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Research Article

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Design, Synthesis, and *In vitro* Antitumor Activity Evaluation of Novel 4-pyrrylamino Quinazoline Derivatives

Xiaoqing Wu[1,2], Mingdong Li[1], Wenhua Tang[2], Youguang Zheng[1], Jiqin Lian[2], Liang Xu[2,*] and Min Ji[1,*]

[1]School of Chemistry and Chemical Engineering, Southeast University, Nanjing 210009, Jiangsu, China
[2]Department of Radiation Oncology, Division of Radiation and Cancer Biology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
**Corresponding authors: Min Ji, jimin@seu.edu.cn and Liang Xu, liangxu@umich.edu*

Here, we describe the design and synthesis of two series of 4-pyrrylamino quinazolines as new analogs of the epidermal growth factor receptor inhibitor gefitinib. *In vitro* antitumor activity of these novel compounds against pancreatic (Miapaca2) and prostate (DU145) cancer cell lines was evaluated. Compared with the parental gefitinib, all 18 derivatives show a greatly increased cytotoxicity to cancer cells. *In vitro* kinase inhibitory activity on epidermal growth factor receptor was also investigated. Among them, compounds GI-6, GII-4, GII-6, GII-8, and GII-9 are more potential receptor tyrosine kinase (RTK) inhibitors. Based on these results, we propose simple structure–activity relationship to provide information for designing and developing more potent antitumor agents.

Key words: 4-pyrrylamino quinazoline, antitumor activity, cancer cells, gefitinib, receptor tyrosine kinase

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Molecular targeted therapy is a new approach in oncotherapy developed in the last 20 years, which selectively targets molecules related to cancers and possesses higher specificity and lower toxicity and side-effect over traditional cancer therapeutics. Receptor tyrosine kinases (RTKs) are a subclass of cell-surface growth factor receptors with an intrinsic, ligand-controlled tyrosine kinase activity (1) and are main regulators of intracellular communication controlling cell proliferation, differentiation, survival, metabolism, and migration (1,2). RTK activity is resting and tightly controlled in normal cell. However, gene application and overexpression of RTKs occur frequently in many human cancers, and abnormal activation of RTKs has shown to be causally associated with the development and progression of many human cancers (1–3). Consequently, RTKs are rational targets for molecular targeted cancer therapy. Strategies toward the attenuation and intervention of RTK signaling include monoclonal antibodies, small-molecule inhibitors, antagonistic ligands, and antisense oligonucleotides.

In 2003, Iressa (gefitinib, Figure 1), a selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, was approved by FDA for locally advanced or metastatic non-small-cell lung cancer therapy (4). In 2004, another EGFR small-molecule inhibitor Tarceva (erlotinib) was launched for same indication (5). Tykerb (lapatinib), a dual tyrosine kinase inhibitor of ErbB-2/EGFR, was approved by FDA for the treatment of patients with advanced or metastatic breast cancer in 2007 (6). These three drugs share the same 4-anilinoquinazoline core structure. Thus, in recent years, 4-anilinoquinazolines have emerged as a versatile template for the inhibition of diverse RTKs and numerous new derivatives have been designed and synthesized.

In this study, using gefitinib as a leading compound, we replaced the benzene ring with pyrrole ring, designed and synthesized two series of novel 4-pyrrylamino quinazoline derivatives. Preliminary *in vitro* antitumor activity and kinase inhibitory potency of these new compounds were also evaluated. Our primary objective was to develop new RTK inhibitors with improved antitumor activity.

Experimental Section

Synthesis

All reagents were purchased from commercial sources and used without further purification. Melting points were measured in open capillaries and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in CDCl3 on a Bruker Avance 500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts (δ) are reported in parts per million (p.p.m.) relative to tetramethylsilane, used as an internal

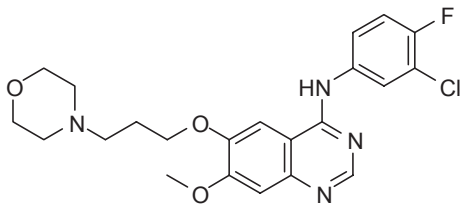


Figure 1: Structure of gefitinib.

standard. Mass spectra (MS) were obtained from Agilent 1100LC/MS. IR spectra were run on FI-IR Spectrometer (Perkin-Elmer, Waltham, MA, USA). Element analysis was run on Elementa Vario ELIII element analyzer. All compounds were routinely checked by TLC with silica gel GF-254 glass plates and viewed under UV light at 254 nm.

Diethyl 2-(2-cyanovinylamino)malonate (1)

To 1.3 L toluene, 62.4 g 50% sodium hydride (1.3 mol) was added in batches with mechanical agitation and ice water bath. A mixture of acetonitrile (49.2 g, 1.2 mol), ethyl formate (74.0 g, 1.00 mol), 200 mL toluene, and 2 mL absolute ethanol was then added dropwise to the aforementioned solution within 1 h. The reaction mixture was allowed to stir at room temperature for 24 h and then concentrated under vacuum.

The residue was dissolved in 1.5 L ethanol, and glacial acetic acid was used to adjust the pH value of this solution to 6–7; 50.0 g sodium acetate and 105 g diethyl 2-aminomalonate (0.5 mol) were added to the aforementioned solution. This mixture was stirred at room temperature for 2 days and then concentrated under vacuum. The residue was dissolved in 2 L water, and sodium bicarbonate was used to adjust the pH value of the mixture to 8–9. This mixture was extracted with ethyl acetate (5 × 200 mL). The combined organic layer was washed with saturated sodium bicarbonate solution (2 × 200 mL) and water (2 × 100 mL) and then dried using Na_2SO_4. The ethyl acetate was evaporated to give dark oil that was then distilled under reduced pressure to collect fraction at 180–183 °C and 2 mmHg as light yellow oil (**1**).

Ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (2)

To 250 mL absolute ethanol, 2.6 g sodium was added in batches with ice water bath; 30.0 g diethyl 2-(2-cyanovinylamino) malonate (**1**, 0.133 mol) in 50 mL absolute ethanol was then added dropwise to the aforementioned solution. The mixture was stirred at room temperature for 24 h and then concentrated under vacuum. The residue was dissolved in 300 mL water, and glacial acetic acid was used to adjust the pH value of the mixture to 7–8. This mixture was extracted with ethyl acetate (4 × 200 mL). The combined organic layer was washed with saturated sodium bicarbonate solution (2 × 200 mL) and water (2 × 100 mL) and then dried using Na_2SO_4. The solvent was evaporated to give 18.1 g light brown oil. The oil was then dissolved in 100 mL acetone, and dried hydrogen chloride gas was led in until no more precipitation was generated. The solid was collected by filtration and washed with acetone. After air-drying, the crude product was recrystallized from acetone to afford 20.8 g product **2** as white powder in 82% yield. Mp: 198–200 °C;^{1}H-NMR δ: 1.32 (t, 3H), 3.95 (b, 1H), 4.30 (q, 2H), 5.02 (b, 2H), 6.42 (d, 1H), 7.08 (d, 1H). Elem. Anal. Calcd: C, 44.10; H, 5.82; N, 14.70. Found C, 44.19; H, 6.15; N, 14.80.

