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Synthesis and biological evaluation of di-aryl urea derivatives as c-Kit inhibitors

Séverine Ravez [a], Stéphane Arsenlis [a,b], Amélie Barczyk [a], Anthony Dupont [a], Raphaël Frédérick [c], Stéphanie Hesse [d], Gilbert Kirsch [d], Patrick Depreux [a,b], Laurence Goossens [a,b,*]

[a] Univ. Lille, Institut de Chimie Pharmaceutique Albert Lespagnol—ICPAL, 3, rue du Professeur Laguesse, F-59006 Lille, France
[b] Univ. Lille, CHU Lille, EA 7365—GRITA—Groupe de Recherche sur les formes Injectables et les Technologies Associées, FED 4260, F-59000 Lille, France
[c] Université Catholique de Louvain, Medicinal Chemistry Research Group (CMFA), B1.73.10, Louvain Drug Research Institute (LDRI), B-1200 Bruxelles, Belgium
[d] Université de Lorraine, UMR CNRS 7565, Structure et Réactivité des Systèmes Moléculaires Complexes—Equipe 3 (HECRIN), F-57070 Metz Technopôle, France

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ABSTRACT

Inhibition of receptor tyrosine kinases (RTKs) continued to be a successful approach for the treatment of many types of human cancers and many potent small molecules kinase inhibitors have been discovered the last decade. In the present study, we describe the synthesis of thienopyrimidine derivatives and their pharmacological evaluation against nine kinases (EGFR, PDGFR-ß, c-Kit, c-Met, Src, Raf, VEGFR-1, -2 and -3). Most of the synthesized compounds showed from moderate to potent activities against c-Kit with IC_{50} values in the nanomolar range. Among them, 4-anilino(urea)thienopyrimidine analogs showed selectivity and potent c-Kit inhibition with IC_{50} values less than 6 nM. Docking simulation was performed for the most promising compound **9** into the c-Kit active site to determine the potential binding mode. This study reveal that the 4-anilino(urea)thienopyrimidine is an interesting scaffold to design novel potent and selective c-Kit inhibitors which may make promising candidates for cancers where c-Kit receptors are overexpressed.

1. Introduction

Protein kinases are critical components of cellular signal transduction cascades. They form one of the most important target classes in drug development, as they are directly involved in many diseases, including cancer. Therefore, small molecule kinase inhibitors have been successfully introduced to the drug market as selective anticancer agents with few side effects.[1]

The c-Kit receptor (also known as CD117) was found to be a subclass III tyrosine kinase receptor (RTK) and its constitutive activation via somatic mutations occurs in several cancers. The c-Kit mutations allow ligand-independent activation of this receptor and the constitutive downstream activation of the mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling cascades. Aberrant expression of c-Kit signaling primarily results mostly in leukemia, tumors of gastrointestinal tract and germ cells.[2,3]

Imatinib mesylate (also known as Gleevec or Glivec) is a tyrosine kinase inhibitor targeting c-Kit, Abl and platelet-derived growth factor receptors (PDGFR) (Fig. 1). It revolutionized the treatment of chronic myeloid leukemia (CML) in 2001. Thus, encouraged by its success in treating CML patients, scientists explored its effect in other cancers and it was found to produce a similar effect in several cancers where c-Kit was overexpressed.[4] For example, the treatment of c-Kit mutant GIST with imatinib mesylate produces responses in 80% of patients with over 90% of these patients remaining progression free at one year.[5] However, the major drawback with imatinib and other c-Kit TKIs is the development of resistance which is therapeutically challenging.[6] Therefore, there is an effort to develop new drugs that inhibit this receptor.

The 4-anilinoquinazoline scaffold has been broadly used as protein kinase inhibitors because quinazoline generally forms two hydrogen bonds with the kinase ATP binding site. The 4-anilinoquinazoline class of inhibitors has led to several commercial compounds.[7] Among them, the EGFR-selective inhibitor gefitinib

Abbreviations: ATP, adenosine 5′-triphosphate; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; FC, flash chromatography; GIST, gastrointestinal stromal tumor; MAPK, mitogen activated protein kinase; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; rt, room temperature; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

* Corresponding author. Tel.: +33 (0)3 20 96 47 02, fax: +33 (0)3 20 96 49 06.
E-mail address: laurence.goossens@univ-lille2.fr (L. Goossens).

