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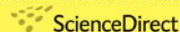
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The effect of the [^{18}F]-PEG group on tracer qualification of [4-(phenylamino)-quinazoline-6-yl]-amide moiety—An EGFR putative irreversible inhibitor

Samar Dissoki^a, Yoel Aviv^{a,b}, Desideriu Laky^a, Galith Abourbeh^{a,b},
Alexander Levitzki^b, Eyal Mishani^{a,*}

^a*Department of Medical Biophysics and Nuclear Medicine, Hadassah Hebrew University, Hadassah University Hospital, P.O.B. 12000, Jerusalem 91120, Israel*

^b*Unit of Cellular Signaling, Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University, Jerusalem 91904, Israel*

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Abstract

Previous reports have designated the labeled derivatives of [4-(phenylamino)-quinazoline-6-yl]-amide group as the most promising EGFR-PET imaging agent candidates. To further improve tracer qualifications and increase stability and solubility, additional derivatives of this group substituted at the 7-position with various lengths of fluoro-polyethyleneglycol (F-PEG) chains were synthesized. These novel derivatives inhibited EGFR autophosphorylation with IC_{50} values of 5–40 nM. The compounds were successfully labeled with fluorine-18 at the PEG chain via a three-step radiosynthesis route. The labeled final products were obtained with a 13–32% decay corrected radiochemical yield, 99% radiochemical purity, and high specific activity.

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Keywords: PET; ^{18}F ; EGFR; Cancer; Molecular imaging

1. Introduction

Overexpression of the EGFR has been demonstrated in numerous human epithelial tumors. Furthermore, correlation between EGFR overexpression and metastasis formation, therapy resistance, poor prognosis and short survival has been recently described (Tokunaga et al., 1995; Shimada and Imamura, 1996; Rae and Lippman, 2004; Levitzki, 2003). Thus, the EGFR-TK has become a major target for the development of specific anticancer drugs (Levitzki and Mishani, 2006). Examples of such therapies include FDA-approved reversible EGFR-TK inhibitors such as Gefitinib (IressaTM, ZD1839; AstraZeneca, Wilmington, PA) and Erlotinib (TarcevaTM; Genentech, San Francisco, CA) (<http://www.fda.gov/cder/drug/advisory/iressa.htm>; <http://www.fda.gov/bbs/topics/news/2004/NEW01139.html>), and

compounds currently under clinical trials such as Lapatinib (GW572016, GlaxoSmithkline), PKI-166, and the irreversible inhibitor CI-1033 (Fig. 1). Erlotinib and Gefitinib yield similar results in that they are both effective only in a small percentage of patients in whom EGFR possesses activating mutations in the kinase domain (Paez et al., 2004; Tsao et al., 2005; Pao et al., 2004; Takano et al., 2005; Lynch et al., 2004). Non-small cell lung cancer patients who initially respond to Gefitinib and Erlotinib become resistant due to secondary mutations in the EGFR, in addition to the primary mutations that made them responsive to these inhibitors (Pao et al., 2005). Concurrent with the multitude of efforts aimed at targeting and inhibiting the EGFR in cancerous cells, the role that EGFR overexpression plays in cancer development is gradually unraveling. Since accurate measurements of EGFR phosphorylation in human tumor are lacking, it is actually not possible to assess whether the poor response to Gefitinib or Erlotinib is indeed due to a lack of the specific activating mutations,

*Corresponding author. Tel.: +972 2 677 7931; fax: +972 2 642 1203.
E-mail address: mishani@md.huji.ac.il (E. Mishani).

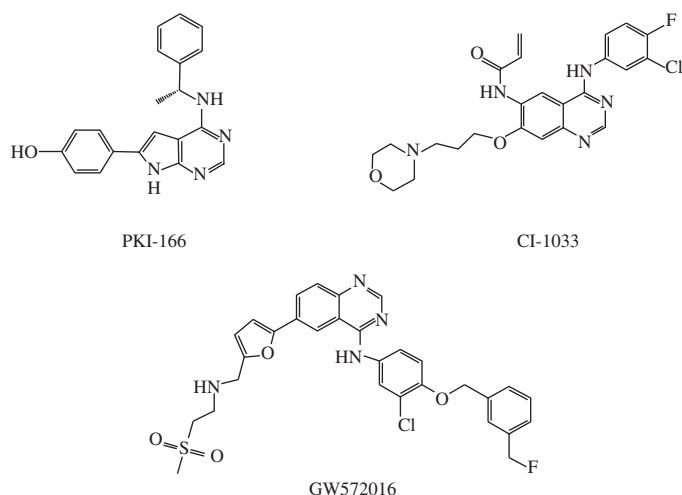


Fig. 1. Chemical structure of PKI-166, CI-1033, GW572016.

the absence of a survival function of EGFR, or to an insufficient long-term occupancy of the receptor by reversible inhibitors. Consequently, 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) (Wang et al., 2006; Pal et al., 2006; Mishani et al., 2005, 1999).

Current pre-clinical data show that cell lines expressing the Gefitinib-resistant mutants are inhibited by irreversible EGFR inhibitors, suggesting that these drugs may have future clinical utility (Gazdar and Minna, 2005; Kobayashi et al., 2005; Kwak et al., 2005; Yoshimura et al., 2006; Tsou et al., 2001). Therefore, in recent reports (Ortu et al., 2002; Mishani et al., 2005; Vasdev et al., 2005; Dissoki et al., 2006; Abourbeh et al., 2007; Pal et al., 2006), we and others have focused on the design and development of novel irreversible inhibitors as PET imaging agent candidates. One group of compounds that was tested, the 4-dimethylamino-but-2-enoic acid [4-(phenylamino)-quinazoline-6-yl]-amide group held a favorable profile, characterized by a remarkable inhibitory potency toward the EGFR, sufficient selectivity with respect to other tested tyrosine kinase receptors (Abourbeh et al., 2007), and elevated chemical and biological stabilities compared with previously studied irreversible inhibitors (Ortu et al., 2002). The lead compound in this group, ML04, was labeled with C-11 (Mishani et al., 2004) and F-18 (Dissoki et al., 2006) (Fig. 2), and its potential as an EGFR-PET imaging agent was evaluated (Abourbeh et al., 2007). Although the biological stability of ML04 was improved relative to previously described irreversible labeled inhibitors, it did not yield adequate PET images in tumor-bearing animal models, probably due to its low bioavailability, solubility under physiological conditions and fast clearance of the labeled inhibitor from blood. We report here the development of novel Fluorine-18 labeled inhibitors 1a–d (Schemes 1–3) based on the 4-anilinoquinazoline structure which

contains the chloroacetamide and dimethylamino-but-2-enoic acid as in ML05 and ML04, respectively (Fig. 2) attached at the 6-position of the quinazoline ring (Mishani et al., 2005). Presently, the chemical modifications that were introduced to the molecule include varied lengths ($n = 2, 4, 6$) of fluorinated polyethyleneglycol (PEG) (Zhang et al., 2005) chains attached at the 7-position of the quinazoline ring of ML05. Since PEG chains of 2 did not improve the biological characteristics of ML05, and PEG chain of 6 resulted in lower affinity of ML05 to the EGFR, only PEG chain of 4 was utilized for the ML04 derivative.

2. Results and discussion

2.1. Chemistry

The main objective of this work was to modify the chemical structures of the potent EGFR irreversible inhibitors, ML05 and ML04, in order to improve their potential as PET imaging agents. In this respect, the deficiencies of ML05 and ML04 were addressed by improving labeling methodology, increasing solubility, decreasing lipophilicity (Wei et al., 2006; Samant et al., 2006; Zhang et al., 2005) to minimize non-specific binding in vivo (Abourbeh et al., 2007), and decreasing blood clearance.

To achieve the tasks described above, we synthesized four different EGFR inhibitors 1a–d based on the 4-(phenylamino)quinazoline ring containing fluorinated PEG chains at the C-7 position while preserving the C-6 position to maintain the potential irreversible nature of the compounds (Mishani et al., 2005). The C-7 position was selected as an anchor to attach the PEG chains. This chemical modification was based on molecular modeling calculations suggesting that a favorable site to attach long-chain functional groups should be at the C-6 and/or C-7 positions, since these positions point toward the outer part of the protein binding pocket and therefore would not cause steric effects and interfere with the binding of the inhibitor to the tyrosine binding domain (Smaill et al., 2000; Tsou et al., 2001). In addition, labeling at the C-7 position was found to be more stable, thus favorable, than the C-6 position (Samen et al., 2006). Various chain lengths of PEG ($n = 2, 4, 6$) were attached at the C-7 position of the quinazoline ring in order to study their impact on binding, solubility and log P. Fluorine atom was attached at the end of the PEG side chain, in order to afford future labeling with fluorine-18 without changing the chemical structure of the non-labeled compound.