Methyl 3-(3-chloropropoxy)-4-methoxybenzoate (3)

A mixture of methyl 3-hydroxy-4-methoxybenzoate (84.6 g, 0.47 mol), 1-bromo-3-chloropropane (101.6 g, 0.65 mol), and potassium carbonate (138.1 g, 1.0 mol) in dimethyl formamide (DMF) (500 mL) was heated at 70 °C for 4 h. The reaction mixture was cooled to room temperature and then poured slowly into ice water (3 L) while stirring constantly. The solid formed was filtered off and washed with cold water. The off-white product was recrystallized from ethyl acetate (200 mL) to give 113.9 g of **3** in 95% yield. Mp: 111–113 °C; ^{1}H-NMR δ: 2.02–2.22 (tt, 2H, -$CH_2C\mathbf{H_2}CH_2$-), 3.65 (t, 2H, -CH_2Cl), 3.79 (s, 3H, -OCH_3), 3.88 (s, 3H, -OCH_3), 4.10 (t, 2H, -CH_2O), 6.84 (d, 1H, HAr), 7.49 (s, 1H, HAr), 7.71 (d, 1H, HAr).

Methyl 5-(3-chloropropoxy)-4-methoxy-2-nitrobenzoate (4)

Nitric acid (84.5 mL, 66%) was added dropwise at 0–5 °C to a solution of methyl 3-(3-chloropropoxy)-4-methoxybenzoate (**3**, 93.0 g, 0.36 mol) in a mixture of acetic acid (300 mL) and acetic anhydride (100 mL). This mixture was stirred at room temperature for 6 h, then slowly poured into ice water (2 L), and extracted with ethyl acetate (4 × 200 mL). The combined organic layer was washed with saturated sodium bicarbonate (2 × 200 mL) and brine (2 × 100 mL) and then dried using Na_2SO_4. The ethyl acetate was evaporated to give a yellow oil that solidified after standing in a refrigerator for 12 h and was then recrystallized from ethyl acetate/petroleum ether to afford the product **4** as light yellow crystals (97.1 g, 89% yield). Mp: 54–56 °C; ^{1}H-NMR δ: 2.03–2.24 (tt, 2H, -$CH_2C\mathbf{H_2}CH_2$-), 3.66 (t, 2H, -CH_2Cl), 3.78 (s, 3H, -OCH_3), 3.89 (s, 3H, -OCH_3), 4.12 (t, 2H, -CH_2O), 7.82 (s, 1H, HAr), 8.01 (d, 1H, HAr).

Methyl 5-(3-chloropropoxy)-2-amino-4-methoxybenzoate (5)

Powdered iron (50 g, 0.89 mol) was added to acetic acid (500 mL). The resulting suspension was stirred for 15 min at 50 °C under an atmosphere of N2, and a solution of methyl 5-(3-chloropropoxy)-4-methoxy-2-nitrobenzoate (**4**, 90.0 g, 0.30 mol) in methanol (300 mL) was added dropwise. The mixture was stirred for another 30 min at 50–60 °C. The catalyst was filtered, and the filtrate was slowly poured into water (4 L) and extracted with ethyl acetate (4 × 200 mL). The organic phase was washed with a saturated solution of sodium carbonate (2 × 100 mL) and brine (2 × 100 mL) and then dried using Na_2SO_4. The solvent was removed under vacuum, and the brown solid residue was recrystallized from ethyl acetate/petroleum ether to give the product **5** as light brown crystals (63.1 g, 77% yield). Mp: 96–98 °C; ^{1}H-NMR δ: 1.98–2.20 (tt, 2H, -$CH_2C\mathbf{H_2}CH_2$-), 3.62 (t, 2H, -CH_2Cl), 3.76 (s, 3H, -OCH_3), 3.85 (s, 3H, -OCH_3), 4.07 (t, 2H, -CH_2O), 5.10–5.35 (b, 2H,-NH_2), 6.09 (d, 1H, HAr), 7.21 (s, 1H, HAr).

6-(3-chloropropoxy)-7-methoxyquinazolin-4(3H)-one (6)

A solution of methyl 5-(3-chloropropoxy)-2-amino-4-methoxybenzoate (**5**, 98.2 g, 0.36 mol) and formamidine acetate (52.6 g, 0.51 mol) in ethanol (800 mL) was heated at reflux for 6 h with overhead stirring. The mixture was allowed to stand in the refrigerator overnight. The precipitate was then collected by filtration, washed with ethanol, and air-dried to give **6** as white powder (88.7 g, 92% yield). Mp: 218–219 °C; ^{1}H-NMR δ: 2.10–2.31 (tt, 2H, -$CH_2C\mathbf{H_2}CH_2$-), 3.72

(t, 2H, -CH_2Cl), 3.83 (s, 3H, -OCH_3), 4.02 (t, 2H, -CH_2O), 6.98 (d, 1H, HAr), 7.89 (s, 1H, HAr), 8.02 (d, 1H, HAr), 9.03–9.42 (b, 1H, -NH-).

4-chloro-6-(3-chloropropoxy)-7-methoxyquinazoline (7)

6-(3-Chloropropoxy)-7-methoxyquinazolin-4(3*H*)-one (**6**, 102 g, 0.38 mol) was added to thionyl chloride (500 mL) with magnetic stirring. DMF (20 mL) was then slowly added dropwise, and the mixture was heated to reflux for 4 h. Most of the excess of thionyl chloride was then removed under reduced pressure, and the yellow residue was dissolved in chloroform (500 mL), washed with a saturated solution of sodium carbonate (2 × 100 mL) and water (2 × 100 mL), and dried using Na_2SO_4. The chloroform was then removed under reduced pressure to give off-white powder, which was recrystallized from ethyl acetate to give the product **7** (93.5 g, 86% yield). Mp: 150**–**152 °C; ^{1}H-NMR δ: 2.43 (tt, 2H, -CH_2C**H_2**CH_2-), 3.85 (t, 2H,-CH_2Cl), 4.09 (s, 3H, -OCH_3), 4.39 (t, 2H, -OCH_2), 7.43 (s, 1H, HAr), 7.47 (s, 1H, HAr), 8.91 (s, 1H, HAr).

Ethyl 3-(6-(3-chloropropoxy)-7-methoxyquinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (8)

A mixture of ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (**2**, 12.1g, 64 mmol) and 4-chloro-6-(3-chloropropoxy)-7-methoxyquinazoline (**7**, 14.9 g, 52 mmol) in isopropanol (300 mL) was heated to reflux for 4 h and then allowed to stand in the refrigerator overnight; the precipitate was collected by filtration, washed with chilled isopropanol (2 × 150 mL), and recrystallized from ethanol to give light yellow powder (19.1 g, 91% yield). Mp: 239–240 °C; ^{1}H-NMR δ: 1.43 (t, 3H), 2.41 (tt, 2H), 3.85 (t, 2H), 4.02 (s, 3H), 3.37 (t, 2H), 4.16–4.46 (m, 2H), 7.24 (s, 1H), 7.29 (s, 1H), 7.46 (d, 1H), 8.58 (b, 1H), 8.72 (d, 1H), 10.18 (b, 1H); *m/z*: 405.1 ([M+H]$^+$, 100%).

