Journal of Enzyme Inhibition and Medicinal Chemistry, 2010; 25(2): 158–171

informa
healthcare

RESEARCH ARTICLE

Quinazoline-urea, new protein kinase inhibitors in treatment of prostate cancer

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Abstract
Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor-2 (VEGFR-2), two protein tyrosine kinases, are involved in pathological disorders and the progression of different types of carcinomas. Concomitant inhibition of both tyrosine kinase activities appears to be an attractive target for cancer chemotherapy. A series of new quinazoline derivatives substituted by amide, urea, or carbamic acid ester groups have been synthesized. The biological activities of these new compounds have been evaluated for their enzyme inhibition and antiproliferative activities.

Keywords: *Quinazoline; EGFR; VEGFR; inhibitors; cancer; prostate*

Introduction

The prostate gland is the most common site for cancer in males within the developed world. Increasingly, amongst the elderly male population, prostate disease is having a significant effect on morbidity, mortality, and health care resources[1]. Although endocrine and cytotoxic treatments remain therapies of choice to induce tumor growth arrest, resistance and recurrence often appear with toxicity side effects[2]. Protein kinases play important roles in regulating most of the cellular functions (proliferation, cell cycle, cell metabolism, survival, apoptosis, DNA damage/repair, etc.), and their overexpression is involved in many cancer cells[3,4]. Most signal transduction pathways are mediated by protein kinases, and aberrant kinase signaling leads to proliferation of cancer cells and also angiogenesis and growth of solid tumors such as prostatic, colon, breast, and gastric cancers[5,6].

Among protein kinases, the epidermal growth factor (EGF)/ErbB family of receptor tyrosine kinases, notably EGFR, is activated following binding with peptide growth factor, inducing the formation of receptor homo- or heterodimers and activation of the intrinsic tyrosine kinase domain. Signal transduction pathways are initiated, such as Ras/MAPK, PI3K/Akt, or JAK/STAT, leading to tumor initiation in the case of overactivation[7–9].

The vascular endothelial growth factor (VEGF) is a potent angiogenic factor produced by tumor cells. This peptide stimulates the formation of new blood vessels, a fundamental event in normal and pathologic angiogenesis. Activation of the VEGF receptor tyrosine kinase (TK) family, notably VEGFR-2, by this growth factor results in endothelial cell survival, mitogenesis, migration, and differentiation[10–12].

Tumor angiogenesis is critically important for cancerous growth, supplying nutrients and oxygen to tumors[13]. It is well-established that tumor cells express EGFR and VEGFR-2, in particular in tumor endothelial cells[14]. These two glycoproteins have been identified in many cancers, and a relationship is established between them: a targeting EGFR strategy inhibits tumor growth, decreasing the product of VEGF, and inhibition of VEGFR-2 increases the antitumoral effect of inhibitors of EGFR[15,16]. According to the knowledge of tumor biology and mechanisms of oncogenesis activation, simultaneous blockade of the intracellular domain of EGFR and VEGFR-2 by small competitive molecules at the adenosine phosphate (ATP) site offers new opportunities for the development of anticancer drugs with a synergic effect[17]. A number of compounds representing a generation of ATP-mimics (Figure 1), such as the 4-anilinoquinazolines gefitinib (ZD1839/Iressa)

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(Received 01 October 2008; accepted 05 February 2009)

ISSN 1475-6366 print/ISSN 1475-6374 online © 2010 Informa UK Ltd
DOI: 10.3109/14756360903169485

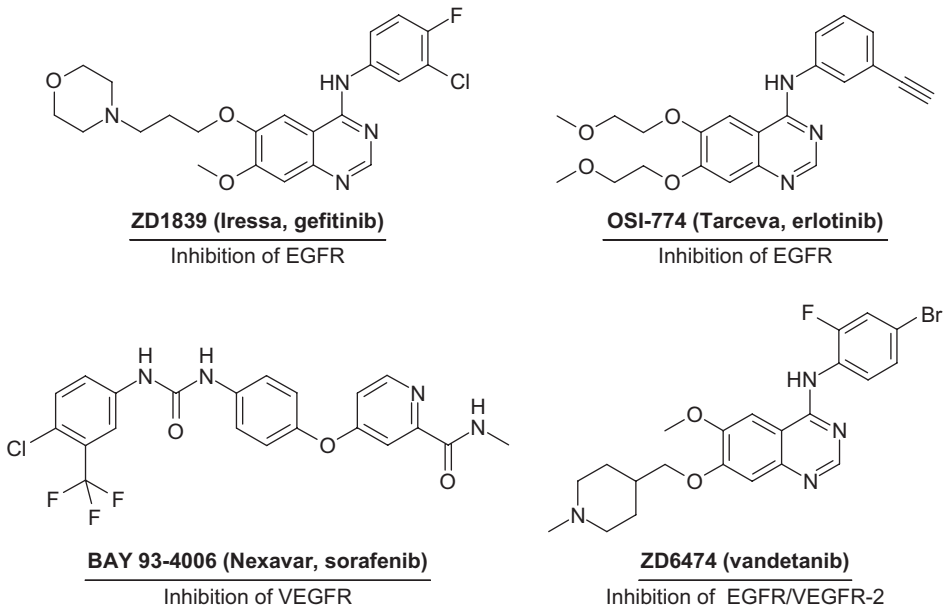


Figure 1. Structures of ATP-mimic inhibitors for treatment of solid tumors.

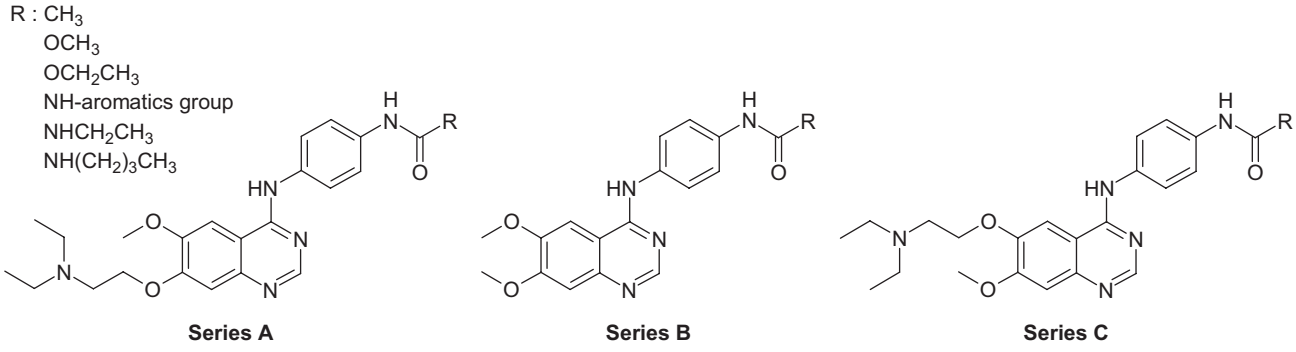


Figure 2. Structures of synthesized compounds.

and erlotinib (OSI-774/Tarceva)[18,19], have been approved by the Food and Drug Administration (FDA).

Some 4-anilinoquinazolines inhibit EGFR and VEGFR-2 TK activities and are described in the literature[20,21]. Vandetanib (ZD6474), an orally bioavailable drug in phase III, is considered to be a dual tyrosine kinase inhibitor targeting EGFR and VEGFR-2[22]. This quinazoline substituted with a halogen at the 2- and 4-positions on the phenyl group showed potent inhibitory activities (IC_{50} = 500 nM for EGFR and 40 nM for VEGFR-2) in enzyme-linked immunosorbent assays (ELISAs) with recombinant enzymes[22,23]. The heterocycle employed appears to be a potent linker to interact with the ATP site of these two proteins. The effects of substituents on the arylamino group and ether linker at the 6- or 7-position of quinazoline influence the inhibitory activities, and it could be interesting to investigate this aryl group. Many compounds which target the VEGFR-2 kinase domain are designed with amide or urea groups[24–26]. The most potent VEGFR inhibitor is represented by the urea derivative sorafenib (BAY 93-4006/Nexavar). Based on the structure–activity relationships established for the described protein tyrosine kinase inhibitors[20–26] and previous work carried out in our laboratory[27–29], we have undertaken the synthesis of a series of amide, carbamic acid ester, and urea derivatives, coupled with a 4-anilinoquinazoline skeleton, leading to new dual EGFR and VEGFR inhibitors (Figure 2).

Three series of compounds, differentiating the ether linker (methoxy or diethylaminoethoxy) at the C-6 and C-7 positions of the quinazoline core, were synthesized. Introduction of the diethylamino side chain may improve physicochemical and pharmacological properties such as water solubility, bioavailability, and cell penetration. According to the position, the inhibitory activity of the 4-anilinoquinazoline might change. Then, the final new compounds reported in this article were evaluated for anticancer activity on hormone-independent PC3 prostate cancer cells and the inhibition of various kinase activities, notably EGFR and VEGFR-2;

the results were compared with the activity of the reference compounds, semaxanib (SU5416) and gefitinib. To evaluate the selectivity of these compounds, they were also studied on Aurora-A, a serine–threonine kinase whose inhibition activity disrupts the cell cycle and blocks proliferation. Vertex[30], a pyrimidine derivative, was used as reference for this test.

Materials and methods

Chemistry

Experimental protocols

Melting points were determined in open capillary tubes on a Büchi reference B-530 digital melting point apparatus and are uncorrected. Kieselgel 60 F-254 commercial plates were used for analytical thin layer chromatography (TLC) with ultraviolet (UV) light and/or iodine to follow the course of the reaction. Silica gel Kieselgel Si 60, 0.063–0.200 mm (Merck), was used for column chromatography. The structures of all compounds were supported by infrared (IR) spectrometry (using a Bruker Vector 22 instrument) ^{1}H-nuclear magnetic resonance (NMR) (300 MHz) spectra were recorded on a Bruker AC300P NMR spectrometer in dimethylsulfoxide (DMSO)-[D_6] or in $CDCl_3$ at room temperature. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS). *J* values are in Hertz and the splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. APCI+ (atmospheric pressure chemical ionization) mass spectra were obtained on a Thermo Electron Surveyor MSQ liquid chromatography-mass spectrometry (LC/MS) system. Gefitinib was synthesized according to described procedures[31]. Semaxanib (SU5416) is a commercial product of Sigma-Aldrich, reference S8442-5MG, lot number 085K4618. These two compounds were references for tests on cellular EGFR and VEGFR-2 activities. Vertex, a product of Huskerchem, reference MK-0457, was the commercial reference for tests on Aurora-A activity.

