m, 1H), 7.43–7.46 (m, 1H), 7.18 (s, 1H), 7.1–7.3 (m, 1H), 7.01 (m, 1H), 6.91 (s, 1H), 4.32–4.35 (m, 2H), 3.93–3.96 (m, 2H), 3.6–3.76 (m, 12H). MS (*m/z*) 555.27 (MH+). HRMS (EI): calcd. for $C_{22}H_{27}IN_4O_5$: 555.1120, found: 555.1104.

*4-Bromo/chloro-but-2-enoic acid[7-(2-2-[2-(2-hydroxy-ethoxy)-ethoxy]- ethoxy-ethoxy) - 4 - (3 -iodo-phenylamino)-quinazolin-6-yl]-amide (**19**)*

Compound **19** was prepared from **18** (0.111 g, 0.2 mmol) similarly to **13** (0.038 g, 27%). ^{1}H-NMR ($CDCl_3$): δ 9.23 (s-br, 1H), 9.07 (s, 1H), 8.65 (s, 1H), 8.2 (s-br, 1H), 7.74 (m, 2H), 7.43–7.45 (m, 1H), 7.09 (s, 1H), 7.07–7.13 (m, 1H), 6.47–6.52 (m, 1H), 4.36 (m, 3H), 4 (m, 3H), 3.59–3.74 (m, 12H). MS (*m/z*) 657.67/701.53. HRMS (EI): calcd. for $C_{26}H_{30}BrClIN_4O_6$: 657.0955/701.0475, found: 657.0977/701.0472.

*4-Dimethylamino-but-2-enoic acid[7-(2-2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy) - 4 - (3 - iodo-phenylamino)-quinazolin-6-yl]-amide (**4**)*

Compound **4** was prepared from **19** (0.038 g, 0.054 mmol) similarly to **3** (0.11 g, 32%). ^{1}H-NMR ($CDCl_3$): δ 9.3 (s-br, 1H), 9.09 (s, 1H), 8.65 (s, 1H), 8.2 (s-br, 1H), 7.74 (m, 2H), 7.43–7.76 (m, 1H), 7.22 (s, 1H), 7–7.06 (m, 1H), 6.41–6.46 (m, 1H), 4.34–4.36 (m, 2H), 3.97 (m, 2H), 3.57–3.75 (m, 12H), 3.16–3.18 (m, 2H). MS (*m/z*) 666.33 (MH+). HRMS (EI): calcd. for $C_{28}H_{36}IN_5O_6$: 666.1805, found: 666.1789.

*2-[2-(2-2-[4-(3-Bromo-phenylamino)-6-nitro-quinazolin-7-yloxy]-ethoxy-ethoxy)-ethoxy]-ethanol (**21**)*

Compound **20** (0.512 g, 1.41 mmol), 2-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethoxy]-ethanol (**9**) (0.654 g, 2.1 mmol), and potassium trimethylsilanolate (0.543 g, 4.23 mmol) were dissolved in dry DMSO (30 mL) and stirred at room temperature for 2 h. The solution was extracted with EtOAc (**4**) and water, washed with sodium bicarbonate (4%) and brine (× 1), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography and eluted with 3% MeOH in CH_2Cl_2 to obtain **21** (0.364 g, 48%). ^{1}H-NMR ($CDCl_3$): δ 8.77 (s, 1H), 8.55

(s, 1H), 8.1 (s-br, 1H), 7.64–7.71 (m, 2H), 7.26–7.35 (m, 3H), 4.33–4.36 (m, 2H), 3.94–3.97 (m, 2H), 3.55–3.8 (m, 12H). MS (*m/z*) 537.93 (MH+). HRMS (EI): calcd. for $C_{22}H_{25}BrN_4O_7$: 537.1004, found: 537.0985.

*2-[2-(2-2-[6-Amino- 4 -(3-bromo-phenylamino)-quinazolin-7-yloxy]-ethoxy-ethoxy)-ethoxy]-ethanol (**22**)*

Compound **22** was prepared from **21** (0.18 g, 0.33 mmol) similarly to **11** (0.095 g, 57%). ^{1}H-NMR ($CDCl_3$): δ 8.56 (s, 1H), 8.03 (s, 1H), 7.63–7.66 (m, 1H), 7.36–7.38 (m, 1H), 7.22 (m, 1H), 7.11 (s, 1H), 6.97 (s, 1H), 4.25–4.28 (m, 2H), 3.89–3.92 (m, 2H), 3.59–3.76 (m, 12H). MS (*m/z*) 507.87 (MH+). HRMS (EI): calcd. for $C_{22}H_{27}BrN_4O_5$: 507.1218, found: 507.1243.