Ethyl 3-(7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-1)

Ethyl 3-(6-(3-chloropropoxy)-7-methoxyquinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (**8**, 0.81 g, 2 mmol) and potassium iodide (0.08 g) were added to the solution of morpholine (5 mL) in DMF (20 mL). The solution was stirred at 70 °C for 30 min. The excess morpholine was then removed under reduced pressure and the residue dissolved in chloroform (60 mL), washed with water (2 × 20 mL), and then dried using Na_2SO_4. The solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel, eluting with ethyl acetate/triethylamine (20:1) to afford white powder (**GI-1**, 0.78 g, 86% yield). Mp: 181**–**183 °C; ^{1}H-NMR δ: 1.39 (t, 3H), 2.04–2.18 (m, 2H), 2.51 (t, 4H), 2.63 (t, 2H), 3.74 (t, 4H), 4.00 (s, 3H), 4.29 (t, 2H), 4.40 (q, 2H), 6. 90 (t, 1H), 7.20 (s, 1H), 7.24 (s, 1H), 7.45 (t, 1H), 8.71 (s, 1H), 9.10 (b, 1H), 9.95 (b, 1H); ^{13}C-NMR δ: 14.68, 26.19, 53.76, 55.44, 56.10, 60.10, 66.98, 67.76, 101.38, 103.73, 107.92, 108.41, 109.35, 122.36, 147.17, 149.01, 153.86, 155.18, 155.30, 161.83; IR (/cm): 3417, 3311, 3147, 2968, 2847, 2193, 1657, 1621, 1559, 1509, 1464, 1393, 1318, 1238, 1208, 1122, 1072, 1040, 934, 855, 780; *m/z*: 456.2 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 60.65; H, 6.42; N, 15.37. Found C, 59.46; H, 6.60; N, 13.69.

Ethyl 3-(7-methoxy-6-(3-(4-methylpiperazin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-2)

As in the procedure described for **GI-1**, compound **GI-2** was prepared as white powder (**GI-2**, 0.65 g, 71% yield). Mp: 175**–**178 °C; ^{1}H-NMR δ: 1.43 (t, 3H), 1.58 (m, 2H), 2.15 (t, 2H), 2.34 (s, 3H), 2.62–2.69 (m, 8H), 4.01 (s, 3H), 4.27 (t, 2H), 4.41 (q, 2H), 6.69 (t, 1H), 7.10 (s, 1H), 7.24 (s, 1H), 7.47 (d, 1Hr), 8.58 (b, 1H), 8.71 (d, 1H), 9.54 (b, 1H); ^{13}C-NMR δ: 14.73, 26.53, 46.01, 53.21, 55.02, 55.20, 56.11, 60.11, 67.89, 101.33, 103.73, 107.95, 108.42, 109.36, 122.35, 147.20, 149.03, 153.87, 155.18, 155.29, 161.78; IR (/cm): 2952, 2936, 2873, 2776, 1659, 1624, 1604, 1585, 1559, 1513, 1471, 1437, 1413, 1398, 1316, 1240, 1212, 1146, 1067, 1044, 987, 926, 844, 777, 617; *m/z*: 469.2 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 61.52; H, 6.88; N, 17.94. Found C, 61.83; H, 6.87; N, 17.32.

Ethyl 3-(7-methoxy-6-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-3)

As in the procedure described for **GI-1**, compound **GI-3** was prepared as yellow powder (**GI-3**, 0.48 g, 64% yield). Mp: 192**–**194 °C; ^{1}H-NMR δ: 1.36 (t, 3H), 1.79 (b, 4H), 2.16 (p, 2H), 2.57 (b, 4H), 2.72 (t, 2H), 4.00 (s, 3H), 4.28 (t, 2H), 4.406 (q, 2H), 6.86 (t, 1H), 7.17 (s, 1H), 7.22 (s, 1H), 7.41 (t, 1H), 8.68 (s, 1H), 9.10 (b, 1H), 9.95 (b, 1H); ^{13}C-NMR δ: 14.69, 23.53, 28.56, 52.97, 54.21, 56.07, 60.05, 67.93, 101.20, 103.68, 107.88, 108.39, 109.34, 122.29, 147.13, 149.02, 153.79, 155.13, 155.24, 161.70; IR (/cm): 3410, 3302, 3043, 2959, 2863, 1665, 1622, 1598, 1557, 1508, 1433, 1309, 1208, 1143, 1072, 1044, 896, 835, 774; *m/z*: 440.2 ([M+H]$^+$, 100%); Elem. Anal. Calcd: C, 62.85; H, 6.65; N, 15.93. Found C, 62.70; H, 6.62; N, 15.07.

Ethyl 3-(7-methoxy-6-(3-(piperidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-4)

As in the procedure described for **GI-1**, compound **GI-4** was prepared as white powder (**GI-4**, 0.58 g, 74% yield). Mp: 188**–**190 °C; ^{1}H-NMR δ: 1.41 (t, 5H), 1.61 (m, 5H), 2.16 (b, 2H), 2.46 (b, 4H), 2.60 (b, 2H), 4.00 (s, 3H), 4.28 (t, 2H), 4.43 (q, 2H), 7.24 (s, 1H), 7.29 (s, 1H), 7.46 (d, 1H), 8.61 (b, 1H), 8.70 (d, 1H), 9.45–10.20 (b, 1H); ^{13}C-NMR δ: 14.76, 24.45, 25.95, 26.45, 54.61, 55.85, 56.15, 60.19, 68.05, 101.12, 103.73, 107.92, 109.34, 109.81, 122.27, 147.18, 149.00, 153.87, 155.10, 155.27, 161.75; IR (/cm): 3324, 2925, 1655, 1623, 1594, 1557, 1513, 1471, 1433, 1395, 1310, 1242, 1211, 1143, 1070, 1046, 980, 926, 777, 628; *m/z*: 452.3 ([M-H]$^+$, 100%); Elem. Anal. Calcd: C, 63.56; H, 6.89; N, 15.44. Found C, 63.63; H, 6.85; N, 14.69.

Ethyl 3-(6-(3-(diethylamino)propoxy)-7-methoxyquinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-5)

As in the procedure described for **GI-1**, compound **GI-5** was prepared as white powder (**GI-5**, 0.62 g, 76% yield). Mp: 195**–**196 °C; ^{1}H-NMR δ: 1.05 (t, 6H), 1.40 (t, 3H), 2.09 (p, 2H), 2.57 (q, 4H), 2.71 (t, 2H), 4.00 (s, 3H), 4.28 (t, 2H), 4.43 (q, 2H), 6.90 (t, 1H), 7.21 (s,

1H), 7.24 (s, 1H), 7.46 (t, 1H), 8.72 (s, 1H), 8.86 (b, 1H), 9.82 (b, 1H); ^{13}C-NMR δ: 11.83, 14.69, 26.62, 47.05, 49.18, 56.10, 60.10, 67.85, 100.93, 103.62, 107.79, 108.34, 109.32, 122.29, 147.07, 149.03, 153.79, 155.04, 155.23, 161.73; IR (/cm): 2959, 2810, 1736, 1665, 1624, 1602, 1559, 1515, 1473, 1413, 1398, 1383, 1317, 1244, 1211, 1147, 1066, 928, 843, 779, 614; *m/z*: 442.2([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 62.57; H, 7.08; N, 15.86. Found C, 62.44; H, 7.22; N, 14.85.

Ethyl 3-(7-methoxy-6-(3-(4-methylpiperidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-6)

As in the procedure described for **GI-1**, compound **GI-6** was prepared as white powder (**GI-6**, 0.52 g, 68% yield). Mp: 186**–**189 °C; ^{1}H-NMR δ: 0.91 (d, 3H), 1.25–1.41 (m, 6H), 1.62 (d, 2H), 1.98 (b, 2H), 2.08 (t, 2H), 2.60 (b, 2H), 2.94 (d, 2H), 4.00 (s, 3H), 4.27 (t, 2H), 4.02 (q, 2H), 6.90 (t, 1H), 7.19 (s, 1H), 7.23 (s, 1H), 7.45 (s, 1H), 8.71 (s, 1H), 8.86 (b, 1H), 9.88 (b, 1H); ^{13}C-NMR δ: 14.72, 21.83, 26.64, 30.83, 34.38, 54.05, 55.47, 56.11, 60.13, 68.06, 101.16, 103.71, 107.89, 108.53, 109.34, 122.29, 147.15, 149.02, 153.83, 155.12, 155.26, 161.71; IR (/cm): 2946, 2911, 1659, 1624, 1603, 1559, 1509, 1471, 1437, 1413, 1385, 1316, 1240, 1212, 1143, 1066, 1040, 923, 844, 777, 649; *m/z*: 467.1 ([M+H]$^+$, 100%); Elem. Anal. Calcd: C, 64.22; H, 7.11; N, 14.98. Found C, 63.96; H, 7.11; N, 14.44.