General procedure for compound 2–6 (series A)

*4-(2-Diethylaminoethoxy)-3-methoxybenzoic acid methyl ester hydrochloride **2*** Potassium carbonate (0.44 mol) was added to a solution of methyl vanillate **1** (0.11 mol) in acetone (300 mL) and was stirred for 5 min. 2-Diethylaminoethyl chloride hydrochloride (0.16 mol) was added and the mixture was refluxed for 16 h. The inorganic solid was filtered off and the filtrate was concentrated *in vacuo*. The oily residue was dissolved in 2-propanol (10 mL), and 2-propanol saturated with HCl was added (15 mL). The resulting white precipitate was filtered, washed with diethyl ether, and dried *in vacuo* to afford **2**. Mp = 90–92°C (toluene); yield = 76%. IR (ν, cm^{-1}): 2579 and 2467 (NH^+), 1695 (C=O). ^{1}H-NMR (DMSO), δ, ppm: 1.25 (m, 6H, $NCH_2\mathit{CH_3}$), 3.20 (m, 4H, $N\mathit{CH_2}CH_3$), 3.50 (t, 2H, $N\mathit{CH_2}CH_2O$, *J* = 4.60 Hz), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.50 (t, 2H, $NCH_2\mathit{CH_2}O$, *J* = 4.60 Hz), 7.15 (d, 1H, ArH, *J* = 8.70 Hz), 7.50 (d, 1H, ArH, *J* = 2.00 Hz), 7.60 (dd, 1H, ArH, *J* = 8.70 Hz and *J* = 2.00 Hz), 12.20 (s, 1H, NH^+). LC/MS (APCI+), *m/z*: 282.3 (M – HCl + H)$^+$.

*4-(2-Diethylaminoethoxy)-3-methoxy-6-nitrobenzoic acid methyl ester hydrochloride **3*** A solution of tin(IV) chloride (28 mmol) and nitric fuming acid (28 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of **2** (9.40 mmol) in CH_2Cl_2 (150 mL) cooled at–70°C. After stirring for 8 h at–70°C and warming to room temperature, the residue was filtered off and dissolved in saturated potassium carbonate solution (100 mL). The aqueous solution was then extracted with ethyl acetate (3 × 70 mL), dried over magnesium sulfate, and concentrated *in vacuo*. The oily residue was dissolved in 2-propanol (3 mL), and 2-propanol saturated with HCl was added (7 mL). The resulting white precipitate was filtered, washed with diethyl ether, and dried *in vacuo* to afford **3**. Mp = 128–131°C (cyclohexane); yield = 88%. IR (ν, cm^{-1}): 2562 (NH^+), 1717 (C=O), 1510 (NO_2). ^{1}H-NMR (DMSO), δ, ppm: 1.10 (m, 6H, $NCH_2\mathit{CH_3}$), 2.60 (m, 4H, $N\mathit{CH_2}CH_3$), 3.00 (t, 2H, $N\mathit{CH_2}CH_2O$, *J* = 4.60 Hz), 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.20 (t, 2H, $NCH_2\mathit{CH_2}O$, *J* = 4.60 Hz), 7.05 (s, 1H, ArH), 7.50 (s, 1H, ArH), 11.90 (s, 1H, NH^+). LC/MS (APCI+), *m/z*: 327.3 (M – HCl + H)$^+$.

*2-Amino-4-(2-diethylaminoethoxy)-3-methoxybenzoic acid methyl ester hydrochloride **4*** The compound **3** (6 mmol) was dissolved in methanol (50 mL), and Raney nickel (0.4 g) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for 16 h. The product was passed through a plug of Celite before being concentrated and purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9/1). Petroleum ether provided the title compound as a brown solid. Mp = 176–178°C; yield = 66%. IR (ν, cm^{-1}): 3480 and 3385 (NH_2), 1707 (C=O). ^{1}H-NMR (DMSO), δ, ppm: 1.25 (m, 6H, $NCH_2\mathit{CH_3}$), 3.20 (m, 4H, $N\mathit{CH_2}CH_3$), 3.00 (t, 2H, $N\mathit{CH_2}CH_2O$, *J* = 4.60 Hz), 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.30 (t, 2H, $NCH_2\mathit{CH_2}O$, *J* = 4.60 Hz), 6.45 (s, 1H, ArH), 6.50 (s, 2H, NH_2), 7.20 (s, 1H, ArH). LC/MS (APCI+), *m/z*: 297.4 (M – HCl + H)$^+$.

*7-(2-Diethylaminoethoxy)-6-methoxyquinazolin-4-one **5*** A mixture of **4** (3 mmol) and ammonium formate (9 mmol) in formamide (1 mL) was heated at 140°C for 16 h. The reaction was hydrolyzed with potassium carbonate solution (1 N, 40 mL) and extracted with ethyl acetate (5 × 30 mL). The combined organic layers were dried over magnesium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9/1) to give a brown solid. Mp = 137–139°C; yield = 68%. IR (ν, cm^{-1}): 1697 (C=O), 1611 (NH). ^{1}H-NMR (DMSO), δ, ppm: 1.05 (m, 6H, $NCH_2\mathit{CH_3}$), 2.70 (m, 4H, $N\mathit{CH_2}CH_3$), 3.00 (t, 2H, $N\mathit{CH_2}CH_2O$, *J* = 4.50 Hz), 3.95 (s, 3H, OCH_3), 4.20 (t, 2H, $NCH_2\mathit{CH_2}O$, *J* = 4.50 Hz), 7.10 (s, 1H, ArH), 7.50 (s, 1H, ArH), 8.00 (s, 1H, ArH), 12.20 (s, 1H, OH). LC/MS (APCI+), *m/z*: 292.3 (M + H)$^+$.

*4-Chloro-7-(2-diethylaminoethoxy)-6-methoxyquinazoline **6*** A mixture of **5** (3 mmol) and phosphorous oxychloride (20 mL) was refluxed for 2 h. After evaporation under vacuum, ice water (50 mL) was added and the mixture was neutralized by ammonium hydroxide. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL) and the extract was washed with saturated aqueous sodium hydrogen

carbonate solution and dried over calcium chloride. The solvent was removed by evaporation and the residue was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9/1) to provide a white solid. Mp = 189–191°C (cyclohexane/toluene); yield = 95%. ^{1}H-NMR (DMSO), δ, ppm: 1.50 (m, 6H, NCH_2CH_3), 3.20 (m, 4H, NCH_2CH_3), 3.45 (t, 2H, NCH_2CH_2O, J = 4.50 Hz), 4.05 (s, 3H, OCH_3), 4.60 (t, 2H, NCH_2CH_2O, J = 4.50 Hz), 7.45 (m, 2H, ArH), 8.90 (s, 1H, ArH). LC/MS (APCI+), *m/z*: 310.8 $(M)^+$ and 312.8 $(M + 2)^+$.

General procedure for compound 14–18 (series C)

*3-(2-Diethylaminoethoxy)-4-methoxybenzoic acid methyl ester hydrochloride **14*** Compound **14** was obtained by the same procedure as used for **2**. Starting from **13** (0.08 mol), a white solid was synthesized. Mp = 103–105°C (toluene); yield = 77%. IR (ν, cm^{-1}): 2580 and 2465 (NH^+), 1697 (C=O). ^{1}H-NMR (DMSO), δ, ppm: 1.25 (m, 6H, NCH_2CH_3), 3.20 (m, 4H, NCH_2CH_3), 3.50 (t, 2H, NCH_2CH_2O, J = 4.60 Hz), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, J = 4.60 Hz), 7.10 (d, 1H, ArH, J = 8.70 Hz), 7.55 (d, 1H, ArH, J = 2.00 Hz), 7.60 (dd, 1H, ArH, J = 8.70 Hz and J = 2.00 Hz), 10.90 (s, 1H, NH^+). LC/MS (APCI+), *m/z*: 282.3 $(M - HCl + H)^+$.

*3-(2-Diethylaminoethoxy)-4-methoxy-6-nitrobenzoic acid methyl ester hydrochloride **15*** A solution of tin(IV) chloride (28 mmol) and nitric fuming acid (28 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of **14** (9.40 mmol) in CH_2Cl_2 (150 mL) cooled at–70°C. After stirring for 8 h at–70°C and warming to room temperature, water was added (100 mL). The aqueous layer was basified with 6 N NaOH and extracted with CH_2Cl_2 (3 × 70 mL). The combined organic layers were washed with saturated potassium carbonate solution and saturated sodium chloride solution and dried over calcium chloride. After concentration *in vacuo*, the oily residue was dissolved in 2-propanol (3 mL), and 2-propanol saturated with HCl was added (7 mL). The resulting white precipitate was filtered, washed with diethyl ether, and dried *in vacuo* to afford **15**. Mp = 134–136°C (cyclohexane); yield = 91%. IR (ν, cm^{-1}): 2560 (NH^+), 1697 (CGENZ 417121O), 1515 (NO_2). ^{1}H-NMR (DMSO), δ, ppm: 0.95 (m, 6H, NCH_2CH_3), 2.60 (m, 4H, NCH_2CH_3), 2.85 (t, 2H, NCH_2CH_2O, J = 4.60 Hz), 3.80 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.20 (t, 2H, NCH_2CH_2O, J = 4.60 Hz), 7.40 (s, 1H, ArH), 7.60 (s, 1H, ArH), 11.90 (s, 1H, NH^+). LC/MS (APCI+), *m/z*: 327.3 $(M - HCl + H)^+$.

*2-Amino-3-(2-diethylaminoethoxy)-4-methoxybenzoic acid methyl ester hydrochloride **16*** The compound **16** was obtained by using the same procedure as that for **4**. Starting from **15** (6 mmol), a brown solid was obtained after purification by column chromatography on silica gel, eluting with CH_2Cl_2/CH_3OH (9/1). Mp = 142–146°C; yield = 77%. IR (ν, cm^{-1}): 3480 and 3385 (NH_2), 1709 (C=O). ^{1}H-NMR (DMSO), δ, ppm: 1.20 (m, 6H, NCH_2CH_3), 3.20 (m, 4H, NCH_2CH_3), 3.40 (t, 2H, NCH_2CH_2O, J = 4.60 Hz), 3.70 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 4.20 (t, 2H, NCH_2CH_2O, J = 4.60 Hz), 6.40 (s, 1H, ArH), 6.50 (s, 2H, NH_2), 7.20 (s, 1H, ArH), 10.60 (s, 1H, NH^+). LC/MS (APCI+), *m/z*: 297.4 $(M - HCl + H)^+$.