*4-Bromo / chloro - but 2-enoic acid[4-(3-bromo-phenylamino)-7-(2-2 - [2 - (2 - hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**23**)*

Compound **23** was prepared from **22** (0.215 g, 0.42 mmol) similarly to **13** (0.063 g, 23%). ^{1}H-NMR ($CDCl_3$): δ 9.61 (s-br, 1H), 9.13 (s, 1H), 8.63 (s, 1H), 8.03 (s, 1H), 7.69–7.77 (m, 2H), 7.28–7.4 (m, 2H), 7.09–7.14 (m, 1H), 6.57 (m, 1H), 4.33 (m, 2H), 3.88 (m, 2H), 3.62–3.77 (m, 14H). MS (*m/z*) 611.53/653.87 (MH+). HRMS (EI): calcd. for $C_{26}H_{30}Br_2ClN_4O_6$: 609.1120/653.0616, found: 609.1115/653.0610.

*4-Dimethylamino-but-2-enoic acid[4-(3-bromo-phenylamino)-7-(2-2 - [2 - (2 - hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**24**)*

Compound **24** was prepared from **23** (0.063 g, 0.096 mmol) similarly to **3** (0.015 g, 25%). ^{1}H-NMR ($CDCl_3$): δ 9.46 (s-br, 1H), 9.08 (s, 1H), 8.65 (s, 1H), 8.1 (s, 1H), 7.97 (s-br, 1H), 7.66–7.7 (m, 1H), 7.2–7.24 (m, 2H), 6.98–7.07 (m, 1H), 6.43–6.49 (m, 1H), 4.35–4.38 (m, 2H), 3.96–3.99 (m, 2H), 3.58–3.77 (m, 12H), 3.15–3.17 (m, 2H), 2.32 (s, 6H). MS (*m/z*) 619.33 (MH+). HRMS (EI): calcd. for $C_{28}H_{36}BrN_5O_6$: 618.1905, found: 618.1927.

*4-Dimethylamino-but-2-enoic acid[4-(3-tributylstannyl-phenylamino) - 7 - (2-2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**25**)*

Compound **24** (0.05 g, 0.08 mmol) was dissolved in dry toluene (3 mL), and *bis*(tributyltin) (0.32 mmol, 163.5 μL) was added, followed by the addition of $(PPh_3)_4Pd$ (0.028 g, 0.024 mmol).[46] The reaction mixture was refluxed for 2 h, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography and eluted with 10% MeOH in CH_2Cl_2 to obtain **25** (0.0183 g, 28%). ^{1}H-NMR ($CDCl_3$): δ 9.31 (s-br, 1H), 9.12 (s, 1H), 8.6 (s, 1H), 7.74–7.77 (m, 1H), 7.68 (m, 1H), 7.59 (s-br, 1H), 7.32–7.39 (m, 2H), 6.98–7.08 (m, 1H), 6.42–6.48 (m, 1H), 4.37 (m, 2H), 3.98 (m, 2H), 3.58–3.75 (m, 12H), 3.15–3.17 (m, 2H), 2.35 (s, 6H), 0.89–1.63 (m, 27H, Bu_3). MS (*m/z*) 829.27 (MH+). HRMS (EI): calcd. for $C_{40}H_{63}N_5O_6Sn$: 828.5412, found: 828.5422.

*4-[Bis-(2-hydroxy-ethyl)-amino]-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenyla-mino) - 7 - (2 - 2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide (**5**)*