Ethyl 3-(7-methoxy-6-(3-(2-methylpiperidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-7)

As in the procedure described for **GI-1**, compound **GI-7** was prepared as off-white powder (**GI-7**, 0.47 g, 57% yield). Mp: 131**–**133 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.11 (d, 3H), 1.30–1.38 (m, 2H), 1.60–1.68 (m, 4H), 2.11 (p, 2H), 2.23 (t, 2H), 2.50 (b, 1H), 2.56–2.62 (m, 2H), 3.00 (p, 1H), 4.01 (s, 3H), 4.31 (t, 2H), 6.58 (t, 1H), 7.25 (s, 1H), 7.47 (d, 1H), 8.10 (s, 1H), 9.53 (s, 1H), 10.79 (b, 1H); ^{13}C-NMR ($CDCl_3$) δ: 18.86, 23.93, 25.47, 25.91, 26.12, 34.58, 50.37, 52.20, 54.69, 56.04, 56.25, 67.80, 101.39, 103.31, 107.69, 108.86, 109.51, 122.52, 147.74, 149.78, 153.87, 154.11, 155.28, 161.69; IR (/cm): 3193, 2932, 1696, 1623, 1609, 1552, 1470, 1441, 1424, 1394, 1299, 1215, 1136, 1115, 1038, 866, 784, 741; *m/z*: 467.1 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 64.22; H, 7.11; N, 14.98. Found C, 65.15; H, 6.43; N, 15.18.

Ethyl 3-(7-methoxy-6-(3-(4-ethylpiperazin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-8)

As in the procedure described for **GI-1**, compound **GI-8** was prepared as white powder (**GI-8**, 0.61 g, 65% yield). Mp: 175**–**177 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.09 (t, 3H), 1.41 (t, 2H), 2.14 (p, 2H), 2.38–2.71 (m, 10H), 4.00 (s, 3H), 4.28 (t, 2H), 4.26 (q, 2H), 6.91 (t, 1H), 7.20 (s, 1H), 7.24 (s, 1H), 7.46 (t, 1H), 8.71 (s, 1H), 8.88 (b, 1H); ^{13}C-NMR ($CDCl_3$) δ: 11.94, 14.73, 26.52, 52.30, 52.87, 53.28, 55.09, 56.27, 60.13, 67.79, 101.28, 103.76, 107.96, 108.41, 109.35, 122.31, 147.21, 149.03, 153.85, 155.17, 155.28, 161.74; IR (/cm): 3441, 2964, 2875, 1812, 1701, 1643, 1624, 1596, 1558, 1512, 1437, 1415, 1382, 1317, 1278, 1213, 1162, 1145, 1068, 1045, 960, 778, 639; *m/z*: 483.3 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 62.22; H, 7.10; N, 17.41;. Found C, 62.12; H, 6.92; N, 62.12.

Ethyl 3-(7-methoxy-6-(3-(4-phenylpiperazin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GI-9)

As in the procedure described for **GI-1**, compound **GI-9** was prepared as white powder (**GI-9**, 0.61 g, 59% yield). Mp: 242**–**243 °C; ^{1}H-NMR δ: 1.37 (t, 3H), 2.16 (p, 2H), 2.68–2.72 (m, 6H), 3.23 (t, 4H), 4.00 (s, 3H), 4.30–4.43 (m, 4H), 6.82–6.93 (m, 4H), 7.25–7.28 (m, 4H), 7.46 (t, 1H), 8.72 (s, 1H), 8.85 (b, 1H); ^{13}C-NMR δ: 14.68, 26.49, 49.16, 53.30, 55.06, 56.15, 60.16, 67.82, 101.31, 103.77, 107.98, 108.43, 109.38, 116.00, 119.63, 122.31, 129.10, 147.22, 149.02, 151.38, 153.89, 155.17, 155.31, 161.79; IR (/cm): 3423, 3334, 2947, 2879, 2822, 2733, 1663, 1624, 1601, 1600, 1472, 1436, 1413, 1397, 1317, 1242, 1211, 1148, 1066, 1044, 930, 845, 780, 690; *m/z*: 531.3 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 65.64; H, 6.46; N, 15.84. Found C, 64.86; H, 6.34; N, 15.53.

Ethyl 3-(7-(3-chloropropoxy)-6-methoxyquinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (9)

A mixture of ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (**2**, 14.0 g, 74 mmol) and 4-chloro-7-(3-chloropropoxy)-6-methoxyquinazoline (16.2 g, 56 mmol) in isopropanol (300 mL) was heated to reflux for 4 h and then allowed to stand in the refrigerator overnight; the precipitate was collected by filtration, washed with chilled isopropanol (2 × 150 mL), and recrystallized from ethanol to give off-white powder (18.9 g, 84% yield). Mp: 244–246 °C; ^{1}H-NMR δ: 1.42 (t, 3H), 2.38 (q, 2H), 3.80 (t, 2H), 4.07 (s, 3H), 4.33–4.47 (m, 4H), 6.90 (s, 1H), 7.20 (s, 1H), 7.45 (d, 1H), 8.54 (b, 1H), 8.72 (d, 1H); *m/z*: 405.1 ([M+H]$^+$, 100%).

Ethyl 3-(7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-1)

Ethyl 3-(7-(3-chloropropoxy)-6-methoxyquinazolin-4-ylamino)-1H-pyrrole-2-carboxyl-ate (**9**, 0.79 g, 2 mmol) and potassium iodide (0.08 g) were added to the solution of morpholine (5 mL) in DMF (20 mL). The solution was stirred at 70 °C for 30 min. The excess morpholine was then removed under reduced pressure and the residue dissolved in chloroform (60 mL), washed with water (2 × 20 mL), and then dried using Na_2SO_4. The solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel, eluting with ethyl acetate/triethylamine (20:1) to afford white powder (**GII-1**, 0.68 g, 75% yield). Mp: 202**–**203 °C; ^{1}H-NMR δ: 1.37 (t, 3H), 2.03–2.14 (m, 2H), 2.47 (t, 4H), 2.56 (t, 2H), 3.71 (t, 4H), 4.05 (s, 3H), 4.23 (t, 2H), 4.40 (q, 2H), 6. 88 (t, 1H), 7.15 (s, 1H), 7.23 (s, 1Hr), 7.44 (t, 1H), 8.70 (s, 1H), 9.11 (b, 1H), 9.90 (b, 1H); ^{13}C-NMR δ: 14.65, 26.02, 53.73, 55.28, 56.25, 60.10, 66.98, 67.43, 99.95, 103.66, 108.36, 108.66, 109.21, 122.29, 147.16, 149.83, 153.82, 154.13, 155.23, 161.80; IR (/cm): 3459, 3089, 2991, 1934, 1659, 1622, 1595, 1513, 1455, 1385, 1312, 1201, 1147, 1118, 1069, 859, 777, 621; *m/z*: 456.2 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 60.65; H, 6.42; N, 15.37. Found C, 60.22; H, 6.40; N, 14.69.