The synthesis of compounds 2–5 have already been described (Mishani et al., 2005). The fluorinated PEG anilinoquinazoline 1a–d were prepared by reactions shown in Schemes 1–3. tert-Butyldimethylsilyl chloride (TBDMSCl) in DMF was added dropwise to a cooled solution of imidazole and PEG ($n = 2, 4, 6$) in DMF during 30 min, and stirred for an additional 2 h at 0 °C to give the protected compounds 6a–c (14–45%). Compounds 6a–c

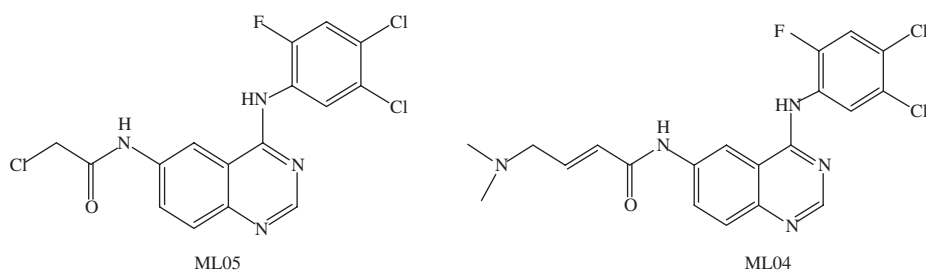
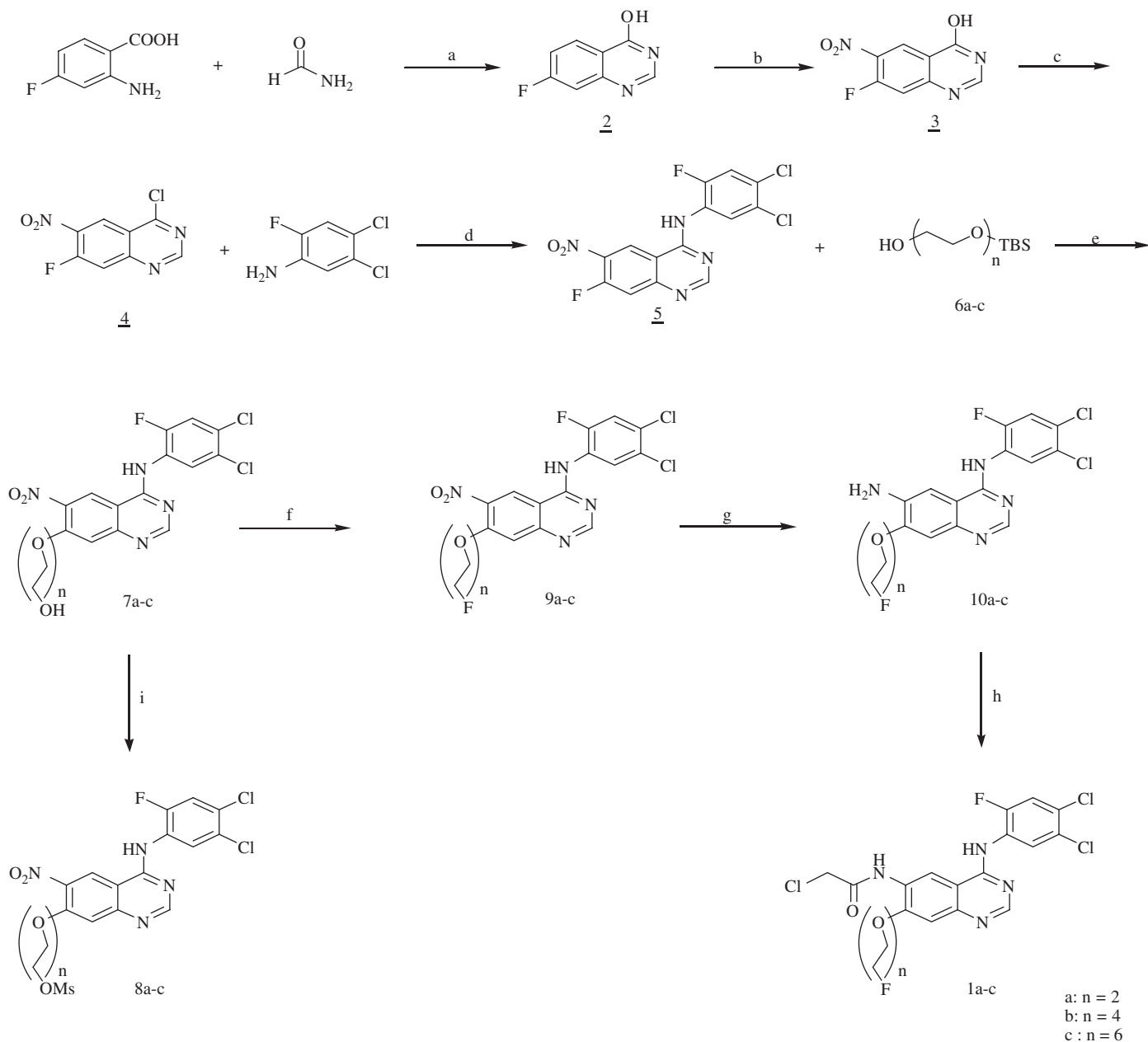


Fig. 2. Chemical structure of ML05, ML04.



Scheme 1. (a) 160 °C, 3 h; (b) H₂SO₄/HNO₃, 100 °C, 2 h; (c) SOCl₂, DMF, reflux, 7 h; (d) i-PrOH/CH₂Cl₂, RT, 30 min; (e) KOSi(CH₃)₃, DMSO, RT; (f) DAST, CH₂Cl₂, 12 h; (g) H₂NNH₂·H₂O/Raney[®] nickel, EtOH/H₂O 9:1, 85 °C, 1 h; (h) ClCOCH₂Cl, DIPEA, THF, 0 °C, 1 h; (i) MsCl, Et₃N, CH₂Cl₂, 4 h.

were coupled with **5** in the presence of potassium trimethylsilanolate to obtain free hydroxy pegylated compounds **7a–c** (24–56%). Compounds **7a–c** were reacted with mesyl chloride (MsCl) in the presence of triethylamine to give **8a–c** (26–55%), precursors for the F-18 radiosynthesis. The fluorinated PEG compounds **9a–c** were obtained (78–86%) by adding (diethylamino)sulfur trifluoride (DAST) dropwise to the **7a–c** solutions. Compounds **9a–c** were dissolved in EtOH : H₂O; an excess of hydrazine monohydrate and Raney® nickel was added to give the reduced compounds **10a–c** (54–75%). Compounds **1a–c** were obtained (21–37%) by reacting **10a–c** with chloroacetyl chloride in the presence of *N,N*-diisopropylethylamine at 0 °C for 30 min. Compound **10b** was reacted with bromo/chloro crotonylchloride to give **2d** (60%), and the latter was reacted with dimethylamine in the presence of *N,N*-diisopropylethylamine to yield **1d** (45%).

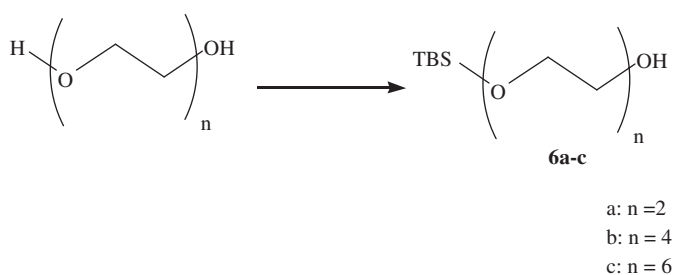
2.2. Radiochemistry

Scheme 4 shows the radiosynthetic route to yield [¹⁸F]**1a–d**. The labeling of compounds **1a–d** with fluorine-18 involves a three-step radiosynthesis. The first step of the radiolabeling was done in an automated GE module, and the crude product was then automatically transferred to a second GE module where the reduction step was performed. The first radiochemical step, the [¹⁸F]-fluorination of each of the compounds, **8a–c**, was performed in DMSO at 120 °C for 10 min, using Kryptofix[®]₂₂₂ to yield the desired [¹⁸F]**9a–c** (radiochemical purity > 90%, decay