Compound **28** (0.087 g, 0.13 mmol) was dissolved in dry THF (0.5 mL) and added to a solution of 2-(2-hydroxy-ethylamino)-ethanol (5.24 mmol, 0.5 mL) in THF (1 mL), followed by the addition of DMF (0.52 mmol, 40.8 μL). The reaction mixture was stirred at 0°C for 1 h, and heated to 60°C for an additional 1 h. The solvent was evaporated, and the residue was extracted with EtOAc (× 2), washed with $NaHCO_3$ (× 1) and brine (× 1), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel chromatography and eluted with 7% MeOH in CH_2Cl_2 to afford **5** (0.0125 g, 14%). ^{1}H-NMR ($CDCl_3$): δ 9.12 (s, 1H), 8.91 (s, 1H), 8.7 (m, 2H), 7.86 (s-br, 1H), 7.31 (s, 1H), 7 (m, 1H), 6.54–6.59 (m, 1H), 4.6–4.63 (m, 1H), 4.44–4.47 (m, 1H), 4.34–4.37 (m, 2H), 3.98–4 (m, 2H), 3.55–3.76 (m, 14 H), 3.42 (m, 2H), 2.71–2.74 (m, 4H). MS (*m/z*) 688.6 (MH+). HRMS (EI): calcd. for $C_{30}H_{37}Cl_2F_2N_5O_7$: 688.2108, found: 688.2116.

*Methanesulfonic acid 2-[2-(2-2-[6-(4-bromo/chloro-but-2-enoylamino)-4-(4,5-dichloro-2-fluoro-phenylamino)-quinazolin-7-y-yloxy]-ethoxy-ethoxy)-ethoxy]-ethyl ester (**29**)*

Methanesulfonyl chloride (0.12 mmol, 9.2 μL) was added to a cooled solution of compound **13** (0.02 g, 0.03 mmol) in dry CH_2Cl_2 (1.8 mL) and triethylamine (0.12 mmol, 21 μL). The reaction mixture was stirred at 0°C for 1.5 h, and extracted from CH_2Cl_2 (× 2) and water, washed with brine (× 1), dried, and evaporated. Compound **29** was obtained with 95% chemical purity (0.017 g, 76%) without any further purification. ^{1}H-NMR ($CDCl_3$): δ 9.18 (s-br, 1H), 9.01 (s, 1H), 8.6 (s, 1H), 8.12 (s-br, 1H), 7.68 (m, 2H), 7.35–7.42 (m, 1H), 7.02 (s, 1H), 6.92–6.98 (m, 1H), 6.4–6.45 (m, 1H), 4.29 (m, 3H), 3.98 (m, 3H), 3.55–3.7 (m, 12H), 2.98 (s, 3H). MS (*m/z*) 695.73/741.2 (MH+). HRMS (EI): calcd. for $C_{27}H_{30}BrCl_3FN_4O_8S$: 694.3584/740.8623, found: 694.3577/740.8630.

*Methanesulfonic acid 2-[2-(2-2-[6-4-[bis-(2-hydroxy-ethyl)-amino]-but-2-enoylamino-4-(4, 5 -dichloro-2-fluoro-phenylamino)-quinazolin-7-yloxy]-ethoxy-ethoxy)-ethoxy]-ethyl ester (**30**)*

Compound **29** (0.017 g, 0.023 mmol) was dissolved in dry THF (0.5 mL), and added dropwise to a cooled solution of diethanolamine (0.46 mmol, 44 μL) in 0.2 mL dry THF, followed by the addition of DMF (0.092 mmol, 7 μL). The reaction mixture was stirred at 0°C for 1 h. The solvent was evaporated and the residue was extracted with EtOAc (× 2), washed with sodium bicarbonate solution (4%) (× 1) and brine (× 1), dried (Na_2SO_4), and evaporated. The residue was purified by silica gel chromatography and eluted with 15% MeOH in CH_2Cl_2 to obtain **30** (0.005 g, 28%). ^{1}H-NMR ($CDCl_3$): δ 9.14–9.17 (m, 1H), 8.71–8.8 (m, 2H), 8.36 (s-br, 1H), 7.65 (m, 1H), 7.28–7.32 (m, 2H), 7.03–7.08 (m, 1H), 6.22–6.27 (m, 1H), 4.28–4.41 (m, 5H), 3.99 (m, 3H), 3.65–3.75 (m, 13H), 3.47 (m, 2H), 3.06 (s, 3H), 2.97–2.73 (m, 3H). MS (*m/z*) 764.87 (MH+). HRMS (EI): calcd. for $C_{31}H_{40}Cl_2FN_5O_{10}S$: 763.2514, found: 763.2509.