Ethyl 3-(6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-2)

As in the procedure described for **GII-1**, compound **GII-2** was prepared as white powder (**GII-2**, 0.56 g, 67% yield). Mp: 183–185 °C; ^{1}H-NMR δ: 1.40 (t, 3H), 2.11 (p, 2H), 2.30 (s, 3H), 2.48–2.61 (m, 1H), 4.06 (s, 3H), 4.23 (t, 2H), 4.40 (q, 2H), 6.89 (s, 1H), 7.15 (s, 1H), 7.21 (d, 1H), 7.44 (t, 1H), 8.70 (s, 1H), 9.21 (b, 1H), 9.94 (b, 1H); ^{13}C-NMR δ: 14.68, 26.36, 45.98, 53.19, 54.81, 55.18, 56.27, 60.10, 67.44, 99.98, 103.68, 108.37, 108.70, 109.22, 122.29, 147.21, 149.87, 153.82, 154.19, 155.25, 161.80; IR (/cm): 3422, 3337, 3113, 2939, 2797, 1656, 1620, 1592, 1513, 1454, 1386, 1314, 1204, 1151, 1074, 1042, 1013, 895, 826, 777; *m/z*: 469.3 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 61.52; H, 6.88; N, 17.94. Found C, 60.91; H, 6.84; N, 17.10.

Ethyl 3-(6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-3)

As in the procedure described for **GII-1**, compound **GII-3** was prepared as light yellow powder (**GII-3**, 0.47 g, 59% yield). Mp: 189–191 °C; ^{1}H-NMR δ: 1.39 (t, 3H), 1.82 (b, 4H), 2.16 (p, 2H), 2.59 (b, 4H), 2.74 (t, 2H), 4.06 (s, 3H), 4.21 (t, 2H), 4.44 (q, 2H), 6.85 (t, 1H), 7.11 (s, 1H), 7.14,(s, 1H), 7.37 (t, 1H), 8.67 (s, 1H), 9.44 (b, 1H), 9.78 (b, 1H); ^{13}C-NMR δ: 14.71, 23.54, 28.35, 52.76, 54.15, 56.27, 60.08, 67.39, 99.89, 103.66, 108.33, 108.67, 109.19, 122.28, 147.20, 149.83, 153.80, 154.11, 155.17, 161.80; IR (/cm): 3423, 3323, 3067, 2952, 2879, 1658, 1622, 1595, 1557, 1511, 1453, 1387, 1314, 1206, 1143, 1072, 1045, 895, 831, 779, 639; *m/z*: 440.3 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 62.85; H, 6.65; N, 15.93. Found C, 62.14; H, 6.64; N, 15.20.

Ethyl 3-(6-methoxy-7-(3-(piperidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-4)

As in the procedure described for **GII-1**, compound **GII-4** was prepared as white powder (**GII-4**, 0.61 g, 72% yield). Mp: 199–202 °C; ^{1}H-NMR δ: 1.38–1.48 (m, 5H), 1.60 (t, 4H), 2.10 (p, 2H), 2.44 (b, 4H), 2.55 (d, 2H), 4.06 (s, 3H), 4.22 (t, 2H), 4.41 (q, 2H), 6.62 (d, 1H), 6.88 (s, 1H), 7.19 (s, 1H), 7.42 (t, 1H), 8.69 (s, 1H), 9.19 (b, 1H), 9.80 (b, 1H); ^{13}C-NMR δ: 14.69, 24.47, 26.00, 26.39, 54.63, 55.62, 56.29, 60.11, 67.63, 99.96, 103.70, 108.34, 108.72, 109.20, 122.27, 147.24, 149.89, 153.81, 154.20, 155.23, 161.77; IR (/cm): 3061, 2932, 1657, 1622, 1595, 1558, 1512, 1454, 1411, 1382, 1312, 1245, 1211, 1143, 1070, 1045, 922, 898, 827, 777, 628; *m/z*: 454.3 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 63.56; H, 6.89; N, 15.44. Found C, 63.57; H, 6.86; N, 14.85.

Ethyl 3-(7-(3-(diethylamino)propoxy)-6-methoxyquinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-5)

As in the procedure described for **GII-1**, compound **GII-5** was prepared as white powder (**GII-5**, 0.63 g, 76% yield). Mp: 175–177 °C; ^{1}H-NMR δ: 1.04 (t, 6H), 1.41 (t, 3H), 2.10 (p, 2H), 2.55 (q, 4H), 2.67 (t, 2H), 4.06 (s, 3H), 4.23 (t, 2H), 4.42 (q, 2H), 6.91 (t, 1H), 7.17 (s, 1H), 7.25 (s, 1H), 7.46 (t, 1H), 8.70 (d, 1H), 8.81 (b, 1H), 9.86 (b, 1H); ^{13}C-NMR δ: 11.87, 14.69, 26.72, 47.03, 49.29, 56.32, 60.15, 67.77, 99.98, 103.78, 108.74, 109.20, 109.79, 122.58, 147.30, 149.94, 153.82, 154.35, 155.30, 161.87; IR (/cm): 3345, 319, 2968, 1697, 1659, 1623, 1597, 1556, 1495, 1453, 1409, 1382, 1313, 1287, 1242, 1199, 1138, 1070, 1044, 930, 863, 775, 632; *m/z*: 442.1 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 62.57; H, 7.08; N, 15.86. Found C, 62.20; H, 6.93; N, 15.46.

Ethyl 3-(6-methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-6)

As in the procedure described for **GII-1**, compound **GII-6** was prepared as white powder (**GII-6**, 0.51 g, 69% yield). Mp: 176–178 °C; ^{1}H-NMR δ: 0.92 (d, 3H), 1.26 (m, 2H), 1.40 (m, 3H), 1.63 (d, 2H), 1.98 (b, 2H), 2.13 (t, 2H), 2.58 (b, 2H), 2.95 (d, 2H), 4.04 (s, 3H), 4.23 (t, 2H), 4.21 (q, 2H), 6.88 (s, 1H), 7.12 (s, 1H), 7.43 (t, 1H), 8.69 (s, 1H), 9.14 (s, 1H), 9.87 (b, 1H); ^{13}C-NMR δ: 14.70, 21.80, 26.50, 30.84, 34.36, 54.03, 55.25, 56.29, 60.10, 67.58, 99.92, 103.66, 108.25, 108.32, 109.18, 122.30, 147.98, 150.87, 153.80, 154.15, 155.20, 161.79; IR (/cm): 3165, 3157, 2945, 2930, 2841, 2071, 1698, 1655, 1623, 1597, 1586, 1558, 1511, 1493, 1452, 1411, 1382, 1313, 1276, 1201, 1143, 1070, 1044, 770; *m/z*: 467.1 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 64.22; H, 7.11; N, 14.98. Found C, 64.06; H, 6.92; N, 15.50.

Ethyl 3-(6-methoxy-7-(3-(2-methylpiperidin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-7)

As in the procedure described for **GII-1**, compound **GII-7** was prepared as off-white powder (**GII-7**, 0.48 g, 58% yield). Mp: 227–230 °C; ^{1}H-NMR δ: 1.10 (d, 3H), 1.57–1.67 (m, 4H), 2.10 (m, 2H), 2.30 (t, 1H), 2.40 (b, 1H), 2.56 (t, 1H), 2.92–3.00 (m, 2H), 3.00 (p, 1H), 4.01 (s, 3H), 4.25 (t, 2H), 4.45 (q, 2H), 6.58 (t, 1H), 7.25 (s, 1H), 7.47 (d, 1H), 8.10 (s, 1H), 9.53 (s, 1H), 10.79 (b, 1H); ^{13}C-NMR δ: 18.61, 23.77, 25.44, 25.93, 26.12, 34.67, 50.28, 52.01, 54.58, 55.89, 56.39, 67.81, 101.20, 103.13, 107.66, 108.78, 109.44, 122.81, 147.64, 149.81, 153.56, 154.06, 155.09, 161.72; IR (/cm): 3125, 2902, 1699, 1622, 1608, 1574, 1489, 1445, 1382, 1300, 1213, 1132, 1116, 1042, 869, 786, 745; *m/z*: 467.1 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 64.22; H, 7.11; N, 14.98. Found C, 65.06; H, 6.73; N, 15.16.