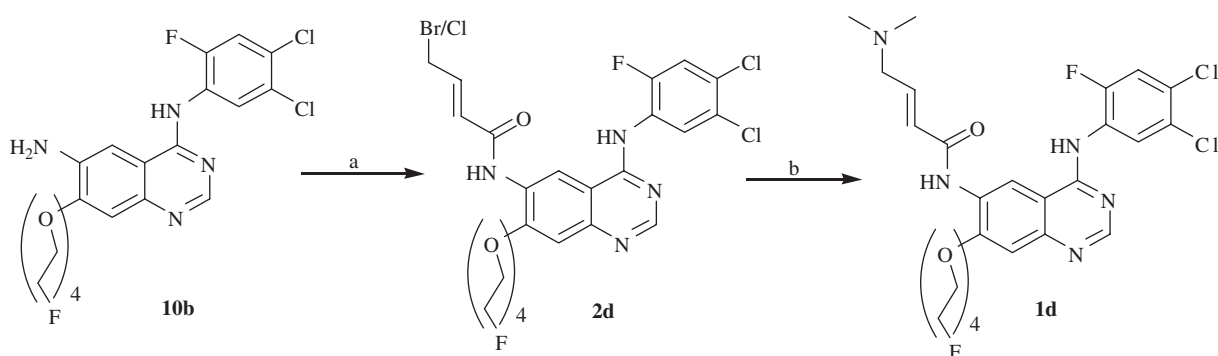
corrected radiochemical yields of 60–65%). The second step, the reduction of the nitro group to amine, was performed with hydrazine monohydrate and Raney® nickel at 60 °C for 7 min, yielding the reduced [¹⁸F]**10a–c** (radiochemical purity > 90%, decay corrected radiochemical yields of 37–50%). In the third and final step, [¹⁸F]**10a–c** were reacted with chloroacetyl chloride in the presence of DIPEA for 20 min at 0 °C in THF to yield [¹⁸F]**1a–c**. Reaction of [¹⁸F]**10b** with bromo/chloro crotonylchloride in the presence of diisopropylethylamine at 0 °C followed by reaction with dimethylamine provided [¹⁸F]**1d**. The crude products were purified by semi-preparative HPLC column to give [¹⁸F]**1a–d** with a radiochemical purity > 99%, decay corrected total radiochemical yield of 13–32%, specific activity of 2000–4000 Ci/mmol (*n* = 15), and a total radiosynthesis time of approx. 3 h, including purification and formulation.

2.3. Log *P* and solubility

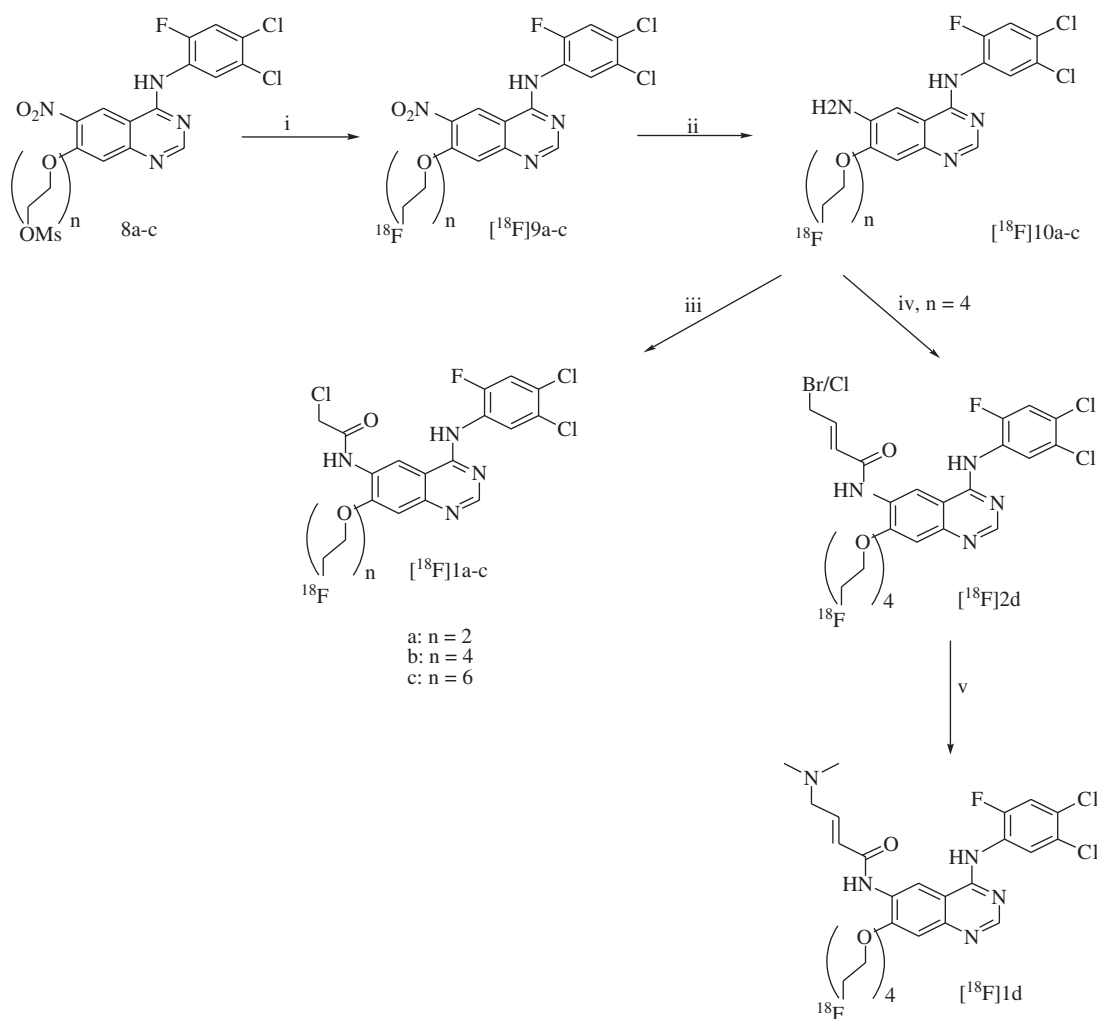
The new fluorinated PEG compounds **1a–c**, had higher lipophilicity (log *P* values were 4.32, 4.64, 4.75 for *n* = 2, 4, 6, respectively), and lower solubility (solubility < 0.05 µg/mL) compared to the parent compound, ML05 (Table 1). Generally, the PEG group with a free hydroxyl functional group at the end of the PEG chain decreases the lipophilicity and increases the solubility and the bioavailability (Zhang et al., 2005). According to our current results, adding longer PEG units (*n* = 2–6) appears to increase the lipophilicity and decrease the solubility of the ML05 derivatives, probably due to the presence of a fluorine atom at the end of the PEG chains. In contrast, the ML04 derivative (**1d**) showed a minor decreased lipophilicity (log *P* = 3.7) and a significantly improved solubility (solubility = 3.5 µg/mL) (Table 1) compared to the parent compound, ML04. Similarly to the ML05 PEG derivatives, this derivative contains the fluorine atom at the end of the PEG chain. We hypothesized that the chloroacetamide/F-PEG functional groups and the dimethylamino-but-2-enoic acid/F-PEG residues of ML05 and ML04 derivatives, respectively, interacted in a dissimilar fashion with water molecules. This interaction resulted in a preserved “positive” effect on solubility of the PEG chain on the ML04



Scheme 2. TBDMSCl, imidazole, DMF, 0 °C.



Scheme 3. (a) Bromo/chlorocrotonylchloride, DIPEA, THF, 0 °C, 1 h; (b) DMA in THF solution (2 M), DIPEA, THF, 0 °C, 1 h.



Scheme 4. (i) Kryptofix, $K^{18}\text{F}$, DMSO, 120 °C, 10 min; (ii) $\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$, Raney[®] nickel, EtOH : H_2O 9:1, 60 °C, 7 min; (iii) ClCOCH_2Cl , DIPEA, THF, 0 °C, 20 min; (iv) Br/Cl-crotonylchloride, DIPEA, THF, 0 °C, 15 min; (v) DMA, 0 °C, 15 min.

Table 1
Log *P* and solubility values

Compounds	Log <i>P</i>	Solubility (μg/mL)
Gefitinib (Iressa)	4.85 (McKillop et al., 2005; Cohen et al., 2004)	3.77 μmol/L (insoluble, pH > 7)
Erlotinib (Tarceva)	4.9 (http://www.gene.com/gene/products/information/oncology/tarceva/insert.jsp)	0.4 mg/mL, pH = 2 (insoluble, pH > 5)
ML05	4.12	0.08
1a	4.3	0.03
1b	4.64	0.01
1c	4.75	0.009
ML04	3.9	0.14
1d	3.7	3.5

derivative only and the impact of the fluorine atom on this derivative was minimally affected, and the solubility was increased.