Radiochemistry

*[^{11}C]4-dimethylamino-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenylamino) - 7 - (2 - 2 - [2 -(2-hydroxy-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide ([**^{11}C**]-**3**)*

[^{11}C]CO_2 was trapped at −160°C, and the activity was transferred into the first reactor, which contained 0.3 mL of 0.25 N $LiAlH_4$ (0.5 mL/1.5 mL THF) at −50°C. The solvent was removed under reduced pressure, followed by the addition of HI (57%) at 160°C. The resultant [^{11}C]MeI was distilled through an NaOH column to the second reactor, containing a solution of the monomethylamine precursor (**14**) (8–10 mg) dissolved in a

mixture of 0.3 mL DMSO, 0.2 mL THF, and 0.1 mL CH_3CN at −15°C. The reaction mixture was heated to 80°C for 5 min. THF and acetonitrile were removed under flow of argon at 80°C, then the reactor was cooled to 40°C, followed by the addition of 1.4 mL CH_3CN/H_2O 50:50 (v/v). The crude product was injected to the HPLC preparative column (Nucleosil C-18, 7 μm, 250 × 16 mm), eluted with ammonium formate 0.1 M:CH_3CN:THF 50:48:2 (v/v/v), flow = 8 mL/min. The labeled product was collected (R_t = 20 min) in a flask containing 50 μL of NaOH (1 M) in 70 mL water. The solution was passed though a 2 × C-18 cartridge (preactivated with 10 mL EtOH and 20 mL of water), then the Sep-Pak was washed with water (4 mL). The product was eluted with EtOH (1 mL) and saline (9 mL), and collected into the product vial. **[^{11}C]-3** was obtained after a total radiosynthesis time of 1 h, 13% decay-corrected radiochemical yield (EOB), 98% radiochemical purity, and specific activity of 2300 Ci/mmol (n = 6).

*[^{124}I]4-dimethylamino-but-2-enoic acid[7-(2-2-[2-(2-hydroxy-ethoxy)-ethoxy]- ethoxy-ethoxy) - 4 - (3 - iodo-phenylamino) - quinazolin- 6 -yl]-amide (**[^{124}I]-4**)*

A total of 0.1 mL of H_2O_2 (3%) was added to a mixture of the tributyltin derivative (**25**) (2 mg/0.2 mL EtOH), 20 μL of [^{124}I]NaI (0.02 M NaOH), and 0.1 mL of HCl (1 N) in a v-vial. The reaction mixture was stirred at room temperature for 10 min and terminated by the addition of 0.1 mL $Na_2S_2O_5$ (10%). The solution was neutralized with saturated $NaHCO_3$ (4 mL), passed though a C-18 cartridge, and eluted with THF (2 mL). The solvent was evaporated to a volume of 0.5 mL, under a stream of argon, followed by the addition of 0.5 mL CH_3CN/H_2O 50:50 (v/v), and injected to the HPLC (semi-preparative column, Nucleosil 100-C-18, 7 μm, 250 × 10 mm), eluted with ammonium formate 0.1 M:acetonitrile 53:47 (v/v), flow = 6 mL/min. The labeled product was collected (R_t = 32 min) in a flask, diluted with 0.137 mL NaOH (1 M) in 195 mL water, passed through a C-18 cartridge, and eluted with 1 mL of EtOH. The final solution of **[^{124}I]-4** was prepared from 0.09 mL EtOH and 0.91 mL of saline. **[^{124}I]-4** was obtained after a total radiosynthesis time of 2.5 h, 31% decay-corrected radiochemical yield, and 100% radiochemical purity.

*[^{18}F]4-[bis-(2-hydroxy-ethyl)-amino]-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenylamino) - 7 - (2 - 2 - [2-(2-fluoro-ethoxy)-ethoxy]-ethoxy-ethoxy)-quinazolin-6-yl]-amide **[^{18}F]-5***