Ethyl 3-(6-methoxy-7-(3-(4-ethylpiperazin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-8)

As in the procedure described for **GII-1**, compound **GII-8** was prepared as white powder (**GII-8**, 0.63 g, 67% yield). Mp: 236–238 °C; ^{1}H-NMR δ: 1.08 (t, 3H), 1.40 (t, 2H), 2.17 (p, 2H), 2.41 (q, 3H), 2.55–2.66 (m, 10), 4.04 (s, 3H), 4.23 (q, 2H), 4.42 (q, 2H), 6.64 (t, 1H), 7.11 (s, 1H), 7.23 (s, 1H), 7.50 (t, 1H), 8.69 (s, 1H), 9.24 (b, 1H); ^{13}C-NMR δ: 11.91, 14.70, 26.33, 52.27, 52.81, 53.24, 54.87, 56.37, 60.11, 67.43, 99.90, 103.66, 108.18, 108.65, 109.67, 122.34, 147.65, 149.84, 153.02, 154.15, 155.20, 161.98; IR (/cm): 3452, 3167, 2929, 1700, 1620, 1602, 1552, 1494, 1394, 1277, 1210, 1107,

948, 897, 780, 738; *m/z*: 483.3 ([M+H]$^+$, 75%). Elem. Anal. Calcd: C, 62.22; H, 7.10; N, 17.41. Found C, 61.90; H, 6.77; N, 17.94.

Ethyl 3-(6-methoxy-7-(3-(4-phenylpiperazin-1-yl)propoxy)quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate (GII-9)

As in the procedure described for **GII-1**, compound **GII-9** was prepared as white powder (**GII-9**, 0.61 g, 59% yield). Mp: 221–223 °C; ^{1}H-NMR δ: 1.41 (t, 3H), 2.17 (t, 2H), 2.63–2.69 (m, 6H), 3.23 (t, 4H), 4.07 (s, 3H), 4.27 (t, 2H), 4.42 (q, 2H), 6.85 (t, 1H), 6.90 (d, 2H), 6.95 (s, 1H), 7.17 (s, 1H), 7.23–7.29 (m, 4H), 7.46 (t, 1H), 8.71 (s, 1H), 8.85 (b, 1H); ^{13}C-NMR δ: 14.69, 26.33, 49.16, 53.28, 54.96, 56.31, 60.16, 67.45, 99.97, 103.77, 105.45, 109.25, 116.09, 119.64, 122.27, 129.08, 147.19, 149.88, 151.37, 153.05, 153.83, 154.18, 155.27, 161.80; IR (/cm): 3423, 3326, 3249, 3136, 2937, 2820, 1693, 1659, 1624, 1599, 1583, 1558, 1499, 1453, 1394, 1383, 1312, 1238, 1211, 1201, 1141, 1071, 1043, 1010, 991, 926, 863, 780, 650; *m/z*: 531.3 ([M+H]$^+$, 100%). Elem. Anal. Calcd: C, 65.64; H, 6.46; N, 15.84. Found C, 64.84; H, 6.13; N, 15.35.

Cell cytotoxicity assay

Human pancreatic cancer cell line Miapaca2 and prostate cancer cell line DU145 were purchased from American Type Culture Collection and cultured in high-glucose DMEM (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone) in a 5% CO_2 humidified incubator at 37 °C. The medium was also supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin.

Cells were seeded in 96-well culture plates (5000 cells/well) and treated with serially diluted testing compounds in triplicate. After 96-h incubation, cell growth medium was removed, and proliferation reagent WST-8 (Sigma) was added to each well and incubated at 37 °C for 1–3 h. Absorbance was measured using a plate reader at 450 nm with correction at 650 nm. The results were expressed as the percentage of absorbance of treated wells versus that of vehicle control. IC_{50}, the drug concentration causing 50% growth inhibition, was calculated via sigmoid curve fitting using GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA) as we described previously (7).

In vitro *kinase assays*

In vitro kinase inhibitory ability was determined using the HTScan EGFR Kinase Assay Kit (Cell Signaling Technology, Danvers, MA, USA), following the manufacturer's instructions.

Results and Discussion

Chemistry

Based on the structure–activity relationships (SAR) and quantitative structure–activity relationships of the 4-anilinoquinazolines reported previously (8–16), and using gefitinib as a leading compound, we designed two series of novel analogs: series A, replacement of the benzene ring with pyrrole ring, different basic side chains at position 6, and methoxy group at position 7 of the quinazoline nucleus; series B, replacement of the benzene ring with pyrrole ring, methoxy group at position 6, and different basic side chains at position 7 of the quinazoline nucleus. For all new compounds, the pyrrole ring had an ethyl formate substitutional group. The reason we chose a carboxylic group but not halides as a substituent was that carboxylic group was also an electron-withdrawing group but rarely reported in previous gefitinib analogs, with the hope to obtain more data for SAR. Structures of the new compounds are illustrated in Figure 2.

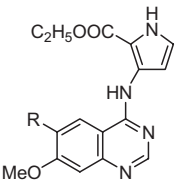
Detailed synthetic routes of 18 4-pyrrylamino quinazoline derivatives in series A (**GI-1-9**) and series B (**GII-1-9**) are depicted in Schemes 1 and 2, respectively. The intermediate 4-chloro-6-(3-chloropropoxy)-7-methoxyquinazoline (**7**) was obtained using the same method we reported previously (7). Briefly, methyl 3-hydroxy-4-methoxybenzoate as starting material was alkylated with 1-bromo-3-chloropropane to give **3** in 95% yield. Nitration of **3** with nitric acid in acetic acid afforded **4**, which was then reduced by powdered iron in acetic acid to give **5** in satisfactory yield (77%). In contrast, catalytic hydrogenation using Raney/Ni or 5% Pd/C gave incomplete conversions, even after a long reaction time. Cyclization of **5** with formamidine acetate and then chlorination with thionyl chloride gave **7**. Then, aminolysis of **7** was performed using excess ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (**2**) to afford **8** in 91% yield. The final step was nucleophilic displacement of the chlorine atom with different aliphatic amines to yield the corresponding target compound series A **GI-1-9**. The material ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (**2**) was synthesized ahead by the route described in Scheme 3: Ethyl formate was condensed with acetonitrile under catalysis of sodium hydride to give 2-formylacetonitrile, which was very active and unstable. Without separation, 2-formylacetonitrile was then condensed with diethyl 2-aminomalonate to afford **1**. Cyclization of **1** under catalysis of sodium ethylate and then salifying obtained **2** in 82% yield.

Starting from methyl 4-hydroxy-3-methoxybenzoate (Scheme 2), series B compounds **GII-1-9** could be obtained via the same synthetic methods as described earlier. We are the first to replace the benzene ring of gefitinib with pyrrole ring to design new compounds. All target compounds are completely new [except compound **GI-1** reported in our previous paper (17)] and obtained in satisfactory yields (Figure 2).