*6-(2-Diethylaminoethoxy)-7-methoxyquinazolin-4-one **17*** A mixture of **16** (3 mmol) and ammonium formate (9 mmol) in formamide (1 mL) was heated at 140°C for 16 h. The reaction was treated with water (50 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The aqueous layer was neutralized by a saturated potassium carbonate solution. The precipitated solid was collected by filtration and washed with water and diethyl ether to provide a white solid after crystallization. Mp = 233–236°C (ethanol/water); yield = 71%. IR (ν, cm^{-1}): 1688 (C=O), 1610 (NH). ^{1}H-NMR (DMSO), δ, ppm: 1.00 (m, 6H, NCH_2CH_3), 2.65 (m, 4H, NCH_2CH_3), 2.80 (t, 2H, NCH_2CH_2O, J = 4.50 Hz), 3.95 (s, 3H, OCH_3), 4.10 (t, 2H, NCH_2CH_2O, J = 4.50 Hz), 7.05 (s, 1H, ArH), 7.45 (s, 1H, ArH), 8.00 (s, 1H, ArH), 12.00 (s, 1H, OH). LC/MS (APCI+), *m/z*: 292.3 $(M + H)^+$.

*4-Chloro-6-(2-diethylaminoethoxy)-7-methoxyquinazoline **18*** The compound **18** was obtained by using the same procedure as that for **6**. Starting from **17** (3 mmol), a white solid was prepared after purification by column chromatography on silica gel eluting with CH_2Cl_2/CH_3OH (95/5) Mp = 200–203°C (heptane); yield = 92%. ^{1}H-NMR (DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.55 (t, 2H, NCH_2CH_2O, J = 4.50 Hz), 4.00 (s, 3H, OCH_3), 4.60 (t, 2H, NCH_2CH_2O, J = 4.50 Hz), 7.50 (m, 2H, ArH), 8.90 (s, 1H, ArH). LC/MS (APCI+), *m/z*: 310.8 $(M)^+$ and 312.8 $(M + 2)^+$.

General procedure for compounds b–c (intermediates)

4-Nitrophenyl isocyanate **20** (6 mmol) was dissolved in a mixture of dichloromethane/methanol (**b**) or ethanol (**c**) (30 mL) and Raney nickel (0.4 g) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for 16 h. The product was filtered through a plug of Celite before being concentrated and purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9/1).

*N-(4-Aminophenyl)carbamic acid methyl ester **b*** Mp = 88–90°C; yield = 80%. IR (ν, cm^{-1}): 3480 and 3385 (NH_2), 2467 (NH–CO), 1695 (C=O). ^{1}H-NMR (DMSO), δ, ppm: 3.70 (s, 3H, OCH_3), 5.80 (s, 2H, NH_2), 7.15 (d, 2H, ArH, J = 8.10 Hz), 7.60 (d, 2H, ArH, J = 8.10 Hz), 9.40 (s, 1H, NH). LC/MS (APCI+), *m/z*: 167.2 $(M + H)^+$.

*N-(4-Aminophenyl)carbamic acid ethyl ester **c*** Mp = 72–74°C (73–74°C, lit.[32]); yield = 85%. IR (ν, cm^{-1}): 3480 and 3375 (NH_2), 2465 (NH–CO), 1695 (C=O). ^{1}H-NMR (DMSO), δ, ppm: 1.70 (m, 3H, CH_2CH_3), 2.85 (m, 2H, CH_2CH_3), 5.90 (s, 2H, NH_2), 7.25 (d, 2H, ArH, J = 8.20 Hz), 7.65 (d, 2H, ArH, J = 8.20 Hz), 9.35 (s, 1H, NH). LC/MS (APCI+), *m/z*: 181.2 $(M + H)^+$.

General procedure for compounds d–j (intermediates)

A solution of appropriate isocyanate (10 mmol) in anhydrous chloroform (15 mL) was added dropwise to a solution of 1,4-phenylenediamine **21** (9 mmol) in anhydrous chloroform (25 mL). After stirring for 2 h at room temperature, the precipitated solid was filtered off, washed with water and diethyl ether, with the exception of **i** which was extracted with ethyl acetate (3 × 15 mL), and obtained without further purification.

N-(4-Aminophenyl)-N′-phenylurea ***d*** Mp = >250°C; yield = 91%. IR (ν, cm^{-1}): 3480 and 3385 (NH_2), 1663 (C=O urea). ^{1}H-NMR (DMSO), δ, ppm: 4.75 (s, 2H, NH_2), 6.50 (d, 2H, ArH, *J* = 8.50 Hz), 6.90 (t, 1H, ArH), 7.10 (d, 2H, ArH, *J* = 8.50 Hz), 7.25 (m, 2H, ArH), 7.45 (m, 2H, ArH), 8.10 (s, 1H, NH), 8.45 (s, 1H, NH). LC/MS (APCI+), *m/z*: 228.3 $(M + H)^+$.

N-(4-Aminophenyl)-N′-(4-methoxyphenyl)urea ***e*** Mp = >250°C; yield = 74%. IR (ν, cm^{-1}): 3480 and 3385 (NH_2), 1665 (C=O urea). ^{1}H-NMR (DMSO), δ, ppm: 3.70 (s, 3H, OCH_3), 4.80 (s, 2H, NH_2), 6.50 (d, 2H, ArH, *J* = 8.60 Hz), 6.80 (d, 2H, ArH, *J* = 9.10 Hz), 7.15 (d, 2H, ArH, *J* = 8.60 Hz), 7.30 (d, 2H, ArH, *J* = 9.10 Hz), 8.10 (s, 1H, NH), 8.25 (s, 1H, NH). LC/MS (APCI+), *m/z*: 258.3 $(M + H)^+$.

N-(4-Aminophenyl)-N′-(2,4-difluorophenyl)urea ***f*** Mp = >250°C; yield = 83%. IR (ν, cm^{-1}): 3480 and 3390 (NH_2), 1655 (C=O urea), 1220 (C-F). ^{1}HNMR (DMSO), δ, ppm: 5.85 (s, 2H, NH_2), 6.50 (d, 2H, ArH, *J* = 8.30 Hz), 6.65–6.80 (m, 2H, ArH), 7.25 (d, 2H, ArH, *J* = 8.30 Hz), 7.55 (m, 1H, ArH), 8.15 (s, 1H, NH), 8.25 (s, 1H, NH). LC/MS (APCI+), *m/z*: 264.3 $(M + H)^+$.

N-(4-Aminophenyl)-N′-(3-chloro-4-fluorophenyl)urea ***g*** Mp = >250°C; yield = 82%. IR (ν, cm^{-1}): 3480 and 3390 (NH_2), 1657 (C=O urea), 1221 (C-F), 1069 (C-Cl). ^{1}H-NMR (DMSO), δ, ppm: 4.85 (s, 2H, NH_2), 6.55 (d, 2H, ArH, *J* = 8.50 Hz), 7.05 (d, 2H, ArH, *J* = 8.50 Hz), 7.25–7.40 (m, 2H, ArH), 7.75 (m, 1H, ArH), 8.20 (s, 1H, NH), 8.70 (s, 1H, NH). LC/MS (APCI+), *m/z*: 280.5 $(M)^+$ and 282.5 $(M + 2)^+$.

N-(4-Aminophenyl)-N′-butylurea ***h*** Mp = 176–178°C; yield = 95%. IR (ν, cm^{-1}): 3470 and 3380 (NH_2), 1650 (C=O urea). ^{1}H-NMR (DMSO), δ, ppm: 1.00 (m, 3H, CH_2CH_3), 1.20–1.40 (m, 6H, $CH_2CH_2CH_2CH_3$), 6.00 (s, 2H, NH_2), 6.20 (s, 1H, NH), 7.50 (d, 2H, ArH, *J* = 8.80 Hz), 8.10 (d, 2H, ArH, *J* = 8.80 Hz), 9.70 (s, 1H, NH). LC/MS (APCI+), *m/z*: 208.3 $(M + H)^+$.

N-(4-Aminophenyl)-N′-ethylurea ***i*** Mp = 149–151°C; yield = 70%. IR (ν, cm^{-1}): 3480 and 3385 (NH_2), 1655 (C=O urea). ^{1}H-NMR (DMSO), δ, ppm: 1.60 (m, 3H, CH_2CH_3), 2.80 (m, 2H, CH_2CH_3), 5.80 (s, 1H, NH), 6.10 (s, 2H, NH_2), 6.50 (d, 2H, ArH, *J* = 8.20 Hz), 7.50 (d, 2H, ArH, *J* = 8.20 Hz), 8.90 (s, 1H, NH). LC/MS (APCI+), *m/z*: 180.2 $(M + H)^+$.

N-(4-Aminophenyl)-N′-cyclohexylurea ***j*** Mp = 199–202°C; yield = 85%. IR (ν, cm^{-1}): 3470 and 3380 (NH_2), 1664 (C=O urea). ^{1}H-NMR (DMSO), δ, ppm: 1.20–1.50 (m, 10H, $CH(CH_2)_5$), 2.55 (m, 1H, $CH(CH_2)_5$), 5.90 (s, 2H, NH_2), 6.15 (s, 1H, NH), 7.45 (d, 2H, ArH, *J* = 8.60 Hz), 7.80 (d, 2H, ArH, *J* = 8.60 Hz), 8.90 (s, 1H, NH). LC/MS (APCI+), *m/z*: 234.2 $(M + H)^+$.

General procedure for compounds 7a–j, 11a–j, and 19a–j

The chloride derivate **6**, **10**, or **18** (0.32 mmol) was dissolved in 2-propanol during refluxing (5 mL) and the synthesized **b–j** or commercial aniline **a** (1.2 eq.) was added. The mixture was refluxed for 3–6 h. Final products were obtained either by filtration and washing with 2-propanol and diethyl ether or by purification by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9/1).

Series A

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}acetamide hydrochloride ***7a*** Mp = 221–223°C; yield = 56%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 2463 (NH^+), 1680 (C=O). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 2.05 (s, 3H, $COCH_3$), 3.20 (m, 4H, NCH_2CH_3), 3.40 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 4.00 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 7.15 (s, 1H, ArH), 7.50 (d, 2H, ArH, *J* = 8.10 Hz), 7.55 (d, 2H, ArH, *J* = 8.10 Hz), 7.90 (s, 1H, ArH), 8.40 (s, 1H, ArH), 9.55 (s, 1H, NH), 9.85 (s, 1H, NH). LC/MS (APCI+), *m/z*: 424.5 (M – HCl + H)$^+$.