[^{18}O]H_2O/$^{18}F^-$ was trapped and then transferred to a reactor through an ion exchange column (UKE F18, Macherey-Nagel, preactivated with 0.8 mL EtOH and 3 mL HPLC grade water) and eluted with 0.5 mL K_2CO_3 (5 mg/1 mL). Kryptofix$_{222}$ (18 mg/1 mL CH_3CN) was added, and the solvent was removed by an azeotropic distillation at 95°C under reduced pressure for 3 min. A solution of mesylate precursor **30** (5 mg/0.4 mL DMSO) was added to the reactor containing the dried complex K^{18}F-kryptofix. The solution was heated to 120°C for 20 min, then it was cooled to 35°C, and 1.6 mL of CH_3CN/H_2O 50:50 (v/v) was added to the reactor, and injected to the HPLC preparative reversed-phase column (Nucleosil 100-C-18, 250 × 16 mm), eluted with ammonium formate 0.1 M/acetonitrile/THF (50:48:2) (v/v/v), flow = 8 mL/min. The labeled product was collected (R_t = 14 min) in a flask diluted with water (35 mL) and loaded onto a C-18 cartridge (Water Sep-Pak, preactivated with 5 mL EtOH and 10 mL of water), and washed with additional 4 mL water, then eluted with 1 mL EtOH and 9 mL saline. **[^{18}F]-5** was obtained with a 5% decay-corrected radiochemical yield, 97% radiochemical purity, and specific activity of 2200 Ci/mmol (n = 3).

Partition coefficient determination

Partition coefficients were measured by mixing compounds **3**–**5** (3 mg) with 1 mL of 1-octanol and 5 mL of phosphate buffer 0.1 M, pH 7.4, in a test tube.[47,48] The test tube was vortexed for 2 min at room temperature, followed by centrifugation for 10 min. Aliquots of 50 μL were taken from the organic phase and the buffer, dissolved in 0.45 mL acetonitrile, and injected to the HPLC (C-18 analytical column, acetate buffer 0.1 M, pH 3.8/CH_3CN 55:45 (v/v), flow = 1 mL/min). The partition coefficient was determined by calculating the ratio of the organic to the aqueous phases.

Water solubility determination

One milligram of each of the compounds (**3**–**5**) was added into two separate test tubes (totally six tubes). Into one of the two tubes, DMSO (1 mL) was added and, to the other, MOPS buffer pH 7.4 (1 mL) was added.[49] The test tubes were sonicated at room temperature for 15 min, and filtered through 0.45-μm filters. The DMSO solution was diluted ten-folds with DMSO. Fifty microliters of each solution was injected onto HPLC system (C-18 analytical, acetate buffer 0.1 M, pH 3.8/CH_3CN 55: 45 (v/v), flow = 1 mL/min). The solubility was calculated according to the equation

$$\text{Solubility} = ((\text{peak area}_{\text{buffer}})/(\text{peak area}_{\text{DMSO}} \times 10)) \times (1000\,\mu\text{g/mL})$$

Biology

Irreversibility test protocol

The inhibitory potency and the extent of irreversible inhibition, which compounds **3**–**5** exerted on the phosphorylation of the EGFR, were evaluated in A431 vulval carcinoma cells. A431 cells (1×10^5 cells/well) were grown in six-well plates for 48 h, and further maintained in serum-free media for an additional 18 h. Duplicate sets of cells were incubated with increasing concentrations of compounds **3**–**5** (0.05% DMSO, 0.1% EtOH) for 3 h, after which the inhibitors were removed, and a serum-free medium was added to the wells. One set of cells was stimulated with EGF (20 ng/mL) immediately after the removal of the inhibitor, while the other set of cells underwent EGF stimulation only 8 h after the removal of the inhibitor from the medium and successive rinsing with phosphate buffer saline. Cell lysates were prepared and loaded onto SDS-PAGE (8% acryl amide) for Western Blot analysis, and the extent of EGFR phosphorylation was evaluated by measuring the signal intensity of the corresponding phosphotyrosine band using a mix of anti-phosphotyrosine antibodies, PY20 (Santa Cruz Biotechnology Inc.) and 4G10 (produced from Su4G10 hybridoma cells).

In vitro blood stability

The stability of compounds **[^{11}C]-3**, **[^{124}I]-4**, and **[^{18}F]-5** was determined by the addition of 100, 25, and 50 μCi, respectively, to 1 mL of human blood, plasma, and saline samples, followed

by incubation at 37°C for different time periods (0, 60, 90, and 120 min), under shaking conditions. All the blood samples were centrifuged (eppendorf-centrifuge 5417C) at 3500 rpm for 5 min to separate RBC from plasma. The radioactive material was extracted by the addition of the solvent THF/CH_3CN 30:70 (v/v) to blood samples, vortexed for 0.5 min, and centrifuged at 10 500*g* for 5 min. The radioactivity was measured by a dose calibrator (Capintec, CRC-35R), and then the upper organic phase was loaded onto TLC plates (Partisil® LK6DF Whatman, reversed-phase silica gel). The total extracted radioactivity was calculated by subtracting the non-extractable radioactivity from the total one. TLC plates were run for 20 min with ammonium formate 0.1 M (20%), CH_3CN (75%), and THF (5%) as a mobile phase. The TLC plates were exposed for 1 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.