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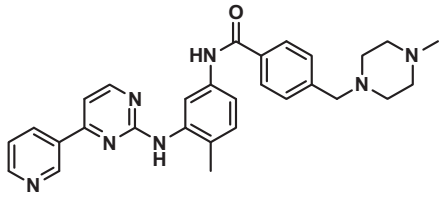


Imatinib (Gleevec, or Glivec)

IC_{50} C-Kit = 0.4 μM
IC_{50} Abl = 0.2 μM
IC_{50} PDGFR = 0.4 μM

Figure 1. Structure and IC_{50} values of imatinib.

(Iressa) and the dual EGFR/VEGFR inhibitor vandetanib (Caprelsa) exhibit potent inhibitory activities against receptors.

Inspired by the potential inhibitory ability of these compounds, quinazoline heterocycle has been chosen for further structural modification in our group. We explored the substitution by several anilino or aryloxy groups in C-4 position of the quinazoline. The anilino compounds substituted by a carbamic acid ester in *para* position show a dual EGFR/VEGFR-2 inhibition,[8] while aryloxy derivatives exhibit selectivity for VEGFR-2 versus EGFR. More precisely, aryloxy compounds substituted by a bis-aryl urea group in *para* position show potent inhibition of VEGFR, PDGFR and also of c-Kit (IC_{50} in the nanomolar range).[9–11]

To determine the importance of quinazoline scaffold in these structures, we envisaged the modulation of quinazoline by other heterocycles containing two nitrogen atoms in position 1 and 3. Indeed, we explored the modulation of 6,7-dimethoxyquinazoline scaffold by thienopyrimidines and tetrahydrobenzothienopyrimidines and we synthesized also quinazoline derivatives without methoxy groups in order to compare results between the different series (Fig. 2). Based on previous results, we designed halogen compounds, carbamic acid ester analogs and urea derivatives in each series with the idea that these molecules could show EGFR, dual EGFR/VEGFR-2, and VEGFR-2 inhibitions, respectively.

Contrary to what was expected, modulations of quinazoline scaffold have led to discover new and potent c-Kit inhibitors. Herein, we describe synthesis, in vitro enzymatic inhibition results and molecular modeling of these compounds.

2. Results and discussion

2.1. Chemistry

The synthesis of target compounds is illustrated in Scheme 1. Displacement of the chloro substituent of heterocycle with commercial or synthesized anilines in refluxing 2-propanol led to the corresponding 4-anilino derivatives. Whereas, reaction of heterocycle with appropriate phenol in presence of tetra-*N*-butylammonium bromide in 2-butanone and 20% solution of sodium hydroxide mixture allowed the access to 4-aryloxy compounds. The target compounds were obtained with yields between 4% and 99% due to their poor solubility in organic solvents which made difficult the purification step.

2.2. In vitro enzyme activity inhibition assay

Firstly, all compounds were evaluated on two tyrosine kinases: EGFR, involved in cellular proliferation and VEGFR-2 which plays a

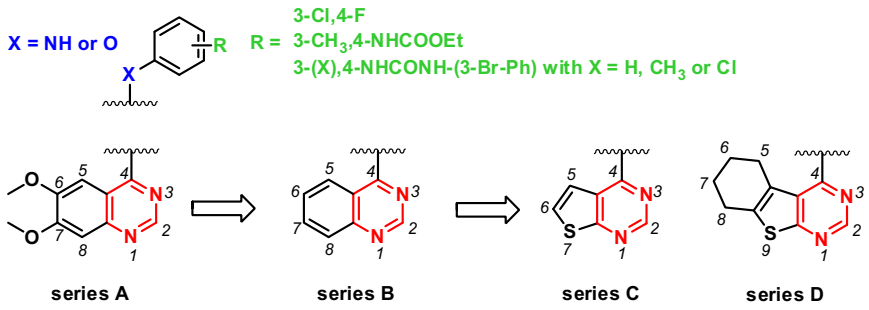
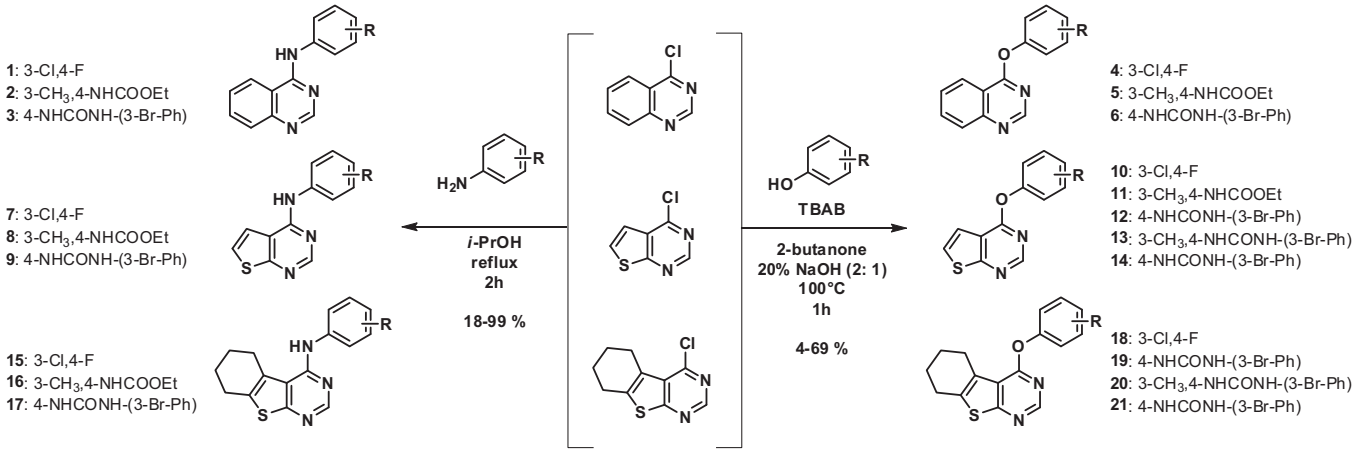