Methyl {4-[7-(2-diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}carbamate hydrochloride ***7b*** Mp = 189–191°C; yield = 48%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 2463 (NH^+), 1682 (C=O). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.20 (m, 4H, NCH_2CH_3), 3.55 (m, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 3.70 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.55 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 7.30 (s, 1H, ArH), 7.50 (d, 2H, ArH, *J* = 8.10 Hz), 7.65 (d, 2H, ArH, *J* = 8.10 Hz), 8.00 (s, 1H, ArH), 8.50 (s, 1H, ArH), 8.70 (s, 1H, NH), 9.85 (s, 1H, NH). LC/MS (APCI+), *m/z*: 440.5 (M – HCl + H)$^+$.

Ethyl {4-[7-(2-diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}carbamate hydrochloride ***7c*** Mp = 198–201°C; yield = 52%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 2465 (NH^+), 1675 (C=O). (1H-NMR DMSO), δ, ppm: 1.30–1.50 (m, 9H, NCH_2CH_3 and OCH_2CH_3), 3.25 (m, 4H, NCH_2CH_3), 3.60 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 4.00 (s, 3H, OCH_3), 4.15 (m, 2H, OCH_2CH_3), 4.50 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 7.25 (s, 1H, ArH), 7.55 (d, 2H, ArH, *J* = 8.30 Hz), 7.70 (d, 2H, ArH, *J* = 8.30 Hz), 8.10 (s, 1H, ArH), 8.55 (s, 1H, ArH), 8.80 (s, 1H, NH), 9.85 (s, 1H, NH). LC/MS (APCI+), *m/z*: 454.5 (M – HCl + H)$^+$.

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}-N′-phenylurea hydrochloride ***7d*** Mp = 215–217°C; yield = 65%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1657 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.65 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 4.00 (s, 3H, OCH_3), 4.55 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 6.90 (t, 1H, ArH), 7.25 (m, 3H, ArH), 7.40–7.55 (m, 4H, ArH), 7.65 (d, 2H, ArH), 8.25 (s, 1H, ArH), 8.70 (s, 1H, ArH), 9.24 (s, 1H, NH), 9.25 (s, 1H, NH), 10.50 (s, 1H, NH). LC/MS (APCI+), *m/z*: 501.3 (M – HCl + H)$^+$.

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}-N′-(4-methoxyphenyl)urea hydrochloride ***7e*** Mp = 207–209°C; yield = 73%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1661 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.55 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 3.85 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.55 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 6.90 (d, 2H, ArH, *J* = 8.70 Hz), 7.20 (s, 1H, ArH), 7.40 (d, 2H, ArH, *J* = 8.70 Hz), 7.50 (d, 2H, ArH, *J* = 8.80 Hz), 7.60 (d, 2H, ArH, *J* = 8.80 Hz), 8.10 (s, 1H, ArH), 8.55 (s, 1H, ArH), 8.70 (s, 1H, NH), 8.80 (s, 1H, NH), 10.55 (s, 1H, NH). LC/MS (APCI+), *m/z*: 531.6 (M – HCl + H)$^+$.

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}-N′-(2,4-difluoro phenyl)urea hydrochloride ***7f*** Mp = 202–204°C; yield = 89%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1657 (C=O urea), 1221 (C–F). (1H-NMR DMSO),

δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.60 (t, 2H, NCH_2CH_2O, J = 4.40 Hz), 4.00 (s, 3H, OCH_3), 4.55 (t, 2H, NCH_2CH_2O, J = 4.40 Hz), 7.00 (d, 1H, ArH), 7.20 (s, 1H, ArH), 7.30 (t, 1H, ArH), 7.50 (d, 2H, ArH, J = 8.80 Hz), 7.65 (d, 2H, ArH, J = 8.80 Hz), 7.95 (s, 1H, ArH), 8.10 (d, 1H, ArH), 8.50 (s, 1H, ArH), 8.60 (s, 1H, NH), 9.15 (s, 1H, NH), 10.50 (s, 1H, NH). LC/MS (APCI+), *m/z*: 537.6 (M – HCl + H)$^+$.

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}-N′-(3-chloro-4-fluorophenyl)urea hydrochloride ***7g*** Mp = 218–220°C; yield = 64%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1664 (C=O urea), 1219 (C–F), 1069 (C–Cl). (1H-NMR DMSO), δ, ppm: 1.25 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.55 (t, 2H, NCH_2CH_2O, J = 4.30 Hz), 3.95 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, J = 4.30 Hz), 7.10–7.30 (m, 3H, ArH), 7.50 (d, 2H, ArH, J = 8.70 Hz), 7.65 (d, 2H, ArH, J = 8.70 Hz), 7.95 (s, 1H, ArH), 8.10 (d, 1H, ArH), 8.45 (s, 1H, ArH), 9.15 (s, 1H, NH), 9.35 (s, 1H, NH), 10.40 (s, 1H, NH). LC/MS (APCI+), *m/z*: 553.6 (M – HCl)$^+$ and 555.6 (M – HCl + 2)$^+$.

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}-N′-butylurea hydrochloride ***7h*** Mp = 222–225°C; yield = 59%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1651 (C=O urea). (1H-NMR DMSO), δ, ppm: 0.95 (m, 3H, CH_2CH_3), 1.20 (m, 6H, NCH_2CH_3), 1.30–1.50 (m, 6H, $CH_2CH_2CH_2CH_3$), 3.20 (m, 4H, NCH_2CH_3), 3.35 (t, 2H, NCH_2CH_2O, J = 4.40 Hz), 3.95 (s, 3H, OCH_3), 4.45 (t, 2H, NCH_2CH_2O, J = 4.40 Hz), 6.20 (s, 1H, NH), 7.20 (s, 1H, ArH), 7.40 (d, 2H, ArH, J = 8.90 Hz), 7.55 (d, 2H, ArH, J = 8.90 Hz), 7.95 (s, 1H, NH), 8.35 (s, 1H, ArH), 8.55 (s, 1H, ArH), 9.55 (s, 1H, NH). LC/MS (APCI+), *m/z*: 481.6 (M – HCl + H)$^+$.

N-{4-[7-(2-Diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}-N′-ethylurea hydrochloride ***7i*** Mp = 207–209°C; yield = 40%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1659 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.05 (m, 3H, CH_2CH_3), 1.30 (m, 6H, NCH_2CH_3), 3.10 (m, 2H, CH_2CH_3), 3.35 (m, 4H, NCH_2CH_3), 3.50 (t, 2H, NCH_2CH_2O, J = 4.30 Hz), 4.00 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, J = 4.30 Hz), 6.30 (s, 1H, NH), 7.30 (s, 1H, ArH), 7.45 (d, 2H, ArH, J = 8.10 Hz), 7.55 (d, 2H, ArH, J = 8.10 Hz), 8.10 (s, 1H, ArH), 8.55 (s, 1H, ArH), 8.80 (s, 1H, NH), 10.45 (s, 1H, NH). LC/MS (APCI+), *m/z*: 453.6 (M – HCl + H)$^+$.

N-Cyclohexyl-N′-{4-[7-(2-diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}urea hydrochloride ***7j*** Mp = 191–194°C; yield = 68%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1657 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.20–1.70 (m, 17H, NCH_2CH_3 and $CH–(CH_2)_5$), 3.25 (m, 4H, NCH_2CH_3), 3.50 (t, 2H, NCH_2CH_2O, J = 4.30 Hz), 4.00 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, J = 4.30 Hz), 6.40 (s, 1H, NH), 7.25 (s, 1H, ArH), 7.50 (d, 2H, ArH, J = 8.80 Hz), 7.65 (d, 2H, ArH, J = 8.80 Hz), 8.00 (s, 1H, ArH), 8.60 (s, 1H, ArH), 8.70 (s, 1H, NH), 10.90 (s, 1H, NH). LC/MS (APCI+), *m/z*: 507.7 (M – HCl + H)$^+$.

Series B

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}acetamide ***11a*** Mp = 211–213°C; yield = 44%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 1668 (C=O). (1H-NMR DMSO), δ, ppm: 2.05 (s, 3H, $COCH_3$), 4.00 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 7.40 (s, 1H, ArH), 7.55–7.75 (m, 4H, ArH), 8.30 (s, 1H, ArH), 8.70 (s, 1H, ArH), 10.20 (s, 1H, NH), 11.30 (s, 1H, NH). LC/MS (APCI+), *m/z*: 339.4 (M + H)$^+$.

Methyl {4-[6,7-dimethoxy-quinazolin-4-ylamino]phenyl} carbamate ***11b*** Mp = >250°C; yield = 72%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 1690 (C=O). (1H-NMR DMSO), δ, ppm: 3.65 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 7.30 (s, 1H, ArH), 7.45 (d, 2H, ArH, J = 8.10 Hz), 7.60 (d, 2H, ArH, J = 8.10 Hz), 8.20 (s, 1H, ArH), 8.80 (s, 1H, ArH), 9.80 (s, 1H, NH), 11.30 (s, 1H, NH). LC/MS (APCI+), *m/z*: 355.4 (M + H)$^+$.

Ethyl {4-[6,7-dimethoxy-quinazolin-4-ylamino]phenyl} carbamate ***11c*** Mp = 198–201°C; yield = 52%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 1669 (C=O). (1H-NMR DMSO), δ, ppm: 1.30 (t, 3H, OCH_2CH_3), 3.94 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.20 (m, 2H, OCH_2CH_3), 7.20 (d, 2H, ArH, J = 9.00 Hz), 7.35 (s, 1H, ArH), 7.55 (d, 2H, ArH, J = 9.00 Hz), 7.65 (s, 1H, ArH), 8.50 (s, 1H, ArH), 8.80 (s, 1H, NH), 9.75 (s, 1H, NH). LC/MS (APCI+), *m/z*: 369.4 (M + H)$^+$.

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}-N′-phenylurea ***11d*** Mp = >250°C; yield = 56%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1662 (C=O urea). (1H-NMR DMSO), δ, ppm: 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.90 (m, 1H, ArH), 7.30 (m, 3H, ArH), 7.45–7.60 (m, 4H, ArH), 7.65 (d, 2H, ArH), 8.25 (s, 1H, ArH), 8.60 (s, 1H, ArH), 9.25 (d, 2H, 2NH), 10.40 (s, 1H, NH). LC/MS (APCI+), *m/z*: 416.6 (M + H)$^+$.