Conclusion

We synthesized and radiolabeled with ^{11}C, ^{18}F, and ^{124}I pegylated anilinoquinazoline ML04 derivatives as candidates for PET imaging of cancers overexpressing EGFR-TK. Compounds **3**–**5** exhibited significant lower lipophilicity and higher solubility compared with the parent compounds ML04 and **2**. Furthermore, these compounds held high EGFR autophosphorylation inhibition potency in A431 cells and their binding effect was irreversible. **[^{11}C]-3**, **[^{124}I]-4**, and **[^{18}F]-5** showed stability in blood samples at different time points. Overall, compounds **3**, **4**, and **5** merit further *in vivo* evaluation as PET bioprobes.

References

[1] C. Arteaga, *Semin. Oncol.* **2003**, *30*, 3–14. DOI: 10.1016/S0093-7754(03)70010-4.
[2] S. Sebastian, J. Settleman, S. J. Reshkin, A. Azzariti, A. Bellizzi, A. Paradiso, *Biochem. Biophys. Acta* **2006**, *1766*, 120–139.
[3] J. Schlessinger, *Cell* **2000**, *103*, 211–225. DOI: 10.1016/S0092-8674(00)00114-8.
[4] N. Normanno, A. De Luca, C. Bianco, L. Strizzi, M. Mancino, M. R. Maiello, *Gene* **2006**, *366*, 2–16. DOI: 10.1016/j.gene.2005.-10.018.
[5] A. Levitzki, *Lung Cancer* **2003**, *41*, S9–S14. DOI: 10.1016/S0169-5002(03)00134-X.
[6] M. Schmidt, M. Maurer-Gebhard, B. G. Groner G. Köhler Brochmann-Santos, W. Wels, *Oncogene* **1999**, *18*, 1711–1721. DOI: 10.1038/sj.onc.1202489.
[7] C. Mamot, D. C. Drummond, U. Greiser, K. Hong, D. B. Kirpotin, J. D. Marks, J. W. Park, *Cancer Res.* **2003**, *63*, 3154–3161.
[8] A. Shir, A. Levitzki, *PLoS Med.* **2006**, *3*, e6. DOI: 10.1371/journal.pmed.0030006.
[9] A. Levitzki, E. Mishani, *Annu. Rev. Biochem.* **2006**, *75*, 93–109. DOI: 10.1146/annurev.biochem.75.103004.142657.
[10] http://www.fda.gov/bbs/topics/news/2004/NEW01139.html.
[11] http://www.fda.gov/cder/drug/advisory/iressa.htm.
[12] J. Baselga, S. D. Averbuch, *Drugs* **2000**, *S1*, 33–40. DOI: 10.2165/00003495-200060001-00004.
[13] R. W. Akita, M. X. Sliwkowski, *Semin. Oncol.* **2003**, *S7*, 15–24. DOI: 10.1016/S0093-7754(03)00187-8.
[14] T. Takano, Y. Ohe, H. Sakamoto, K. Tsuta, Y. Matsuno, U. Tateishi, S. Yamamoto, H. Nokihara, N. Yamamoto, I. Sekine, H. Kunitoh, T. Shibata, T. Sakiyama, T. Yoshida, T. Tamura, *J. Clin. Oncol.* **2005**, *23*, 6829–6837. DOI: 10.1200/jco.2005.01.0793.
[15] E. Mishani, G. Abourbeh, M. Eiblmaier, C. Anderson, CPD **2008**, *14*, 2983–2998.
[16] J. G. Paez, P. A. Janne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson, M. Meyerson, *Science* **2004**, *304*, 1497–1500. DOI: 10.1126/science.1099314.
[17] T. J. Lynch, D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. W. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman, D. A. Haber, *N. Engl. J. Med.* **2004**, *350*, 2129–2139. DOI: 10.1056/nejmoa040938.
[18] M. S. Tsao, A. Sakurada, J. C. Cutz, C. Q. Zhu, S. Kamel-Reid, J. Squire, I. Lorimer, T. Zhang, N. Liu, M. Daneshmand, P. Marrano, G. da Cunha Santos, A. Lagarde, F. Richardson, L. Seymour, M. Whitehead, K. Ding, J. Pater, F. A. Shepherd, *N. Engl. J. Med.* **2005**, *352*, 133–144. DOI: 10.1056/nejmoa050736.
[19] W. Pao, V. Miller, M. Zakowski, J. Doherty, K. Politi, I. Sarkaria, B. Singh, R. O. Heelan, V. Rusch, L. Fulton, F. Mardis, D. O. Kupfer, R. I. Wilson, M. Kris, H. Varmus, *Proc. Natl. Acad. Sci.* **2004**, *101*, 13306–13311. DOI: 10.1073/pnas.0405220101.