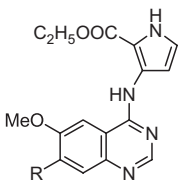
Cytotoxicity

The cytotoxicity of 18 compounds, as well as the parental compound gefitinib, in human pancreatic cancer cell line Miapaca2 and human prostate cancer cell line DU145, was investigated by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)-based cytotoxicity assay. As shown in Table 1, the 50% inhibitory concentration (IC_{50}) of all new compounds against the two cancer cell lines was much lower than that of gefitinib, indicating that the *in vitro* antitumor activity of these compounds was significantly improved. Interestingly, all compounds show greater inhibitory effect on Miapaca2 cells compared with DU145 cells, while gefitinib shows minor effect between the two cell lines (both IC_{50} values around 40 μM).

With further inspection, we found that the cytotoxicity of compound **GI-1** was the weakest among the 18 compounds but still more



series A



series B

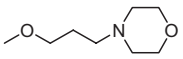
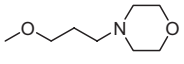
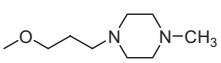
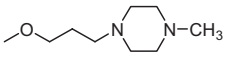
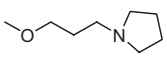
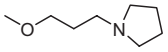
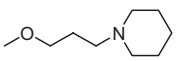
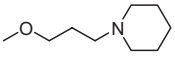
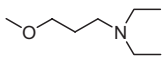
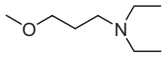
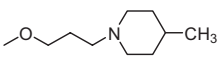
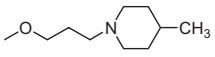
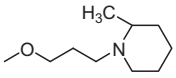
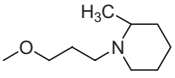
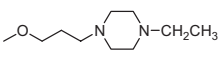
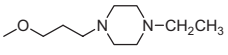
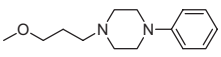
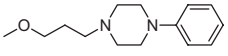
Cpd.	R	Yield (%)	Cpd.	R	Yield (%)
GI-1		86	GII-1		75
GI-2		71	GII-2		67
GI-3		64	GII-3		59
GI-4		74	GII-4		72
GI-5		76	GII-5		76
GI-6		68	GII-6		69
GI-7		57	GII-7		58
GI-8		65	GII-8		67
GI-9		59	GII-9		59

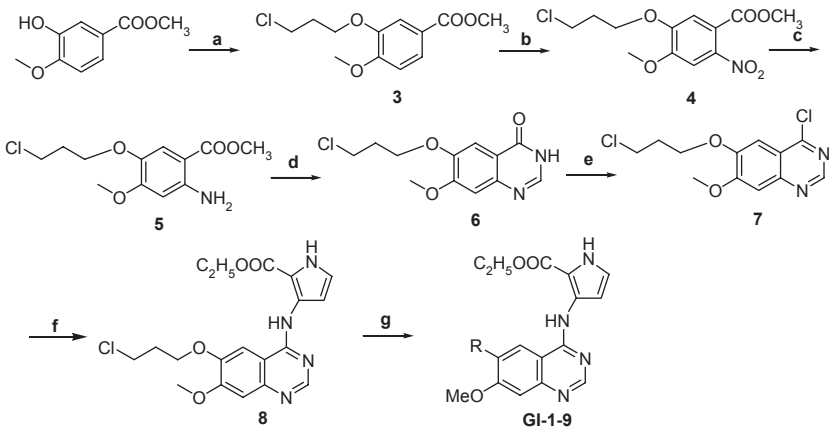
Figure 2: Structure and yield of new compounds.

Table 1: Cytotoxicity against cancer cell lines and EGFR inhibitory activity of gefitinib and new compounds

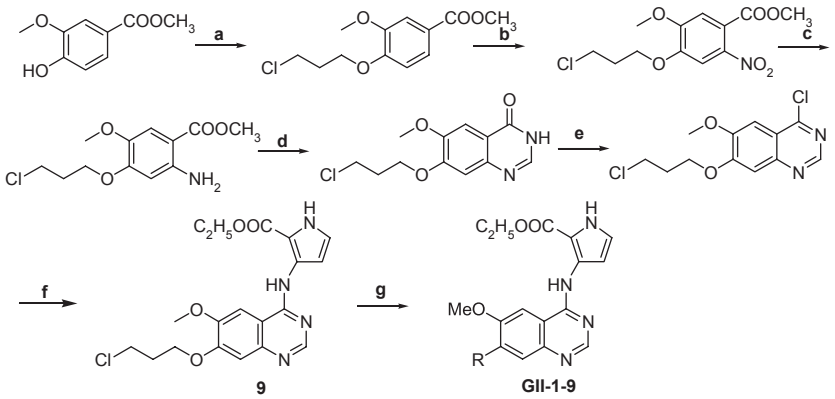
	Cytotoxicity (IC_{50}, μM)[a]				Cytotoxicity (IC_{50}, μM)[a]		
Compound	Miapaca2	DU145	EGFR inhibition (%)[b,c]	Compound	Miapaca2	DU145	EGFR inhibition (%)[b,c]
Gefitinib	38.81	42.17	99.18				
GI-1	15.36	39.28	18.32	**GII-1**	14.35	27.52	23.27
GI-2	9.897	16.09	31.46	**GII-2**	7.273	16.83	38.72
GI-3	7.392	27.13	26.58	**GII-3**	10.51	14.63	36.57
GI-4	5.855	21.41	33.91	**GII-4**	8.721	10.59	53.20
GI-5	7.914	17.02	40.50	**GII-5**	7.752	16.58	43.28
GI-6	7.241	12.89	51.23	**GII-6**	6.498	7.947	66.54
GI-7	7.670	18.34	32.67	**GII-7**	13.17	20.09	27.64
GI-8	9.206	16.87	37.81	**GII-8**	5.844	13.45	58.53
GI-9	8.690	19.24	29.83	**GII-9**	5.460	12.94	56.31

EGFR, epidermal growth factor receptor.
[a]Values are averages of three independent experiments, SD < 10%.
[b]Values are averages of two independent experiments, SD < 10%.
[c]Compounds tested at concentration of 10 μM.

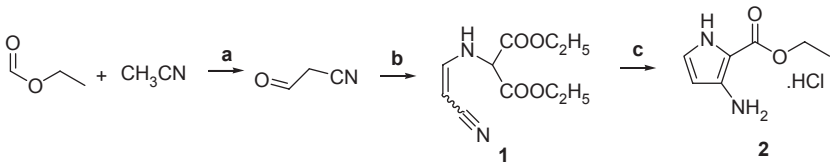
potent than gefitinib. The structure modification of gefitinib to design **GI-1** is that pyrrole ring was substituted for the benzene ring. This result reveals that replacement of benzene ring with pyrrole ring increases *in vitro* antitumor activity. The ethyl formate substitutional group may also have synergic effect. The structure difference among **GI-1** and other compounds in series A was the basic side chain at



Scheme 1: Reagents and conditions: (a) $ClCH_2CH_2CH_2Br$, K_2CO_3, 70 °C; (b) HNO_3, AcOH, Ac_2O, 0–5 °C; (c) Fe, AcOH, MeOH, N_2, 50 °C; (d) formamidine acetate, EtOH, reflux; (e) $SOCl_2$, DMF, reflux; (f) **2**, i-PrOH, reflux; (g) aliphatic amine, KI, DMF, 70 °C.



Scheme 2: Reagents and conditions: (a) $ClCH_2CH_2CH_2Br$, K_2CO_3, 70 °C; (b) HNO_3, AcOH, Ac_2O, 0–5 °C; (c) Fe, AcOH, MeOH, N_2, 50 °C; (d) formamidine acetate, EtOH, reflux; (e) $SOCl_2$, DMF, reflux; (f) **2**, i-PrOH, reflux; (g) aliphatic amine, KI, DMF, 70 °C.