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}-N′-(4-methoxyphenyl)urea ***11e*** Mp = 193–196°C; yield = 54%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1655 (C=O urea). (1H-NMR DMSO), δ, ppm: 3.70 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.85 (d, 2H, ArH, J = 8.80 Hz), 7.20 (s, 1H, ArH), 7.30 (d, 2H, ArH, J = 8.80 Hz), 7.50 (d, 2H, ArH, J = 9.00 Hz), 7.60 (d, 2H, ArH, J = 9.00 Hz), 8.00 (s, 1H, ArH), 8.55 (s, 1H, ArH), 8.65 (s, 1H, NH), 8.80 (s, 1H, NH), 10.10 (s, 1H, NH). LC/MS (APCI+), *m/z*: 446.6 (M + H)$^+$.

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}-N′-(2,4-difluorophenyl)urea ***11f*** Mp = 168–171°C; yield = 58%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1657 (C=O urea), 1220 (C-F). (1H-NMR DMSO), δ, ppm: 3.95 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 7.00 (m, 1H, ArH), 7.20 (s, 1H, ArH), 7.30 (m, 1H, ArH), 7.50 (d, 2H, ArH, J = 9.00 Hz), 7.60 (d, 2H, ArH, J = 9.00 Hz), 7.95 (s, 1H, ArH), 8.10 (m, 1H, ArH), 8.55 (s, 1H, ArH), 8.60 (s, 1H, NH), 9.20 (s, 1H, NH), 10.10 (s, 1H, NH). LC/MS (APCI+), *m/z*: 452.5 (M + H)$^+$.

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}-N′-(3-chloro-4-fluorophenyl)urea ***11g*** Mp = 241–243°C; yield = 71%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1658 (C=O urea), 1220 (C-F), 1069 (C-Cl). (1H-NMR DMSO), δ, ppm: 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.20–7.40 (m, 3H, ArH), 7.55 (s, 4H, ArH), 7.80 (m, 1H, ArH), 8.15 (s, 1H, ArH), 8.80 (s, 1H, ArH), 9.35 (s, 1H, NH), 9.45 (s, 1H, NH), 11.20 (s, 1H, NH). LC/MS (APCI+), *m/z*: 468.9 (M)$^+$and 470.9 (M + 2)$^+$.

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}-N′-butylurea ***11h*** Mp = 196–198°C; yield = 50%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1662 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.00 (t, 3H, CH_2CH_3), 1.20–1.40 (m, 6H, $CH_2CH_2CH_2CH_3$), 4.00 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.30 (s, 1H, NH), 7.25 (s, 1H, ArH), 7.40 (d, 2H, ArH, J = 8.70 Hz), 7.60 (d, 2H, ArH, J = 8.70 Hz),

8.20 (s, 1H, ArH), 8.60 (s, 1H, ArH), 8.90 (s, 1H, NH), 10.60 (s, 1H, NH). LC/MS (APCI+), *m/z*: 396.5 $(M + H)^+$.

N-{4-[6,7-Dimethoxy-quinazolin-4-ylamino]phenyl}-N′-ethylurea ***11i*** Mp = 189–192°C; yield = 40%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1657 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.15 (t, 3H, CH_2CH_3), 3.20 (m, 2H, CH_2CH_3), 3.94 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.35 (s, 1H, NH), 7.20 (s, 1H, ArH), 7.45 (d, 2H, ArH, *J* = 8.80 Hz), 7.55 (d, 2H, ArH, *J* = 8.80 Hz), 8.00 (s, 1H, ArH), 8.40 (s, 1H, ArH), 8.90 (s, 1H, NH), 10.30 (s, 1H, NH). LC/MS (APCI+), *m/z*: 368.4 $(M + H)^+$.

N-Cyclohexyl-N′-{4-[6,7-dimethoxy-quinazolin-4-ylamino]phenyl}urea ***11j*** Mp = 151–153°C; yield = 53%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1659 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.20 (m, 5H, $(CH_2)_5$), 1.40 (m, 1H, CH–$(CH_2)_5$), 1.65 (m, 5H, $(CH_2)_5$), 3.95 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.30 (s, 1H, NH), 7.25 (s, 1H, ArH), 7.50 (m, 4H, ArH), 8.10 (s, 1H, ArH), 8.70 (s, 1H, ArH), 8.70 (s, 1H, NH), 11.00 (s, 1H, NH). LC/MS (APCI+), *m/z*: 422.5 $(M + H)^+$.

Series C

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}acetamide hydrochloride ***19a*** Mp = 239–241°C; yield = 60%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 2461 (NH^+), 1671 (C=O). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 2.05 (s, 3H, $COCH_3$), 3.25 (m, 4H, NCH_2CH_3), 3.40 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 3.95 (s, 3H, OCH_3), 4.45 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 7.20 (s, 1H, ArH), 7.45 (d, 2H, ArH, *J* = 8.10 Hz), 7.55 (d, 2H, ArH, *J* = 8.10 Hz), 7.90 (s, 1H, ArH), 8.35 (s, 1H, ArH), 9.75 (s, 1H, NH), 9.95 (s, 1H, NH). LC/MS (APCI+), *m/z*: 424.5 $(M - HCl + H)^+$.

Methyl {4-[6-(2-diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}carbamate hydrochloride ***19b*** Mp = 239–241°C; yield = 56%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 2461 (NH^+), 1680 (C=O). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.25 (m, 4H, NCH_2CH_3), 3.50 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 3.65 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 7.20 (s, 1H, ArH), 7.45 (d, 2H, ArH, *J* = 8.10 Hz), 7.65 (d, 2H, ArH, *J* = 8.10 Hz), 8.00 (s, 1H, ArH), 8.40 (s, 1H, ArH), 8.65 (s, 1H, NH), 9.85 (s, 1H, NH). LC/MS (APCI+), *m/z*: 440.5 $(M - HCl + H)^+$.

Ethyl {4-[7-(2-diethylaminoethoxy)-6-methoxy-quinazolin-4-ylamino]phenyl}carbamate hydrochloride ***19c*** Mp = 201–203°C; yield = 49%. IR (ν, cm^{-1}): 3480 and 3385 (NH), 2462 (NH^+), 1679 (C=O). (1H-NMR DMSO), δ, ppm: 1.30–1.50 (m, 9H, NCH_2CH_3 and OCH_2CH_3), 3.25 (m, 4H, NCH_2CH_3), 3.55 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 3.95 (s, 3H, OCH_3), 4.15 (m, 2H, OCH_2CH_3), 4.45 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 7.25 (s, 1H, ArH), 7.50 (d, 2H, ArH, *J* = 8.50 Hz), 7.70 (d, 2H, ArH, *J* = 8.50 Hz), 8.15 (s, 1H, ArH), 8.55 (s, 1H, ArH), 8.80 (s, 1H, NH), 9.85 (s, 1H, NH). LC/MS (APCI+), *m/z*: 454.5 $(M - HCl + H)^+$.

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}-N′-phenylurea hydrochloride ***19d*** Mp = 177–179°C; yield = 81%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1665 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.60 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 3.95 (s, 3H, OCH_3), 4.65 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 7.00 (t, 1H, ArH), 7.20 (m, 3H, ArH), 7.40–7.55 (m, 4H, ArH), 7.70 (m, 2H, ArH), 8.30 (s, 1H, ArH), 8.60 (s, 1H, ArH), 9.10 (s, 1H, NH), 9.12 (s, 1H, NH), 10.45 (s, 1H, NH). LC/MS (APCI+), *m/z*: 501.6 $(M - HCl + H)^+$.

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}-N′-(4-methoxy phenyl)urea hydrochloride ***19e*** Mp = 145–147°C; yield = 82%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1661 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.20 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.60 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 3.80 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.60 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 6.90 (d, 2H, ArH, *J* = 8.70 Hz), 7.20 (s, 1H, ArH), 7.35 (d, 2H, ArH, *J* = 8.70 Hz), 7.55 (d, 2H, ArH, *J* = 8.80 Hz), 7.65 (d, 2H, ArH, *J* = 8.80 Hz), 8.00 (s, 1H, ArH), 8.50 (s, 1H, ArH), 8.70 (s, 1H, NH), 8.80 (s, 1H, NH), 10.50 (s, 1H, NH). LC/MS (APCI+), *m/z*: 531.6 $(M - HCl + H)^+$.

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}-N′-(2,4-difluoro phenyl)urea hydrochloride ***19f*** Mp = 143–146°C; yield = 66%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1660 (C=O urea), 1221 (C–F). (1H-NMR DMSO), δ, ppm: 1.25 (m, 6H, NCH_2CH_3), 3.30 (m, 4H, NCH_2CH_3), 3.55 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 3.85 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 7.00 (d, 1H, ArH), 7.20 (s, 1H, ArH), 7.30 (m, 1H, ArH), 7.50 (d, 2H, ArH, *J* = 8.80 Hz), 7.60 (d, 2H, ArH, *J* = 8.80 Hz), 8.00 (s, 1H, ArH), 8.15 (m, 1H, ArH), 8.50 (s, 1H, ArH), 8.60 (s, 1H, NH), 9.20 (s, 1H, NH), 10.50 (s, 1H, NH). LC/MS (APCI+), *m/z*: 537.6 $(M - HCl + H)^+$.

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}-N′-(3-chloro-4-fluorophenyl)urea hydrochloride ***19g*** Mp = 215–217°C; yield = 59%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1658 (C=O urea), 1220 (C–F), 1067 (C–Cl). (1H-NMR DMSO), δ, ppm: 1.30 (m, 6H, NCH_2CH_3), 3.25 (m, 4H, NCH_2CH_3), 3.55 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 3.95 (s, 3H, OCH_3), 4.55 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 7.10–7.30 (m, 3H, ArH), 7.55 (d, 2H, ArH, *J* = 8.70 Hz), 7.65 (d, 2H, ArH, *J* = 8.70 Hz), 7.95 (s, 1H, ArH), 8.10 (m, 1H, ArH), 8.45 (s, 1H, ArH), 9.25 (s, 1H, NH), 9.45 (s, 1H, NH), 10.45 (s, 1H, NH). LC/MS (APCI+), *m/z*: 553.6 $(M - HCl)^+$and 555.6 $(M - HCl + 2)^+$.