[20] G. J. Riely, K. A. Politi, V. A. Miller, W. Pao, *Clin. Cancer Res.* **2006**, *12*, 7232–7241. DOI: 10.1158/1078-0432.ccr-06-0658.
[21] W. Pao, V. A. Miller, K. A. Politi, G. J. Riely, R. Somwar, M. F. Zakowski, M. G. Kris, H. Varmus, *PLoS Med.* **2005**, *2*, e73. DOI: 10.1371/journal.pmed.0020073.
[22] D. Cunningham, Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, M. Mueser, A. Harstrick, C. Verslype, I. Chau, E. Van Cutsem, *N. Engl. J. Med.* **2004**, *351*, 337–345. DOI: 10.1056/nejmoa033025.
[23] J. Bernier, *Expert Rev. Anticancer Ther.* **2006**, *6*, 1539–1552. DOI: 10.1586/14737140.6.11.1539.
[24] K. Y. Chung, J. Shia, N. E. Kemeny, M. Shah, G. K. Schwartz, A. Tse, A. Hamilton, D. Pan, D. Schrag, L. Schwartz, D. S. Klimstra, D. Fridman, D. P. Kelsen, L. B. Saltz, *J. Clin. Oncol.* **2005**, *23*, 1803–1810. DOI: 10.1200/jco.2005.08.037.
[25] S. M. Hoy, A. J. Wagstaff, *Drugs* **2006**, *66*, 2005–2014. DOI: 10.2165/00003495-200666150-00011.
[26] J. Berlin, J. Posey, S. Tchekmedyian, *Clin. Colorectal Cancer* **2007**, *6*, 427–432.
[27] M. Cohenuram, M. W. Saif, *Anticancer Drugs* **2007**, *18*, 7–15. DOI: 10.1097/CAD.0b013e32800feecb.
[28] R. Bianco, G. Daniele, F. Ciardiello, G. Tortora, *Curr. Drug Targets* **2005**, *6*, 275–287. DOI: 10.2174/1389450053765842.
[29] S. S. Sridhar, L. Seymour, F. A. Shepherd, *Lancet Oncol.* **2003**, *4*, 397–406. DOI: 10.1016/S1470-2045(03)01137-9.
[30] E. Mishani, G. Abourbeh, *Curr. Top. Med. Chem.* **2007**, *7*(18), 1755–1772. DOI: 10.2174/156802607782507457.
[31] T. A. Bonasera, G. Ortu, Y. Rozen, R. Krais, N. M. Freedman, R. Chisin, A. Gazit, A. Levitzki, E. Mishani, *Nucl. Med. Biol.* **2001**, *28*, 359–374. DOI: 10.1016/S0969-8051(01)00200-1.
[32] W. Cai, G. Niu, X. Chen, *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, 186–188. DOI: 10.1007/s00259-007-0560-9.
[33] G. Abourbeh, S. Dissoki, O. Jacobson, A. Litchi, R. Ben Daniel, D. Laki, A. Levitzki, E. Mishani, *Nucl. Med. Biol.* **2007**, *34*, 55–70. DOI: 10.1016/j.nucmedbio.2006.10.012.
[34] J. Q. Wang, M. Gao, K. D. Miller, G. W. Sledge, Q. H. Zheng, *Bioorg. Med. Chem.* **2006**, *16*, 4102–4106. DOI: 10.1016/j.bmcl.2006.04.080.
[35] A. Pal, A. Glekas, M. Doubrovin, J. Balatoni, T. Beresten, D. Maxwell, S. Soghomonyan, A. Shavrin, L. Ageyeva, R. Finn, S. M. Larson, W. Bornmann, J. G. Gelovani, *Mol. Imaging Biol.* **2006**, *8*, 262–277. DOI: 10.1007/s11307-006-0049-0.
[36] E. Mishani, G. Abourbeh, O. Jacobson, S. Dissoki, R. Ben-Daniel, A. Levitzki, *J. Med. Chem.* **2005**, *48*(16), 5337–5348. DOI: 10.1021/jm0580196.
[37] W. Cai, K. Chen, L. He, Q. Cao, A. Koong, X. Chen, *Eur. J. Nucl. Med. Mol. Imaging* **2007**, *34*, 850–858. DOI: 10.1007/s00259-006-0361-6.
[38] W. P. Li, L. A. Meyer, D. A. Capretto, C. D. Sherman, C. J. Anderson, *Cancer Biother. Radiopharm.* **2008**, *23*, 158–171. DOI: 10.1089/cbr.2007.0444.
[39] H. Su, Y. Seimbille, G. Z. Ferl, C. Bodenstein, B. Fueger, K. J. Kim, Y.-T. Hsu, S. M. Dubinett, M. E. Phelps, J. Czernin, W. A. Weber, *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, 1089–1099. DOI: 10.1007/s00259-007-0636-6.
[40] E. L. Kwak, R. Sordella, D. W. Bell, N. Godin-Heymann, R. A. Okimoto, B. W. Brannigan, P. L. Harris, D. R. Driscoll, P. Fidias, T. J. Lynch, S. K. Rabindran, J. P. McGinnis, A. Wissner, S. V. Sharma,