2.4. Biological evaluations

The inhibitory potency and the extent of irreversible inhibition (Smaill et al., 1999), which compounds 1a–d exert on the phosphorylation of the EGFR, were evaluated in A431 vulval carcinoma. A431 cells 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 1a–d for 1 h, after which the inhibitor was removed, and a serum-free medium was added to the wells. One set of cells was stimulated with EGF immediately after removal of the inhibitor, while the other set of cells underwent EGF stimulation only 8 h after removal of the inhibitor from the medium and successive rinsing with phosphate buffer saline (PBS). 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. According to this assay, an 80% inhibition of phosphorylation (or more), 8 h after removal

Table 2
EGFR binding

Compounds	IC ₅₀ (A431 cells) (nM)	Binding
ML05	10	Irreversible
1a	5–10	Reversible
1b	20–25	Reversible
1c	35–40	Reversible
ML04	10	Irreversible
1d	5	Irreversible

of the inhibitor, compared to the 1 h control, suggested that the compound was irreversible, whilst a 20–80% inhibition classified the compound as a partially irreversible one.

The IC₅₀ values of compounds 1a–c, vis-à-vis the level of EGFRs phosphotyrosine content, were 5–40 nM, a decreasing potency pattern commensurate with the PEG chain length (Table 2). Moreover, in contrast to ML05, its derivatives, compounds 1a–c, exhibited a reversible inhibitory characteristic. When the PEG chain was attached at the C-7 position of ML04 in compound 1d, the potency was preserved yielding an IC₅₀ of 5 nM, and an irreversible inhibitory characteristic.

The partial positive carbon center which serves as a potential target for the nucleophilic attack of the cys-773 on the receptor binding site is at a distance of 3 and 4 atom chains from the quinazoline ring in ML05 and ML04, respectively. We hypothesized that in compounds 1a–c (the PEG derivative of ML05), as the PEG chain became longer at the C-7 position, it led to a steric effect and disabled the proximity of the positive carbon center to the cys-773, disallowing formation of covalent binding. In contrast, this phenomenon was observed to a lesser extent in the PEG derivatives of ML04 (compound 1d) since the partial positively charged carbon at the C-6 position was at a distance of 4 atom chains from the quinazoline ring, allowing for a better proximity to the cys-773 and preserving the irreversible binding characteristic of this derivative.

2.5. Conclusion

We have synthesized, radiolabeled (F-18) and evaluated four fluorinated PEG anilinoquinazoline derivatives as EGFR-TK inhibitors. Compounds 1a–c showed higher lipophilicity ($\log P = 4-5$), lower solubility compared to the parent compound, ML05, and a reversible inhibitory potency. Furthermore, commensurate to the increased length of the PEG chains, these compounds showed a decreased potency of the EGFR autophosphorylation inhibition. In contrast, compound 1d showed a moderate decreased lipophilicity ($\log P = 3.7$) and a significantly improved solubility compared to the parent compound, ML04. In addition, compound 1d displayed a high potency of EGFR autophosphorylation inhibition and an irreversible inhibition characteristic in vitro, indicating that pegylated derivatives of ML04 have better potential to be

used as radiotracers for molecular imaging of EGFR-positive tumors.

3. Experimental section

3.1. General

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific, Merck or J.T. Baker. Chemicals were used as supplied, excluding THF, which was refluxed over sodium and benzophenone and freshly distilled prior to its use. Mass spectroscopy was performed in EI mode on a Thermo Quest—Finnigan Trace MS—mass spectrometer at the Hadassah-Hebrew University Mass Spectroscopy Facility. ¹H-NMR spectra were obtained on a Bruker AMX 300 MHz apparatus using the hydrogenated residue of the deuterated solvents (DMSO-d₆, $\delta = 2.5$ ppm, CDCl₃, $\delta = 7.25$ ppm) and TMS as internal standard for ¹H-NMR. Elemental analysis was performed on a Perkin-Elmer 2400 series II analyzer at the Hebrew University Microanalysis Laboratory. Thin-layer chromatography (TLC) was run on silica gel 60F₂₅₄ plates (Merck). The compounds were localized at 254 nm using an UV lamp. Reversed phase HPLC: analytical and semi-preparative column C-18 μ Bondapak[®] Waters mobile phase system was composed of 45:55 and 50:50 (v/v) acetonitrile: acetate buffer 0.1 M pH 3.8, and 53:47 (v/v) ammonium formate 0.1 M: acetonitrile for the semi-preparative column. 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 were used. Specific radioactivities were determined by HPLC, using cold mass calibration curves. Radiosyntheses, using fluorine-18, were carried out on a [¹⁸F] module (GE). Fluorine-18 was produced on an IBA 18/9 cyclotron by irradiation of 2 mL water target (97%-enriched [¹⁸O]water) by the [¹⁸O(*p,n*)¹⁸F] nuclear reaction and was transferred to the appropriate hot cell.

3.2. Chemistry

Compounds 2–5 were prepared according to literature procedures (Mishani et al., 2005).

2-[2-(tert-butyl-dimethyl-silyloxy)-ethoxy]-ethanol (6a): A solution of imidazole (2.11 g, 31 mmol) and diethylene glycol (5 g, 47 mmol) in dry DMF (11 mL) was cooled to 0 °C and stirred for 30 min under argon (MacMahon et al., 2001). To this solution, tertbutyldimethylsilyl chloride (4.72 g, 31 mmol) in dry DMF (15 mL) was added dropwise. After 2 h at 0 °C, the reaction mixture was allowed to warm up to RT. Water (180 mL) was added and the resulting solution was extracted with ethyl acetate (4 × 80 mL). The combined organic extracts were washed with brine. After solvent evaporation, the crude product was purified on silica gel chromatography by elution with ethylacetate to yield 6a as an oil (4.65 g, 45% yield).

$^1\text{H-NMR}$ (CDCl_3): δ 3.7–3.78 (m, 4H), 3.53–3.63 (m, 4H), 0.9 (s, 9H), 0.07 (s, 6H). MS (m/z) 221.47 (MH⁺).

2-{2-[2-(2-(tert-butyl-dimethyl-silyloxy)-ethoxy)-ethoxy]-ethoxy}-ethoxy-ethanol (6b): Compound 6b was prepared from tetraethylene glycol (6 g, 30 mmol), TBDMSCl (3 g, 20 mmol), and imidazole (1.4 g, 20 mmol) in DMF (24 mL), using the same procedure as described for compound 6a (3.79 g, 41% yield). $^1\text{H-NMR}$ (CDCl_3): δ 3.53–3.78 (m, 16H), 0.9 (s, 9H), 0.058 (s, 6H). MS (m/z) 309.05 (MH⁺).

2-[2-(2-[2-[2-(2-(tert-butyl-dimethyl-silyloxy)-ethoxy]-ethoxy)-ethoxy]-ethoxy)-ethoxy]-ethoxy-ethanol (6c): Compound 6c was prepared from hexaethylene glycol (5 g, 17.7 mmol), (1.65 g, 11 mmol), and imidazole (0.748 g, 11 mmol) in DMF (26 mL), using the same procedure as described for compounds 6a and 6b (1 g, 14% yield). $^1\text{H-NMR}$ (CDCl_3): δ 3.49–3.7 (m, 24H), 0.83 (s, 9H), 0.045 (s, 6H). MS (m/z) 420.73 (M+Na).

2-[2-[4-(4,5-dichloro-2-fluoro-phenylamino)-6-nitro-quinazoline-7-yloxy]-ethoxy]-ethoxy-ethanol (7a): (4,5-dichloro-2-fluoro-phenyl-(7-fluoro-6-nitro-quinazolin-4-yl)-amine (5, 0.723 g, 1.94 mmol), 2-[2-(tert-butyl-dimethyl-silyloxy)-ethoxy]-ethoxy-ethanol (6a, 0.64 g, 2.9 mmol) and potassium trimethylsilylanolate (0.75 g, 5.83 mmol) were dissolved in dry DMSO (42 mL) and stirred under nitrogen for 5 h at 25 °C (Smail et al., 2000). The deep pink mixture was extracted with EtOAc and water; the organic phase was washed with NaHCO_3 (4%) and brine, then it was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified on silica gel column, eluted with 2% MeOH in CH_2Cl_2 to obtain 7a (0.217 g, 24% yield). $^1\text{H NMR}$ (DMSO): δ 10.26 (s, 1H), 9.14 (s, 1H), 8.58 (s, 1H), 7.92 (m, 1H), 7.84 (d, 1H, $J = 9.6$ Hz), 7.52 (s, 1H), 4.6 (m, 1H), 4.42 (m, 2H), 3.8 (m, 2H), 3.49 (m, 3H). MS (m/z) 457.6 (MH⁺). HRMS (EI): calcd. for $\text{C}_{18}\text{H}_{15}\text{Cl}_2\text{FN}_4\text{O}_5$: 457.0476, found: 457.0482. $\text{Mp} = 149$ °C.