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}-N′-butylurea hydrochloride ***19h*** Mp = 102–105°C; yield = 49%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1661 (C=O urea). (1H-NMR DMSO), δ, ppm: 0.95 (m, 3H, CH_2CH_3), 1.15 (m, 6H, NCH_2CH_3), 1.25–1.45 (m, 6H, $CH_2CH_2CH_2CH_3$), 3.10 (m, 4H, NCH_2CH_3), 3.30 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 3.90 (s, 3H, OCH_3), 4.30 (t, 2H, NCH_2CH_2O, *J* = 4.40 Hz), 6.10 (s, 1H, NH), 7.15 (s, 1H, ArH), 7.40 (d, 2H, ArH, *J* = 9.05 Hz), 7.60 (d, 2H, ArH, *J* = 9.05 Hz), 7.90 (s, 1H, NH), 8.40 (s, 1H, ArH), 8.50 (s, 1H, ArH), 9.50 (s, 1H, NH). LC/MS (APCI+), *m/z*: 481.6 $(M - HCl + H)^+$.

N-{4-[6-(2-Diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}-N′-ethylurea hydrochloride ***19i*** Mp = 194–196°C; yield = 33%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1660 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.10 (m, 3H, CH_2CH_3), 1.20 (m, 6H, NCH_2CH_3), 2.90 (m, 4H, NCH_2CH_3), 3.05 (m, 2H, CH_2CH_3), 3.40 (t, 2H, NCH_2CH_2O,

J = 4.30 Hz), 3.90 (s, 3H, OCH_3), 4.30 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 6.20 (s, 1H, NH), 7.20 (s, 1H, ArH), 7.40 (d, 2H, ArH, *J* = 8.80 Hz), 7.60 (d, 2H, ArH, *J* = 8.80 Hz), 7.90 (s, 1H, ArH), 8.40 (s, 1H, ArH), 8.50 (s, 1H, NH), 9.50 (s, 1H, NH). LC/MS (APCI+), *m/z*: 453.6 $(M - HCl + H)^+$.

N-Cyclohexyl-N'-{4-[6-(2-diethylaminoethoxy)-7-methoxy-quinazolin-4-ylamino]phenyl}urea hydrochloride ***19j*** Mp = 184–186°C; yield = 64%. IR (ν, cm^{-1}): 3200 and 2800 (NH), 1661 (C=O urea). (1H-NMR DMSO), δ, ppm: 1.20–1.70 (m, 17H, NCH_2CH_3 *and* $CH-(CH_2)_5$), 3.25 (m, 4H, NCH_2CH_3), 3.50 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 4.00 (s, 3H, OCH_3), 4.50 (t, 2H, NCH_2CH_2O, *J* = 4.30 Hz), 6.40 (s, 1H, NH), 7.25 (s, 1H, ArH), 7.50 (d, 2H, ArH, *J* = 8.80 Hz), 7.65 (d, 2H, ArH, *J* = 8.80 Hz), 8.00 (s, 1H, ArH), 8.60 (s, 1H, ArH), 8.70 (s, 1H, NH), 10.90 (s, 1H, NH). LC/MS (APCI+), *m/z*: 507.7 $(M - HCl + H)^+$.

Pharmacology

Cell culture and cell proliferation assay

Human prostate cancer cells PC3 were grown at 37°C in a humidified atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 IU/mL), and streptomycin (100 μg/mL). In the cell proliferation assay, cells were plated in triplicate on 96-well plates (3×10^3 cells per well) and incubated for 72 h. The cell medium was changed to serum-free medium, and the cells were starved for 24 h for culture synchronization. Cells were then incubated in culture medium that contained various concentrations of tested compounds, each dissolved in less than 0.1% DMSO. After 72 h, cell growth was estimated by the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test.

In vitro kinase assays

Kinase assays were performed in 96-well plates (Multiscreen Durapore, Millipore) using [γ-^{32}P]ATP (Amersham Biosciences) and the synthetic polymer poly(Glu_4/Tyr) (Sigma Chemicals) as a phosphoacceptor substrate. Tested compounds were dissolved in 1H-NMR DMSO, and the final concentration of 1H-NMR DMSO in assay solutions was 0.1% (total kinase activity) or 0.01% (EGFR kinase activity), which was shown to have no effect on kinase activity.

EGFR tyrosine kinase activity 20 ng of EGFR (purified from human carcinoma A431 cells; Sigma Chemicals) was incubated for 1 h at 28°C using various concentrations of tested compounds in kinase buffer (HEPES 50 mM pH 7.5, bovine serum albumin (BSA) 0.1 mg/mL, $MnCl_2$ 10 mM, $MgCl_2$ 5 mM, Na_3VO_4 100 μM, dithiothreitol (DTT) 0.5 mM, poly(Glu_4/Tyr) 250 μg/mL, ATP 5 μM, [γ-^{32}P]ATP 0.5 μCi).

VEGFR-2 tyrosine kinase activity 10 ng of VEGFR-2 (recombinant human protein; Invitrogen) was incubated for 1 h at 28°C using various concentrations of tested compounds in kinase buffer (Tris 50mM pH 7.5, BSA 25 μg/mL, $MnCl_2$ 1.5 mM, $MgCl_2$ 10 mM, DTT 2.5 mM, Na_3VO_4 100 μM, β-glycerophosphate 5 mM, poly(Glu_4/Tyr) 250 μg/mL, ATP 5 μM, [γ-^{32}P]ATP 0.5 μCi).

The reaction was stopped by adding 20 μL of trichloroacetic acid 100%. Wells were screened out and washed 10 times with trichloroacetic acid 10%. Plates were counted in a Top Count for 1 min per well.

Aurora-A kinase activity 2 μg of Aurora-A$(His)_6$ was incubated in 15 μL of kinase buffer (50 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM DTT, 10 mM $MgCl_2$) in the presence of 5 μCi [γ-^{32}P]ATP at 3000 Ci/mmol and 10 μg histone H3 with or without the compounds. The control was performed with a solution of 1H-NMR DMSO in water corresponding to the dilution used to prepare the chemical compounds. After 20 min incubation at 30°C, the incubations were spotted into 2.5 cm × 3 cm pieces of Whatman P81 phosphocellulose paper, and, after 20 s, the filters were washed five times (for at least 10 min each time) in a solution of phosphoric acid in water (1.2%). The wet filters were transferred into 6 mL plastic scintillation vials, and 5 mL ACS scintillation fluid (Amersham) was added prior to measuring the radioactivity in a Packard counter. The kinase activity was expressed as percent of the control assay.

Molecular modeling

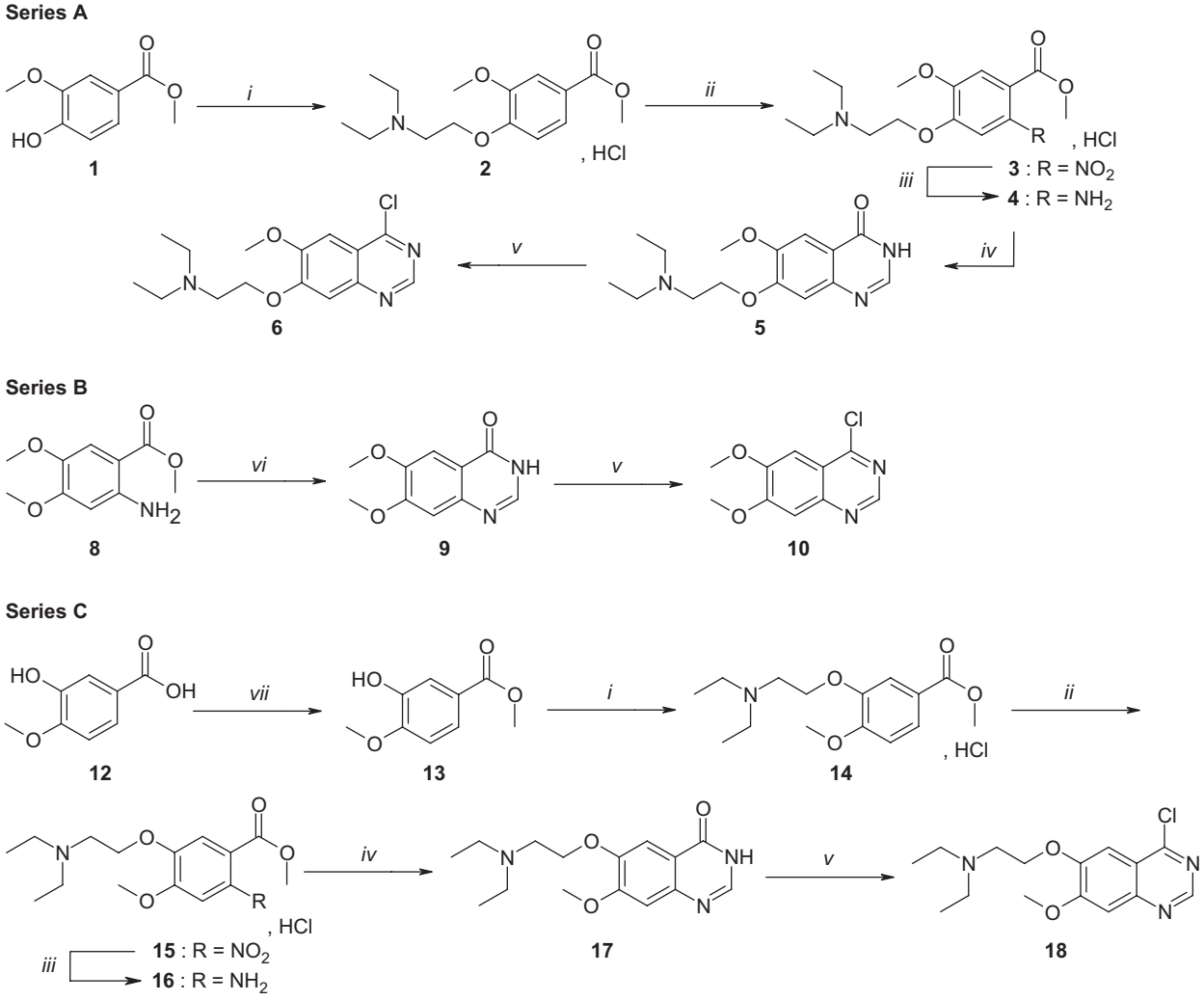
All the calculations were carried out using the Sybyl 6.9.1 molecular modeling package[33] running on Silicon Graphics Octane 2 workstations. The ligands were built from the internal fragments library of Sybyl and their geometry was optimized by the Powell method available in the Maximin2 procedure to a gradient of 0.001 kcal/mol/Å. The dielectric constant was set to 4 to implicitly represent a biological middle, the atomic charges were attributed following the Gasteiger–Hückel method, and energy minimization was run using the Tripos force field[34]. The structure of the protein co-crystallized with an inhibitor was obtained from the Protein Data Bank (http://www.pdb.org)[35] under the entries 1M17[36] and 1YWN[37]. The co-crystallized inhibitor and water molecules were removed and hydrogens were added to the protein, taking care to be as close as possible to the biological protonation state of the residues. The binding mode of the compounds was investigated by a two-step process. They were first docked into the two binding sites of EGFR and VEGFR-2 by Gold 3.2[38]. The multiple conformations generated were then ranked by a consensus scoring based on Goldscore[38] and X-Score[39]. The consistency of the best ranked conformation was visually assessed to insure it was the most stable binding mode prediction.