K. J. Isselbacher, J. Settleman, D. A. Haber, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7665–7670. DOI: 10.1073/pnas.0502860102.

[41] G. Ortu, I. Ben-David, Y. Rozen, N. M. T. Freedman, R. Chisin, A. Levitzki, E. Mishani, *Int. J. Cancer* **2002**, *101*(4), 360–370. DOI: 10.1002/ijc.10619.

[42] E. Mishani, G. Abourbeh, Y. Rozen, O. Jacobson, D. Laky, I. Ben David, A. Levitzki, M. Saul, *Nucl. Med. Biol.* **2004**, *31*, 469–476. DOI: 10.1016/j.nucmedbio.2003.12.005.

[43] S. Dissoki, Y. Aviv, D. Laky, G. Abourbeh, A. Levitzki, E. Mishani, *Appl. Radiat. Isot.* **2007**, *65*, 1140–1151. DOI: 10.1016/j.apradiso.2007.04.014.

[44] S. Dissoki, D. Laky, E. Mishani, *J. Labelled Compd. Radiopharm.* **2006**, *49*, 533–543. DOI: 10.1002/jlcr.1071.

[45] C. Crouzel, B. Langstrom, V. W. Pike, H. H. Coenen, *Int. J. Appl. Radiat. Isot.* **1987**, *38*, 601–604. DOI: 10.1016/0883-2889(87)90123-7.

[46] M. Shaul, G. Abourbeh, O. Jacobson, Y. Rozen, D. Laky, A. Levitzki, E. Mishani, *Bioorg. Med. Chem.* **2004**, *12*, 3421–3429. DOI: 10.1016/j.bmc.2004.04.044.

[47] L. Cai, F. T. Chin, V. W. Pike, H. Toyama, J. S. Liow, S. S. Zoghbi, K. Modell, E. Briard, H. U. Shetty, K. Sinclair, S. Donohue, D. Tipre, M. P. Kung, C. Dagostin, D. A. Widdowson, M. Green, W. Gao, M. M. Herman, M. Ichise, R. B. Innis, *J. Med. Chem.* **2004**, *47*, 2208–2218. DOI: 10.1021/jm030477w.

[48] Z. P. Zhaung, M. P. Kung, C. Hou, D. M. Skovronsky, T. L. Gur, K. Plössl, J. Q. Trojanowski, V. M. Y. Lee, H. F. Kung, *J. Med. Chem.* **2001**, *44*, 1905–1914. DOI: 10.1021/jm010045q.

[49] B. C. Bookser, B. G. Ugarkar, M. C. Matelich, R. H. Lemus, M. Allan, M. Tsuchiya, M. Nakane, A. Nagahisa, J. B. Wiesner, M. D. Erion, *J. Med. Chem.* **2005**, *48*, 7808–7820. DOI: 10.1021/jm050394a.