2-[2-[2-(2-[4-(4,5-dichloro-2-fluoro-phenylamino)-6-nitro-quinazoline-7-yloxy]-ethoxy)-ethoxy]-ethoxy]-ethoxy-ethanol (7b): Compound 7b was prepared from (5, 0.247 g, 0.8 mmol), 2-[2-[2-(2-(tert-butyl-dimethyl-silyloxy)-ethoxy)-ethoxy]-ethoxy]-ethoxy-ethanol (6b, 0.37 g, 1.19 mmol), potassium trimethylsilylanolate (0.307 g, 2.39 mmol) dissolved in DMSO (15 mL), using the same procedure as described for 7a. The crude product was purified on silica gel column and eluted with 3% methanol in dichloromethane to obtain 7c (0.181 g, 42% yield). $^1\text{H-NMR}$ (CDCl_3): δ 8.82 (s, 1H), 8.77 (d, 1H, $J = 0.9$ Hz), 8.47 (s, 1H), 7.65 (s, 1H), 7.45 (s, 1H), 7.36 (d, 1H, $J = 10.2$ Hz), 4.4 (t, 2H, $J = 4.2$ Hz), 3.97 (t, 2H, $J = 4.5$ Hz), 3.58–3.99 (m, 12H). MS (m/z) 546.44 (MH⁺). HRMS (EI): calcd. for $\text{C}_{22}\text{H}_{23}\text{Cl}_2\text{FN}_4\text{O}_7$: 545.0995, found: 545.1006.

2-[2-(2-[2-[2-(2-[4-(4,5-dichloro-2-fluoro-phenylamino)-6-nitro-quinazoline-7-yloxy]-ethoxy)-ethoxy]-ethoxy]-ethoxy)-ethoxy]-ethoxy-ethanol (7c): Compound 7c was prepared from (5, 0.596 g, 1.6 mmol), 2-[2-(2-[2-[2-(2-(tert-butyl-dimethyl-silyloxy)-ethoxy)-ethoxy]-ethoxy)-ethoxy]-ethoxy]-ethoxy-ethanol (6c, 0.957 g, 2.4 mmol), potassium trimethylsilylanolate (0.621 g, 4.74 mmol) dissolved in DMSO (37 mL), using the

same procedure as described for 7a and 7b. The crude product was purified on silica gel column, eluted with 2% methanol in dichloromethane to obtain 7c (0.568 g, 56% yield). $^1\text{H-NMR}$ (CDCl_3): δ 8.64–8.82 (m, 2H), 8.5–8.55 (m, 1H), 7.46 (s, 1H), 7.34 (d, 1H, $J = 10.5$ Hz), 4.39–4.41 (m, 2H), 3.96–3.98 (m, 2H), 3.58–3.78 (m, 20H). MS (m/z) 635.78 (MH⁺). HRMS (EI): calcd. for $\text{C}_{26}\text{H}_{31}\text{Cl}_2\text{FN}_4\text{O}_9$: 633.1522, found: 633.1530.

Methanesulfonic acid 2-[2-[4-(4,5-dichloro-2-fluoro-phenylamino)-6-nitro-quinazolin-7-yloxy]-ethoxy]-ethyl ester (8a): Compound 7a (0.035 g, 0.076 mmol) was dissolved in dichloromethane (4.3 mL) and triethylamine (0.032 g, 3.82 mmol) (Zhang et al., 2005). Methanesulfonyl chloride (0.025 g, 0.022 mmol) was then added. The solution was stirred at room temperature for 3 h. The crude product was extracted with CH_2Cl_2 ($\times 2$), washed with brine ($\times 1$) and dried over sodium sulfate. The residue was purified on silica gel column, eluted with 2% methanol in dichloromethane to afford 8a (0.012 g, 29% yield). $^1\text{H-NMR}$ (CDCl_3): δ 8.82–8.84 (m, 2H), 8.45 (s, 1H), 7.56 (m, 1H), 7.46 (s, 1H), 7.35 (d, 1H, $J = 10.2$ Hz), 4.38–4.41 (m, 4H), 3.99–4.02 (m, 2H), 3.88–3.99 (m, 2H), 3.06 (s, 3H). MS (m/z) 536.81 (MH⁺). HRMS (EI): calcd. for $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{FN}_4\text{O}_7\text{S}$: 535.0215, found: 535.0219.

Methanesulfonic acid 2-[2-(2-[2-[4-(4,5-dichloro-2-fluoro-phenylamino)-6-nitro-quinazoline-7-yloxy]-ethoxy]-ethoxy)-ethoxy]-ethyl ester (8b): Compound 8b was prepared from 7b (0.023 g, 0.043 mmol), methanesulfonyl chloride (0.014 g, 0.12 mmol) and triethylamine (0.021 g, 0.021 mmol) in dichloromethane (2.3 mL), using the same procedure as described for 8a (0.015 g, 55% yield). $^1\text{H-NMR}$ (CDCl_3): δ 8.83 (s, 1H), 8.76 (d, 1H, $J = 7.8$ Hz), 8.48 (s, 1H), 7.65 (s-br, 1H), 7.44 (s, 1H), 7.34 (d, 1H, $J = 10.5$ Hz), 4.33–4.4 (m, 4H), 3.97 (t, 2H, $J = 4.5$ Hz), 3.65–3.77 (m, 10H), 3.06 (s, 3H). MS (m/z) 625.53 (MH⁺). HRMS (EI): calcd. for $\text{C}_{23}\text{H}_{25}\text{Cl}_2\text{FN}_4\text{O}_9\text{S}$: 623.0725, found: 623.0731.

Methanesulfonic acid 2-(2-[2-[2-(2-[2-[4-(4,5-dichloro-2-fluoro-phenylamino)-6-nitro-quinazolin-7-yloxy]-ethoxy]-ethoxy)-ethoxy]-ethoxy]-ethyl ester (8c): Compound 8c was prepared from 7c (0.067 g, 0.1 mmol), methanesulfonyl chloride (0.036 g, 0.32 mmol) and triethylamine (0.053 g, 0.53 mmol) in dichloromethane (6.7 mL), using the same procedure as described for 8a and 8b (0.02 g, 26% yield). $^1\text{H-NMR}$ (CDCl_3): δ 8.82 (s-br, 1H), 8.68 (d, 1H, $J = 7.8$ Hz), 8.53 (s, 1H), 7.75 (s-br, 1H), 7.42 (s, 1H), 7.36 (d, 1H, $J = 10.2$ Hz), 4.34–4.4 (m, 4H), 3.96 (t, 2H, $J = 4.5$ Hz), 3.61–3.66 (m, 18H), 3.073 (s, 3H). MS (m/z) 713.89 (MH⁺). HRMS (EI): calcd. for $\text{C}_{27}\text{H}_{33}\text{Cl}_2\text{FN}_4\text{O}_{11}\text{S}$: 711.1242, found: 711.1248.

(4,5-dichloro-2-fluoro-phenyl)-{7-[2-(2-fluoro-ethoxy)-ethoxy]-6-nitro-quinazolin-4-yl}-amine (9a): (diethylamino)sulfur trifluoride (DAST) (0.16 g, 1 mmol) was added slowly to a cold solution (-78 °C) of compound 7a (0.047 g, 0.1 mmol) in dichloromethane (2 mL) (Yoo et al., 2005). The mixture was stirred and allowed to warm up to RT for 12 h. The crude product was poured onto a basic

solution (saturated sodium bicarbonate), diluted with water and extracted with dichloromethane ($\times 3$), washed with brine ($\times 1$), dried over sodium sulfate and evaporated to afford 9a (0.036 g, 78% yield). Compound 9a was obtained with 94% purity, without any further purification. $^1\text{H-NMR(DMSO)}$: δ 10.3 (s-br, 1H), 9.18 (s-br, 1H), 8.6 (s-br, 1H), 7.82–7.95 (m, 2H), 7.58 (s-br, 1H), 4.59–4.62 (m, 1H), 4.4–4.44 (m, 3H), 3.75–3.3.85 (m, 3H), 3.7–3.72 (m, 1H). MS (m/z) 460.37 (MH⁺). Mp = 162–164 °C. HRMS (EI): calcd. for $\text{C}_{18}\text{H}_{14}\text{Cl}_2\text{F}_2\text{N}_4\text{O}_4$: 459.0423, found: 459.0419.