Results and discussion

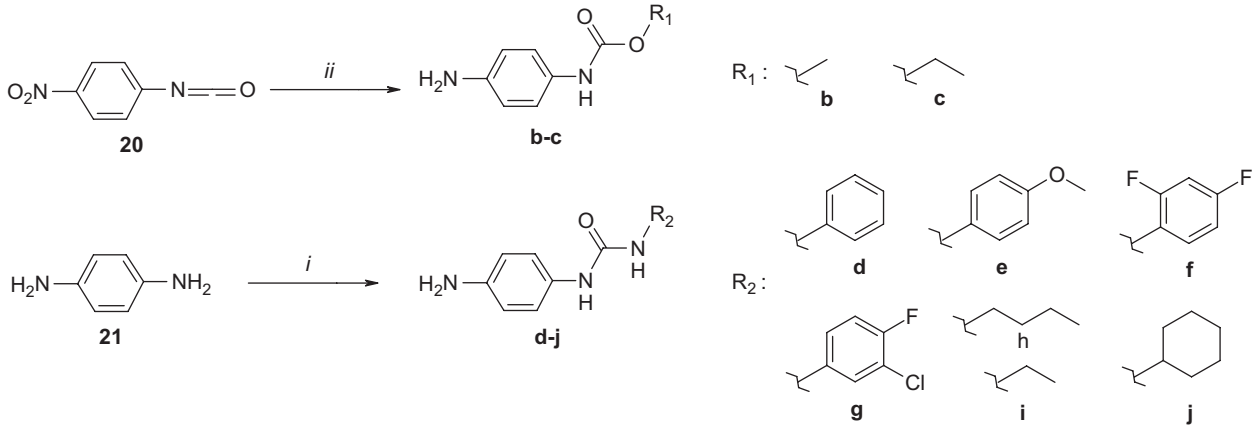
Chemistry

The synthesis of the three series (A, B, C) of quinazolines **7a–j**, **11a–j**, and **19a–j** is presented in Schemes 1, 2, and 3, respectively, according to a parallel synthesis route. We have described the synthesis of 4-chloroquinazoline derivates **6**, **10**, and **18**, differentiating the ether linker at the C-6 and C-7 positions (Scheme 1).

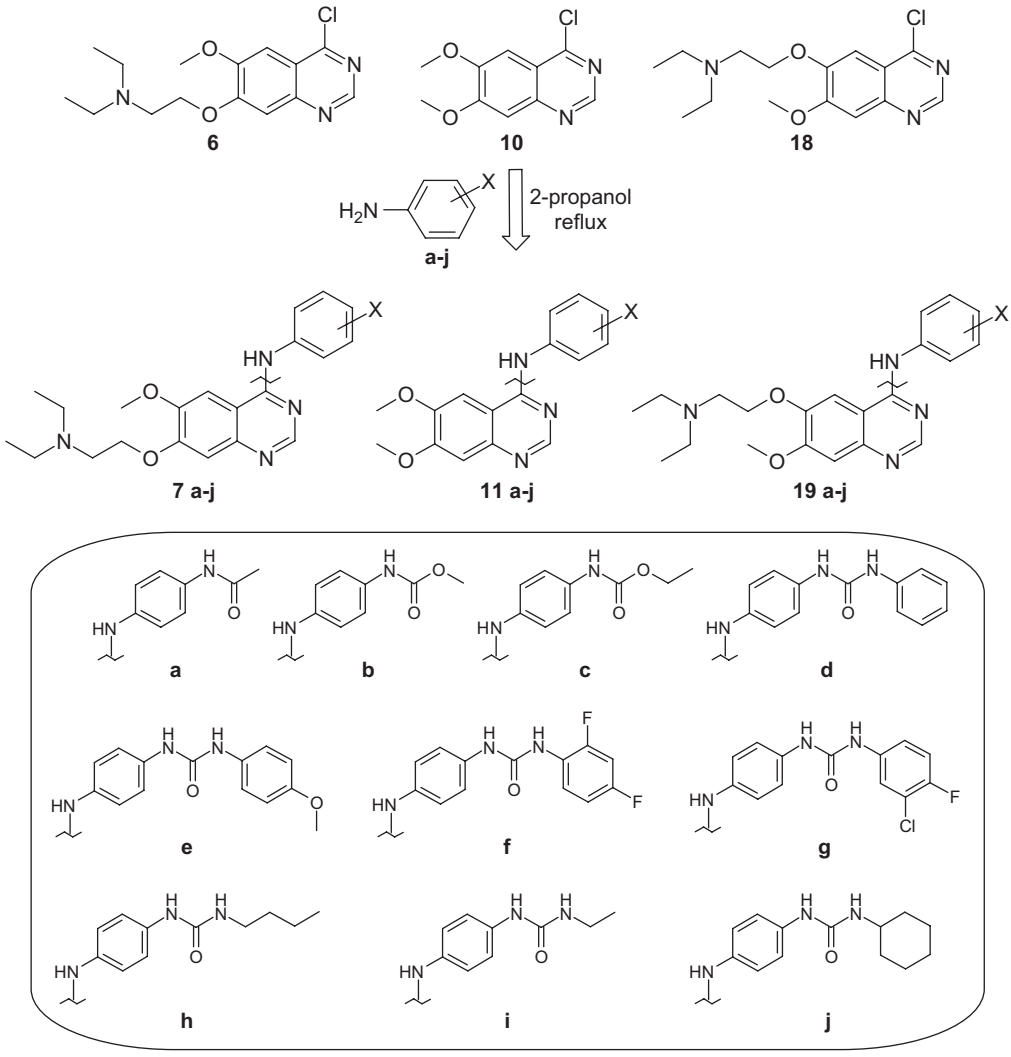
These compounds were synthesized with high yields using an optimized and potent synthesis route. For series A,



Scheme 1. Synthesis of 4-chloroquinazoline derivatives **6**, **10**, and **18**. Reagents and conditions: (*i*) $ClCH_2CH_2N(CH_2CH_3)_2$.HCl, K_2CO_3, acetone, reflux, 3 h; (*ii*) $SnCl_4$, HNO_3, CH_2Cl_2,–25°C, 4 h; (*iii*) Raney Ni, H_2, MeOH, rt, 16 h; (*iv*) $HCOONH_4$, $HCONH_2$, 140°C, 16 h; (*v*) $POCl_3$, 120°C, 2 h; (*vi*) NaOMe, formamide, DMF/MeOH, 110°C, 4 h; (*vii*) $SOCl_2$, MeOH, 0°C at refluxing, 3 h.



Scheme 2. Synthesis of aniline intermediates b–j. Reagents and conditions: (i) Raney Ni, H_2, CH_2Cl_2/MeOH or EtOH, rt, 10 h; (ii) isocyanate (R2–N=C=O) diluted in $CHCl_3$, $CHCl_3$, rt, 2 h.



Scheme 3. Synthesis of targeted derivatives **7a–j**, **11a–j**, and **19a–j**.

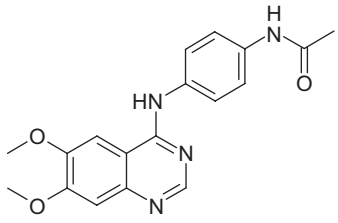
we used a synthesis route according to previously described procedures[40] to synthesize compounds **2** and **3**. Catalytic hydrogenation of the nitro group led to amino derivative **4** (67% yield), which was converted, by heating at 140°C in formamide in the presence of ammonium formate, to the corresponding substituted quinazolinone derivative **5**. Treatment of quinazolinone **5** in refluxing phosphorus oxychloride afforded the chloroquinazoline derivative in good yield (>90%). In series B, compound **10** was prepared according to procedures previously described by Furuta *et al.*[41] using methyl 2-amino-4,5-dimethoxybenzoate **8** as starting material. To prepare series C, the same synthesis route was employed as for series A. After a step in which 3-hydroxy-4-methoxybenzoic acid **12** was converted into its methyl ester **13**[40], we synthesized intermediates **14–18** with high yields. These chloride derivatives **6**, **10**, and **18** were then engaged in a nucleophilic substitution reaction in the presence of aniline (**a–j**).

The synthesis of non-commercially available aniline derivatives **b–j** is depicted in Scheme 2. During these preparations, we also report a novel beneficial (80–85%) approach to synthesizing (4-aminophenyl)carbamic acid methyl and ethyl ester (**b**, **c**). For this, we used 4-nitrophenylisocyanate **20** in the presence of a dichloromethane/alcohol mixture under a hydrogen atmosphere and Raney nickel as catalyst. Condensation of 1,4-phenylenediamine **21** and the corresponding isocyanate in $CHCl_3$[42] afforded the desired ureas **d–j** in high yields and with short reaction times.

Obtaining of the final 4-anilinoquinazoline derivatives **7**, **11**, and **19** was performed by the same synthesis strategy: nucleophilic displacement of the chlorine atom with various arylamino groups in 2-propanol. The final products were crystallized in various solvents (Scheme 3).

Molecular modeling

Molecular modeling of many compounds described in the literature was realized in order to understand their binding modes with the ATP site of EGFR and VEGFR-2. The models used the reported crystal structure of the EGFR domain in complex with erlotinib[36] and the VEGFR-2 domain with a compound from the literature, a 4-amino-furo[2,3-*d*]pyrimidine derivative[37].