Scheme 3: Reagents and conditions: (a) NaH, $C_6H_5CH_3$; (b) $NH_2CH(COOC_2H_5)_2$, AcONa; (c) (i) EtOH, EtONa, (ii) HCl, CH_3COCH_3.

position 6 of quinazoline nucleus, which was reported to affect the physical properties of compound *in vivo* (16). As cytotoxicity assay was carried out *in vitro*, the inhibitory effect variance among these compounds could be attributed to the hydrophobicity and permeability changes resulting from different basic side chains.

Kinase inhibitory activity

Because gefitinib is a selective EGFR inhibitor, we determined the inhibitory activity on EGFR of these gefitinib analogs. As also shown in Table 1, the inhibitory potency of the new compounds is much lower than that of gefitinib, which indicates that these compounds are no longer specific EGFR tyrosine kinase inhibitors. We are currently carrying out more experiments to access inhibitory activity on other RTKs, hoping to figure out the exact target(s) and details on the mechanism of action.

Taken the biological data together, we noticed that compounds with stronger cytotoxicity also show better EGFR inhibitory potency. This data may suggest that *in vitro* antitumor activity of these compounds results from their kinase inhibitory activity. We also found that shifting basic amine side chain at position 6 (series A) to position 7 (series B) results in a minor increased cytotoxicity and more obviously higher EGFR inhibitory potency in most cases.

Compounds **GI-6**, **GII-4**, **GII-6**, **GII-8**, and **GII-9** that possess relatively stronger cytotoxicity against the two cancer cell lines are more potent RTK inhibitors and will be chosen for further evaluation of *in vitro* and *in vivo* activities.

Conclusion

In our study, using gefitinib as a leading compound, two series of novel 4-pyrrylamino quinazoline derivatives were designed and synthesized. Cytotoxicity against two cancer cell lines Miapaca2 and DU145 and EGFR inhibitory activity of these compounds were examined. The preliminary evaluation result demonstrates that all new compounds possess improved *in vitro* antitumor activity compared with the parental gefitinib, especially the compounds **GI-6**, **GII-4**, **GII-6**, **GII-8**, and **GII-9**. Based on the investigation result, certain SAR were proposed: Replacement of benzene ring with pyrrole ring contributes to the improvement of antitumor activity; basic side chain at position 6 or 7 of quinazoline nucleus also affects the cytotoxicity of the compound. Our study provides useful information for developing new and more potent antitumor agents.

Acknowledgments

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References

1. Gschwind A., Fischer O.M., Ullrich A. (2004) The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer;4:361–370.
2. Zwick E., Bange J., Ullrich A. (2002) Receptor tyrosine kinases as targets for anticancer drugs. Trends Mol Med;8:17–23.
3. Brunelleschi S., Penengo L., Santoro M.M., Gaudino G. (2002) Receptor tyrosine kinases as target for anti-cancer therapy. Curr Pharm Des;8:1959–1972.
4. Cohen M.H., Williams G.A., Sridhara R., Chen G., Pazdur R. (2003) FDA drug approval summary: gefitinib (ZD1839) (Iressa) tablets. Oncologist;8:303–306.
5. Cohen M.H., Johnson J.R., Chen Y.F., Sridhara R., Pazdur R. (2005) FDA drug approval summary: erlotinib (Tarceva) tablets. Oncologist;10:461–466.
6. Ryan Q., Ibrahim A., Cohen M.H., Johnson J., Ko C.W., Sridhara R., Justice R., Pazdur R. (2008) FDA drug approval summary: lapatinib in combination with capecitabine for previously treated metastatic breast cancer that overexpresses HER-2. Oncologist;13:1114–1119.
7. Wu X., Li M., Qu Y., Tang W., Zheng Y., Lian J., Ji M., Xu L. (2010) Design and synthesis of novel Gefitinib analogues with improved anti-tumor activity. Bioorg Med Chem;18:3812–3822.
8. Rewcastle G.W., Denny W.A., Bridges A.J., Zhou H.R., Cody D.R., Mcmichael A., Fry D.W. (1995) Tyrosine kinase inhibitors .5. Synthesis and structure-activity-relationships for 4-[(Phenylmethyl)Amino]-quinazolines and 4-(Phenylamino)quinazolines as potent adenosine 5′-triphosphate binding-site inhibitors of the tyrosine kinase domain of the epidermal growth-factor receptor. J Med Chem;38:3482–3487.
9. Bridges A.J., Zhou H., Cody D.R., Rewcastle G.W., McMichael A., Showalter H.D.H., Fry D.W., Kraker A.J., Denny W.A. (1996) Tyrosine kinase inhibitors .8. An unusually steep structure-activity relationship for analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor.. J Med Chem;39:267–276.
10. Smaill J.B., Palmer B.D., Rewcastle G.W., Denny W.A., McNamara D.J., Dobrusin E.M., Bridges A.J., *et al.* (1999) Tyrosine kinase inhibitors. 15. 4-(phenylamino)quinazoline and 4-(phenylamino)pyrido[d] pyrimidine acrylamides as irreversible inhibitors of the ATP binding site of the epidermal growth factor receptor. J Med Chem;42:1803–1815.
11. Smaill J.B., Rewcastle G.W., Loo J.A., Greis K.D., Chan O.H., Reyner E.L., Lipka E., Showalter H.D., Vincent P.W., Elliott W.L., Denny W.A. (2000) Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-d]-pyrimidine-6-acrylamides bearing additional solubilizing functions (vol 43, pg 1380, 2000). J Med Chem;43:3180–1397.
12. Wissner A., Berger D.M., Boschelli D.H., Floyd M.B. Jr, Greenberger L.M., Gruber B.C., Johnson B.D., *et al.* (2000) 4-Anilino-6,7-dialkoxyquinoline-3-carbonitrile inhibitors of epidermal growth factor receptor kinase and their bioisosteric relationship to the 4-anilino-6,7-dialkoxyquinazoline inhibitors. J Med Chem;43:3244–3256.
13. Assefa H., Kamath S., Buolamwini J.K. (2003) 3D-QSAR and docking studies on 4-anilinoquinazoline and 4-anilinoquinoline epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. J Comput Aided Mol Des;17:475–493.
14. Zhang Y.M., Cockerill S., Guntrip S.B., Rusnak D., Smith K., Vanderwall D., Wood E., Lackey K. (2004) Synthesis and SAR of potent EGFR/erbB2 dual inhibitors. Bioorg Med Chem Lett;14:111–114.
15. Petrov K.G., Zhang Y.M., Carter M., Cockerill G.S., Dickerson S., Gauthier C.A., Guo Y., Mook R.A. Jr, Rusnak D.W., Walker A.L., Wood E.R., Lackey K.E. (2006) Optimization and SAR for dual ErbB-1/ErbB-2 tyrosine kinase inhibition in the 6-furanylquinazoline series. Bioorg Med Chem Lett;16:4686–4691.
16. Ballard P., Bradbury R.H., Harris C.S., Hennequin L.F., Hickinson M., Kettle J.G., Kendrew J., Klinowska T., Ogilvie D.J., Pearson S.E., Williams E.J., Wilson I. (2006) Inhibitors of epidermal growth factor receptor tyrosine kinase: optimisation of potency and in vivo pharmacokinetics. Bioorg Med Chem Lett;16:4908–4912.
17. Zheng Y.G., Wu X.Q., Sun M., Feng L.H., Li M.D., Ji M. (2009) Design, synthesis, crystal structure and antitumor activity of a new ethyl 3-(quinazolin-4-ylamino)-1H-pyrrole-2-carboxylate derivative. Lett Drug Des Discov;6:538–541.