4,5-dichloro-2-fluoro-phenyl-[7-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-6-nitro-quinazolin-4-yl)-amine (9b): Compound 9b was prepared from 7b (0.05 g, 0.09 mmol): DAST (0.147 g, 0.9 mmol), in dichloromethane (1.5 mL), using the same procedure as described for 9a (0.042 g, 83% yield). The product was obtained with 90% purity, without any further purification. $^1\text{H-NMR (CDCl}_3)$: δ 8.8 (s, 1H), 8.64 (d, 1H, $J = 7.5$ Hz), 8.53 (s, 1H), 7.83 (s-br, 1H), 7.35 (s, 1H), 7.32 (s, 1H), 4.58 (m, 1H), 4.42 (m, 1H), 4.3 (m, 2H), 3.95 (m, 2H), 3.68–3.75 (m, 12H). MS (m/z) 548.47 (MH⁺). HRMS (EI): calcd. for $\text{C}_{22}\text{H}_{22}\text{Cl}_2\text{F}_2\text{N}_4\text{O}_6$: 547.0948, found: 547.0952.

(4,5-dichloro-2-fluoro-phenyl)-(7-{2-[2-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-6-nitro-quinazolin-4-yl)-amine (9c): Compound 9c was prepared from 7c (0.072 g, 0.11 mmol): DAST (0.182 g, 0.11 mmol), in dichloromethane (3 mL), using the same procedure as described for 9a, 9b (0.06 g, 86%). The product was obtained with 89% purity, without any further purification. $^1\text{H-NMR (CDCl}_3)$: δ 8.82 (s-br, 1H), 8.68 (d, 1H, $J = 7.8$ Hz), 8.54 (s, 1H), 7.76 (s-br, 1H), 7.44 (s, 1H), 7.34 (d, 1H, $J = 10.2$ Hz), 4.6–4.63 (m, 1H), 4.38–4.47 (m, 3H), 3.95–3.98 (m, 2H), 3.95–3.69 (m, 18H). MS (m/z) 637.49 (MH⁺). HRMS (EI): calcd. for $\text{C}_{26}\text{H}_{30}\text{Cl}_2\text{F}_2\text{N}_4\text{O}_8$: 635.1416, found: 635.1422.

N^4 -(4,5-dichloro-2-fluoro-phenyl)-7-[2-(2-fluoro-ethoxy)-ethoxy]-quinazoline-4,6-diamine (10a): Compound 9a (0.036 g, 0.078 mmol) was dissolved in EtOH/H₂O (9:1) (6 mL) at 85 °C, hydrazine monohydrate (0.31 mmol, 15.18 μL) and Raney[®] nickel solution (500 μL) were added. The reaction mixture was stirred for 1 h. The solution was cooled and filtered over Celite[®]; the filtrate was evaporated under reduced pressure to afford 10a (0.018 g, 54% yield). Compound 10a was obtained with 92% purity, without any further purification. $^1\text{H-NMR(DMSO)}$: δ 9.3 (s-br, 1H), 8.4 (s, 1H), 8.25 (s-br, 1H), 7.93 (m, 1H), 7.74 (d, 1H, $J = 9.9$ Hz), 7.27 (s, 1H), 7.09 (s, 1H), 5.39 (s-br, 2H), 4.62–4.64 (m, 1H), 4.26–4.29 (m, 3H), 3.88–3.9 (m, 3H), 3.79–3.81 (m, 1H). MS (m/z) 430.45 (MH⁺). Mp = 126 °C. HRMS (EI): calcd. for $\text{C}_{18}\text{H}_{16}\text{Cl}_2\text{F}_2\text{N}_4\text{O}_2$: 429.0652, found: 429.0646.

N^4 -(4,5-dichloro-2-fluoro-phenyl)-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinazoline-4,6-diamine (10b): Compound 10b was prepared from 9b (0.12 g, 0.21 mmol), hydrazine monohydrate (0.87 mmol, 42.56 μL) and Raney[®] nickel solution (700 μL) in ethanol/water 9:1 (23 mL), using the

same procedure as described for 10a (0.085 g, 75% yield). Compound 10b was obtained with 90% purity without any further purification. $^1\text{H-NMR(CDCl}_3)$: δ 8.62 (s, 1H), 8.58 (s, 1H), 8.3 (s, 1H), 7.5 (s-br, 1H), 7.29 (d, 1H, $J = 10.8$ Hz), 5.2 (s-br, 2H), 4.34–4.36 (m, 2H), 3.9–3.93 (m, 2H), 3.39–3.68 (m, 12H). MS (m/z) 518 (MH⁺). HRMS (EI): calcd. for $\text{C}_{22}\text{H}_{24}\text{Cl}_2\text{F}_2\text{N}_4\text{O}_4$: 517.1163, found: 517.1157.

N^4 -(4,5-dichloro-2-fluoro-phenyl)-7-{2-[2-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-quinazoline-4,6-diamine (10c): Compound 10c was prepared from 9c (0.045 g, 0.07 mmol), hydrazine monohydrate (0.28 mmol, 13.7 μL) and Raney[®] nickel solution (400 μL) in ethanol/water 9:1 (8.8 mL), using the same procedure as described for 10a and 10b (0.029 g, 68% yield). Compound 10c was obtained with 88% purity without any further purification. $^1\text{H-NMR(CDCl}_3)$: δ 8.97 (d, 1H, $J = 8.1$ Hz), 8.61 (s, 1H), 7.26 (d, 1H, $J = 9.3$ Hz), 7.19 (s, 2H), 7.03 (s, 1H), 4.6–4.63 (m, 1H), 4.45–4.47 (m, 1H), 4.3–4.33 (m, 3H), 3.93–3.96 (m, 3H), 3.65–3.79 (m, 16H). MS (m/z) 607.62 (MH⁺). HRMS (EI): calcd. for $\text{C}_{26}\text{H}_{32}\text{Cl}_2\text{F}_2\text{N}_4\text{O}_6$: 605.1726, found: 605.1721.

2-chloro- N -[4-(4,5-dichloro-2-fluoro-phenylamino)-7-[2-(2-fluoro-ethoxy)-ethoxy]-quinazoline-6-yl]-acetamide (1a): Compound 10a (0.07 g, 0.16 mmol) was dissolved in dry THF (7 mL) and stirred in an ice bath for 10 min. Diisopropylethylamine (DIPEA) (0.326 mmol, 56.8 μL) and chloroacetylchloride (0.326 mmol, 25.9 μL) were added. The reaction mixture was stirred for 1 h at 0 °C. The solvent was evaporated and the residue was extracted with EtOAc (3 \times 25 mL) and washed with NaHCO₃ (4%) (3 \times 10 mL). The combined extracts were dried (Na₂SO₄), filtered and evaporated. The crude product was purified by silica gel chromatography, eluting with 1.5% MeOH in CH₂Cl₂ to obtain 1a (0.03 g, 37% yield). $^1\text{H NMR (DMSO)}$: δ 9.89 (s-br, 1H), 9.66 (s-br, 1H), 8.85 (s, 1H), 8.42 (s-br, 1H), 7.75–7.84 (m, 2H), 7.33 (s-br, 1H), 4.6–4.63 (m, 1H), 4.47 (s, 2H), 4.38 (m, 3H), 3.88–3.91 (m, 2H), 3.79–3.82 (m, 1H), 3.69–3.71 (m, 1H). MS (m/z) 507.07 (MH⁺). Mp = 167–169 °C. HRMS (EI): calcd. for $\text{C}_{20}\text{H}_{17}\text{Cl}_3\text{F}_2\text{N}_4\text{O}_3$: 505.0412, found: 505.0413.

2-chloro- N -[4-(4,5-dichloro-2-fluoro-phenylamino)-7-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinazoline-6-yl]-acetamide (1b): Compound 10b (0.085 g, 0.16 mmol), DIPEA (0.33 mmol, 57.8 μL) and chloroacetylchloride (0.33 mmol, 26.4 μL) in THF (11 mL), using the same procedure as described for compound 1a (0.025 g, 26% yield). $^1\text{H-NMR(CDCl}_3)$: δ 9.43 (s, 1H), 9.02 (s, 1H), 8.76 (s, 1H), 7.3–7.34 (m, 2H), 4.63 (m, 1H), 4.48 (m, 1H), 4.41 (m, 2H), 4.3 (s, 2H), 4 (m, 2H), 3.68–3.77 (m, 10H). MS (m/z) 594.8 (MH⁺). Mp = 135–137 °C. HRMS (EI): calcd. for $\text{C}_{24}\text{H}_{25}\text{Cl}_3\text{F}_2\text{N}_4\text{O}_5$: 593.0920, found: 593.0937.