Compound 11a (series B)

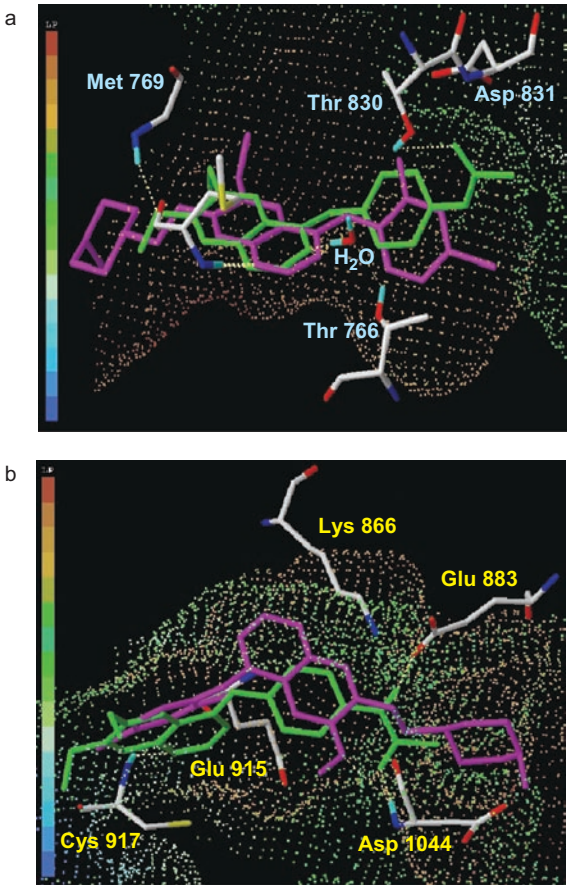


Figure 3. Docking mode of **ZD6474** (vandetanib) and compound **11a** to the ATP site of EGFR and VEGFR-2. In each case, hydrophobic regions are represented in brown. (a) Active site of EGFR with docked vandetanib (magenta) and compound **11a** (green). (b) Active site of VEGFR-2 with docked vandetanib (magenta) and compound **11a** (green).

Preliminary docking simulations were carried out in order to predict the binding mode of the first synthesized compound **11a** into the ATP active site of EGFR and VEGFR occupied by vandetanib, a dual EGFR/VEGFR kinase inhibitor. The most stable docking model in EGFR (Figure 3a) shows a binding mode very similar to the orientation of vandetanib. Compound **11a** interacts via a H-bonding interaction with Met769 (between a methionine NH and the N-1 nitrogen of quinazoline from **11a**), Thr766 (via a bridge between a water molecule, the OH threonine side chain, and the N-3 nitrogen of quinazoline from **11a**), and Thr830 (between a threonine OH and the sp2 oxygen from the amide group of **11a**).

We also compared the volume occupied by vandetanib and **11a** in the ATP active site of VEGFR-2. Both compounds interact differently with this active site. The quinazoline cores adopt essentially volumes and conformations in opposite directions. We observed new interactions between, respectively, the CO and NH amide groups from **11a** and the backbone of Asp1044 and the Lys866–Glu883 salt bridge (Figure 3b). This observation led us to introduce different bulky substituents, such as amide, carbamate, or urea, which are a common moiety in EGFR and VEGFR inhibitors, in order to strengthen the ligand–receptor interactions and by the way probably increase the affinity.

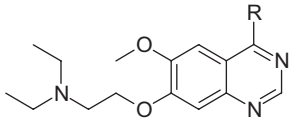
Biological evaluation

The substituted anilinoquinazoline derivatives **7a–j** (series A), **11a–j** (series B), and **19a–j** (series C) were evaluated for both their antiproliferative activity toward the hormone-independent PC3 prostate cancer cell and their *in vitro* EGFR and VEGFR-2 kinase inhibition. The results are reported in Table 1. Urea derivatives were slightly less active in the cell proliferation assays than the reference compound gefitinib, while other quinazolines were found to be poorly cytotoxic with IC_{50} >10 μM. Substitution of urea with a phenyl or 2,4-difluorophenyl group seemed to be most tolerant of the steric bulk of substituents for *in vitro* antiproliferative activities. Replacement of one of the methoxy groups at the C-6 or C-7 position of the quinazoline core with a diethylaminoethyl resulted in a clear decrease in activity. Also, the antiproliferative activities of quinazoline substituted by carbamic acid ester (**b** and **c**) and aliphatic-urea (**h**, **i**, and **j**) were decreased.

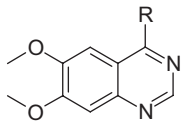
All the compounds tested exhibited weak to moderate *in vitro* EGFR and VEGFR kinase inhibitions (Table 1). The best results were obtained when the quinazoline derivatives were substituted on the aniline moiety by an alkyl or arylurea group, excepted for compounds **11f** and **11j**, leading to selective VEGFR inhibitors with IC_{50} values from 4.31 to 6.62 μM (**7h, 11d**, **11e**, **11g**, **11h**, **11i**). This suggests that the oxygen of urea and the NH moiety interact with the binding site of VEGFR-2 as described in previous investigations. Surprisingly, amide derivative **11a** was found to be inactive in both *in vitro* antiproliferative and enzymatic assays. Replacement of the urea with a carbamic acid methyl ester derivative led to dual EGFR/VEGFR active compound **11b**, while its ethyl (**11c)** counterpart was found to be inactive on EGFR enzymatic activity. These observations demonstrated that the introduction of bulky substituents suh as ethyl carbamate (compared to methyl) or urea derivatives is the key modification to increase VEGFR-2 kinase inhibition, while those changes in the aniline substitution resulted in a total loss of EGFR activity.

We investigated the inhibitory activity of many compounds toward Aurora-A to demonstrate selectivity against this serine–threonine kinase. These compounds did not significantly impact on the enzyme-inhibitory activity (IC_{50} >10 μM) compared to the reference Vertex[30] (80% inhibition

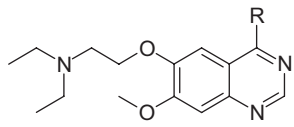
Table 1. Antiproliferative activity toward hormone-independent PC3 prostate cancer cells and *in vitro* EGFR and VEGFR-2 kinase inhibition.



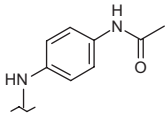
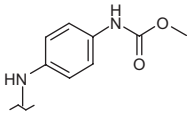
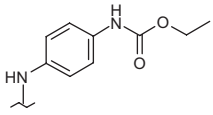
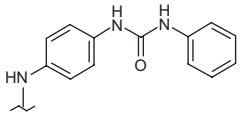
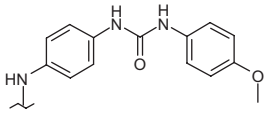
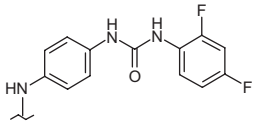
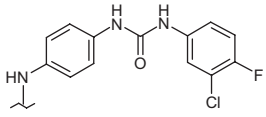
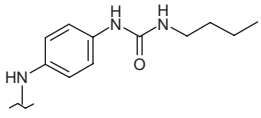
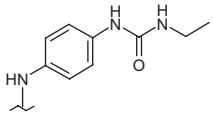
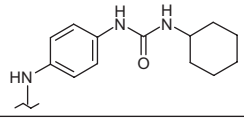
Series A
7 a-j



Series B
11 a-j



Series C
19 a-j

Compounds			% Inhibition[a] (IC_{50}, μM)[b]		
			PC3 cancer cells[c]	EGFR[d]	VEGFR-2[e]
Gefitinib		/	81.9 (*7.31*)	92.3 (*0.072*)	10.8
Semaxanib		/	35.2	16.6	80.2 (*3.60*)
R					
a		**7a**	7.2	0	1.3
		11a	13.4	31.3	14.7
		19a	26.1	0	2.3
b		**7b**	2.2	1.7	45.2
		11b	15.9	72.4 (*6.87*)	79.8 (*5.79*)
		19b	16.1	11.1	26.7
c		**7c**	10.2	9.4	34.2
		11c	22.7	47.9	81.2 (*5.62*)
		19c	21.2	18.6	29.2
d		**7d**	0	9.1	44.1
		11d	78.1 (*8.80*)	28.7	77.8 (*5.12*)
		19d	19.1	4.3	26.4
e		**7e**	30.2	17.1	33.2
		11e	29.1	34.5	75.6 (6.18)
		19e	28.7	21.5	28.3
f		**7f**	27.9	8.3	30.7
		11f	69.1 (*9.45*)	2.1	22.2
		19f	31.2	1.7	20.9
g		**7g**	10.4	43.2	35.4
		11g	33.2	15.2	81.9 (*4.31*)
		19g	27.8	29.2	29.7
h		**7h**	17.4	5.2	72.1 (*6.62*)
		11h	28.2	30.6	80.3 (*4.71*)
		19h	0.8	3.4	24.5
i		**7i**	17.3	29.1	40.3
		11i	0	36.3	78.3 (*5.82*)
		19i	0	0	19.2
j		**7j**	11.3	28.2	1.9
		11j	35.1	51.3 (*9.81*)	30.4
		19j	6.2	32.3	8.2

[a]Compounds tested at a concentration of 10 μM.
[b]Values correspond to $n = 3$ (SD <10%).
[c]Cell proliferation was realized by MTT assay at 10 μM from at least three independent determinations.
[d]Inhibition of EGFR (purified from human carcinoma A431 cells) tyrosine kinase activity.
[e]Inhibition of VEGFR-2 (recombinant human protein) tyrosine kinase activity.

at 1 μM). The quinazoline core employed is not a favorable structure to provide a potent inhibitory activity on Aurora-A, caused by the conformational restriction.

Conclusion

Three series of new quinazolines, differentiating ether linkers at the 6- and 7-positions of the core, incorporating a donor/acceptor group such as acetamide, carbamic acid ester, or urea substituted on an arylamino moiety, have been designed using structure–activity relationships. Suppression of the basic side chain on the quinazoline core confers an increase of cellular and enzymatic inhibitory activity. Our results show that some derivates of series B (6,7-dimethoxyquinazoline) have highly selective activity on EGFR and VEGFR-2 compared to series A and C. The introduction of a urea group was demonstrated by the increase of VEGFR-2 enzymatic activity but not EGFR. Also, replacement of the urea entity by a carbamic acid methyl ester group such as in **11b** presented a dual EGFR/VEGFR-2 activity. According to the investigations, substitution by halogens on the middle phenyl group of carbamic acid ester and urea derivatives (**b–j**) appears to be an opportunity to develop a new dual inhibitor for EGFR/VEGFR-2. This suggests that this type of compound could bind in the ATP pocket of these two enzymes with better affinity leading to a synergic growth inhibition of the tumors.

Declaration of interest

The authors are grateful to the “Ligue Contre le Cancer” for its financial support.

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