2-chloro- N -(4-(4,5-dichloro-2-fluoro-phenylamino)-7-{2-[2-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-quinazoline-6-yl)-acetamide (1c): Compound 1c was prepared from 10c (0.13 g, 0.21 mmol), DIPEA (0.43 mmol, 75.1 μL) and chloroacetylchloride (0.43 mmol,

34.3 μL) in THF (18 mL), using the same procedure as described for compounds 1a and 1b (0.03 g, 21% yield). $^1\text{H-NMR}(\text{CDCl}_3)$: δ 9.45 (s, 1H), 9.01 (s, 1H), 8.85 (d, 1H, $J = 7.8$ Hz), 8.75 (s, 1H), 7.53 (s-br, 1H), 7.33 (s, 1H), 4.61–4.64 (m, 1H), 4.45–4.48 (m, 1H), 4.37–4.41 (m, 2H), 4.3 (s, 2H), 3.97–4 (m, 2H), 3.64–3.75 (m, 18H). MS (m/z) 684.07 (MH⁺). Mp = 109–111 °C. HRMS (EI): calcd. for $\text{C}_{28}\text{H}_{33}\text{Cl}_3\text{F}_2\text{N}_4\text{O}_7$: 681.1484, found: 681.1461.

4-bromo/Chloro-but-2-enoic acid[4-(4,5-dichloro-2-fluorophenylamino)-7-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinazoline-6-yl]-amide (2d): Compound 10b (0.036 g, 0.069 mmol) was dissolved in dry THF (1 mL), and added dropwise to a solution of 4-bromo/chloro-but-2-enoyl chloride (Mishani et al., 2005) (0.034 g, 0.2 mmol) in THF (0.5 mL) at 0 °C. Diisopropylethylamine (DIPEA) (0.2 mmol, 36.2 μL) was added to the cooled solution, and stirred for 1 h. The solvent was evaporated and the residue was extracted with EtOAc (3 \times 25 mL) from water and washed with brine (5 mL). The combined extracts were dried (Na_2SO_4), filtered and evaporated. The crude product was purified by silica gel chromatography, eluting with 2% MeOH in dichloromethane to obtain 10 (0.028 g, 60% yield). $^1\text{H NMR}(\text{CDCl}_3)$: δ 9.15(s, 1H), 8.86 (d, 1H, $J = 7.8$ Hz), 8.73 (s, 1H), 8.65 (s-br, 1H), 7.56 (s-br, 1H), 7.32 (t, 1H), 7.06–7.13 (dt, 1H, $J = 5.7$; 6 Hz), 6.41–6.47 (dm, 1H), 4.6–4.63 (m, 1H), 4.44–4.47 (m, 1H), 4.38–4.41 (m, 3H), 3.99–4.02 (m, 3H), 3.65–3.78 (m, 10H). MS (m/z) 621.3/665.2 (MH⁺). Mp = 95–96 °C. HRMS (EI): calcd. for $\text{C}_{26}\text{H}_{27}\text{BrCl}_3\text{F}_2\text{N}_4\text{O}_5$: 619.1103, 663.061 found: 619.1093, 663.0599.

4-dimethylamino-but-2-enoic acid [4-(4,5-dichloro-2-fluorophenylamino)-7-(2-{2-[2-(2-fluoro-ethoxy)ethoxy]-ethoxy}-ethoxy)-quinazoline-6-yl]-amide (1d): Compound 2d (0.028 g, 0.042 mmol) dissolved in THF (1 mL), was added dropwise to a cooled solution of dimethylamine (2 mL) in THF (2 M), then diisopropylethylamine (0.18 mmol, 31.4 μL) was added. The reaction mixture was stirred at 0 °C for 30 min, and heated at 60 °C for an additional 30 min. The solvent was evaporated, and extracted with ethylacetate (3 \times 20 mL), washed with sodium bicarbonate solution (4%) (1 \times 10 mL), brine (1 \times 5 mL), dried over Na_2SO_4 , filtered and evaporated. The crude product was purified by silica gel chromatography, eluting with 10% MeOH in Dichloromethane to obtain 11 (0.012 g, 45% yield). $^1\text{H NMR}(\text{CDCl}_3)$: δ 9.17(s, 1H), 8.8–8.83 (m, 1H), 8.72 (s, 1H), 8.5 (s, 1H), 7.6 (s-br, 1H), 7.3 (s, 1H) 7.037 (m, 1H), 6.27–6.32 (m, 1H), 4.62 (m, 2H), 4.46 (m, 1H), 4.4 (m, 2H), 4.0 (m, 2H), 3.68–3.76 (m, 10H), 3.16–3.18 (m, 2H), 2.31 (s, 6H). MS (m/z) 628.93 (MH⁺). Mp = 119–120 °C. HRMS (EI): calcd. for $\text{C}_{28}\text{H}_{33}\text{Cl}_2\text{F}_2\text{N}_5\text{O}_5$: 628.1893 found: 628.1905. Anal. ($\text{C}_{28}\text{H}_{33}\text{Cl}_2\text{F}_2\text{N}_5\text{O}_5 \cdot 1.5\text{H}_2\text{O}$) calcd. C, 51.29; H, 5.34; N, 10.68; found. C, 51.71; H, 5.67; N, 9.76.

3.3. Radiochemistry

^{18}F fluoride ion was produced with IBA cyclotron by irradiation of $^{18}\text{O}(\text{p,n})^{18}\text{F}$ nuclear

reaction. Synthesis of ^{18}F -9a–c and ^{18}F -10a–c was performed in two automated modules (GE).

^{18}F [(4,5-dichloro-2-fluoro-phenyl)-{7-[2-(2-fluoro-ethoxy)-ethoxy]-6-nitro-quinazolin-4-yl]-amine (^{18}F]-9a): $^{18}\text{O}(\text{H}_2\text{O}/^{18}\text{F}^-)$ was trapped and then transferred to a reactor through an ion exchange column (preactivated with 0.8 mL EtOH and 3 mL HPLC grade water) and by elution with 0.5 mL potassium carbonate (2.5 mg/0.5 mL) and 1 mL Kryptofix[®] 222 (18 mg/mL CH_3CN). The solvent was removed by azeotropic distillation at 95 °C under reduced pressure for 3 min. A solution of mesylate precursor 8a (6 mg/0.4 mL DMSO) was added to the reactor containing the dried ^{18}F . The solution was heated to 120 °C for 10 min. The reaction mixture was cooled to 30 °C, and 13 mL of water was added to the solution, and loaded onto a C-18 cartridge (Waters Sep-Pak, preactivated with 5 mL EtOH and 10 mL of sterile water). Compound ^{18}F -9a was eluted with EtOH (2 mL) to a collect vial. The product was obtained after a 35 min synthesis, with a 60% radiochemical yield (decay corrected). To confirm its identity, ^{18}F -9a was co-injected with standard onto a reversed-phase HPLC C-18 analytical column (acetate buffer 0.1 M/pH = 3.8: CH_3CN , 55:45 (v/v), flow = 1 mL/min).

^{18}F [(4,5-dichloro-2-fluoro-phenyl)-[7-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-6-nitro-quinazolin-4-yl]-amine (^{18}F]-9b): Using a similar procedure, ^{18}F -9b was obtained from 8b, with a radiochemical yield of 62% (decay corrected). To confirm its identity, ^{18}F -9b was co-injected with standard onto a reversed-phase HPLC C-18 analytical column (acetate buffer 0.1 M/pH = 3.8: CH_3CN , 55:45 (v/v), flow = 1 mL/min).

^{18}F [(4,5-dichloro-2-fluoro-phenyl)-(7-{2-[2-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-6-nitro-quinazolin-4-yl)-amine (^{18}F]-9c): Using a similar procedure, ^{18}F -9c was obtained from 8c with a radiochemical yield of 65% (decay corrected). To confirm its identity, ^{18}F -9c was co-injected with standard onto a reversed-phase HPLC C-18 analytical column (acetate buffer 0.1 M/pH = 3.8: CH_3CN , 55:45 (v/v), flow = 1 mL/min).

^{18}F N^4 -(4,5-dichloro-2-fluoro-phenyl)-7-[2-(2-fluoro-ethoxy)-ethoxy]-quinazoline-4,6-diamine (^{18}F]-10a): To a reactor containing 200 μL of EtOH/ H_2O (9 : 1), Raney[®] nickel (400 μL), and hydrazine monohydrate (200 μL), compound ^{18}F -9a in ethanol was added and the solution was heated to 60 °C for 7 min to yield ^{18}F -10a, with a 37% radiochemical yield (decay corrected). 10a was diluted with water (10 mL) and passed through a C-18 cartridge; the column was dried under argon for 5 min. ^{18}F -10a was eluted with 2 mL of dry THF to a v-vial for the next step. The identity of the product was analyzed by co-injection with standard onto a reversed-phase HPLC C-18 analytical column (acetate buffer 0.1 M/pH = 3.8: CH_3CN , 55:45 (v/v), flow = 1 mL/min).

^{18}F N^4 -(4,5-dichloro-2-fluoro-phenyl)-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinazoline-4,6-diamine (^{18}F]-10b): Using a similar procedure, ^{18}F -10b was obtained from 9b. Radiochemical yield was 40% (decay corrected).

[¹⁸F]*N*⁴-(4,5-dichloro-2-fluoro-phenyl)-7-{2-[2-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-quinazoline-4,6-diamine ([¹⁸F]-10c): Using a similar procedure, [¹⁸F]-10c was obtained from 9c. Radiochemical yield was 50% (decay corrected).

[¹⁸F]2-chloro-*N*-{4-(4,5-dichloro-2-fluoro-phenylamino)-7-[2-(2-fluoro-ethoxy)-ethoxy]-quinazoline-6-yl}-acetamide ([¹⁸F]-1a): [¹⁸F]-10a in THF (2 mL) was cooled to 0 °C for 5 min, 200 μL of *N,N*-diisopropylethylamine (DIPEA) (40 μL/mL THF), and 600 μL of chloroacetylchloride (100 μL/0.5 mL THF) were added to the v-vial. The reaction mixture was stirred for 20 min at 0 °C. THF was evaporated to a volume of 300 μL; the residue was dissolved in CH₃CN : H₂O 50:50 (v/v) and injected onto HPLC for purification (reversed-phase C-18 semi-preparative column, ammonium formate 0.1 M: acetonitrile, 53:47 (v/v), flow = 6 mL/min). Retention time of [¹⁸F]-1a was 25 min under these particular HPLC conditions. The entire radiochemical synthesis, including purification and formulation of [¹⁸F]-1a, lasted 3 h. The radiochemical yield was 22% (decay corrected). To determine the radiochemical purity and specific activity, [¹⁸F]-1a was injected onto a C-18 analytical column eluted once with ammonium formate 0.1 M: acetonitrile 53:47 (v/v), flow = 0.7 mL/min; the retention time of [¹⁸F]-1a was 8.8 min and secondly with acetate buffer 0.1 M: acetonitrile 55:45 (v/v), flow = 1 mL/min; the retention time was of 21.3 min. In both cases, the radiochemical purity was 99.5%. Specific activity was determined by calibration curve of the standard injections and found to be 4000 Ci/mmol (*n* = 3).

[¹⁸F]2-chloro-*N*-[4-(4,5-dichloro-2-fluoro-phenylamino)-7-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinazoline-6-yl]-acetamide ([¹⁸F]-1b): Using a similar procedure, [¹⁸F]-1b was obtained from [¹⁸F]-10b. The entire radiochemical synthesis, including purification and formulation of [¹⁸F]-1b, lasted 3 h. The radiochemical yield was 25% (decay corrected). To determine the radiochemical purity and specific activity, [¹⁸F]-1b was injected onto a C-18 analytical column eluted once with ammonium formate 0.1 M: acetonitrile 53:47 (v/v), flow = 0.7 mL/min; the retention time of [¹⁸F]-1b was 9 min and secondly with acetate buffer 0.1 M: acetonitrile 55:45 (v/v), flow = 1 mL/min; the retention time was 24 min. In both cases, the radiochemical purity was 99.5%. Specific activity was determined by calibration curve of the standard injections and found to be 3000 Ci/mmol (*n* = 3).

[¹⁸F]2-chloro-*N*-(4-(4,5-dichloro-2-fluoro-phenylamino)-7-{2-[2-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-quinazoline-6-yl)-acetamide ([¹⁸F]-1c): Using a similar procedure, [¹⁸F]-1c was obtained from [¹⁸F]-10c with 32% decay corrected radiochemical yield, radiochemical purity of 99.4%, and specific activity of 2000 Ci/mmol (*n* = 3).

[¹⁸F]4-bromo/chloro-but-2-enoic acid[4-(4,5-dichloro-2-fluoro-phenylamino)-7-(2-{2-[2-(2-fluoro-ethoxy)-ethoxy]-ethoxy}-ethoxy)-quinazoline-6-yl]-amide ([¹⁸F]2d: [¹⁸F]-10b

was eluted with dry THF (2 mL) onto a conical vial maintained at 0 °C. 300 μL of *N,N*-diisopropylethylamine in THF (40 μL/mL), and 1.5 mL of Br/Cl-crotonylchloride in THF (150 mg/1.5 mL) were added. The reaction mixture was stirred for 15 min at 0 °C, and used for the next step without any further treatment. The identity of the product was analyzed by injection of an aliquot onto a C-18 analytical column (acetate buffer 0.1 M, pH = 3.8: CH₃CN 50:50 (v/v), flow = 1 mL/min): RT = 22.67 min (first peak), RT = 25.04 min (second peak).

[¹⁸F]4-dimethylamino-but-2-enoic acid [4-(4,5-dichloro-2-fluoro-phenylamino)-7-(2-{2-[2-(2-fluoro-ethoxy)ethoxy]-ethoxy}-ethoxy)-quinazoline-6-yl]-amide [¹⁸F]1d: Dimethylamine (1.5 mL) in THF (2.0 M) was added to the solution of compound [¹⁸F]2d at 0 °C and the reaction proceeded for 15 min. The solution was evaporated under argon to a volume of 500 μL, then a mixture of CH₃CN: H₂O 50:50 (v/v) was added. [¹⁸F]-1d was purified by a HPLC reversed-phase C-18 column (RT = 18 min, ammonium formate 0.1 M: acetonitrile 53:47 (v/v), flow = 7 mL/min), and was obtained after a total synthesis time, including purification and formulation of 3.5 h, with a 15% radiochemical yield and specific activity of 1890 Ci/mmol, and 98% radiochemical purity (*n* = 15).

3.4. Partition coefficient determination—Log *P*

Compounds 1a–d, ML04 and ML05 (3 mg) were dissolved in sodium phosphate buffer (0.1 M, pH 7.4, 5 mL) and 1-octanol (1 mL) (Cai et al., 2004; Zhaung et al., 2001). The mixture was vortexed for 2 min, filtered, stood at RT for 10 min, and centrifuged at 4000 rpm for 10 min. Aliquots of 50 μL were taken from the 1-octanol and buffer layers, and dissolved in 0.45 mL acetonitrile. Each of the two samples was injected onto HPLC system (C-18 analytical column, acetonitrile: acetate buffer 0.1 M, pH 3.8, 55:45 (v/v), flow = 1 mL/min). The partition coefficients were determined by the concentration ratio of the organic phase to the aqueous phase, and the measurement was done in triplicate (Table 1).

3.5. Water solubility determination

One mg of each of the compounds (1a–d, ML04, ML05), was added into two separate test tubes (12 tubes for the six compounds), one containing DMSO (1 mL) and the other MOPS buffer pH 7.4 (1 mL) (Bookser et al., 2005). The tubes were sonicated at RT for 20 min, and 10 min post sonication, filtered through 0.45 μm filters. The DMSO solution was diluted 10-folds with DMSO. A 50 μL sample of each solution was injected onto HPLC (C-18 analytical column, CH₃CN: acetate buffer 0.1 M, pH 3.8, 55:45 (v/v), flow = 1 mL/min, UV detector set at 254 nm). Water solubility was calculated according to the equation: solubility = ((peak area_{buffer})/ (peak area_{DMSO} × 10)) × (1000 μg/mL). The water solubilities were done in triplicate (*n* = 2) (Table 1).

3.6. Biology

The inhibitory potency and the extent of irreversible inhibition, which compounds 1a–d exert 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 1a–d (0.05% DMSO, 0.1% EtOH) for 1 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 immediately after removal of the inhibitor, while the other set of cells underwent EGF stimulation only 8 h after removal of the inhibitor from the medium and successive rinsing with phosphate buffer saline (PBS). 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).

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