Figure 2. Structures of designed compounds.



Scheme 1. Synthetic step for the quinazoline and thienopyrimidine derivatives (**1**–**21**).

crucial role in angiogenesis process. Inhibition assay was performed by our team by measuring the levels of phosphorylation of the tyrosine-specific peptides (poly(Glu Tyr)substrate) in vitro using [γ-^{32}P]ATP (Table 1).

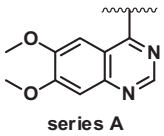
The 4-anilino derivatives substituted by halogens (**1**, **7** and **15**) show interesting affinity for EGFR and selectivity for this receptor versus VEGFR-2, like the reference compound **P1**. However, modulation of quinazoline by thienopyrimidine heterocycles decreases the enzyme inhibition potential. Unlike the dual EGFR/VEGFR-2 inhibitor **P2**, the carbamic acid esters (**2**, **8** and **16**) exhibit no or low inhibition for both receptors. Insertion of bis-aryl urea group on thienopyrimidine scaffolds led to keep inhibition of VEGFR-2 and selectivity for this kinase. Indeed, urea analogs **9** and **17** exhibit IC_{50} values of 1160 and 310 nM, respectively. These values show a decrease in activity against VEGFR-2 compared to the quinazoline compounds.

Concerning the 4-aryloxy compounds, we observe an excellent inhibitory activity and a general selectivity for VEGFR-2 versus EGFR for most of the compounds. More exactly, urea analogs selectively inhibit the VEGF receptor with IC_{50} values in nanomolar range.

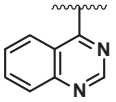
As a result of the substantial sequence homology shared by three isoforms of VEGFR and other RTKs (such as platelet-derived growth factor receptor, PDGFR and c-Kit) in their catalytic domains, VEGFR inhibitors are rarely selective for one kinase.[12] For example, sorafenib and sunitinib inhibit VEGFR-2 and also other kinases such as VEGFR-1 and -3, PDGFR and Raf... For this reason, we investigated a selectivity profile of newly synthesized urea analogs. Thus, the selected urea compounds (**3**, **6**, **9**, **12**, **17** and **19**) were tested on a panel of seven additional isolated kinase receptors including: VEGFR-1, VEGFR-3, PDGFR-ß, c-Kit, C-Met, Src and Raf. This evaluation was performed at ProQinase GmbH (Freiburg, Germany) by a radiometric protein kinase assay (^{33}PanQinase® activity assay). The inhibition data are summarized in Table 2.

Most of the compounds show no or low inhibition of EGFR, C-Met, Raf and Src. However, selected ureas and more particularly aryloxy derivatives, (**6**, **12** and **19**) exhibit higher affinities for the three isoforms of VEGFR. All compounds also inhibit the PDGFR kinase activity (IC_{50} between 320 and 2820 nM) and contrary to what was expected, they show potent inhibitions of c-Kit. Indeed, all compounds show IC_{50} values on this kinase in the nanomolar range and anilino derivatives (**3**, **9**, **17**) exhibit better results than their aryloxy analogs (**6**, **12** and **19**). Additionally, we can observe a selectivity profile for c-Kit versus the other tested kinase for aniline derivatives, and especially for compound

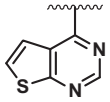
Table 1
Structures and enzymatic results (EGFR, VEGFR-2) of targeted compounds



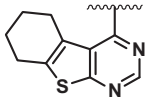
series A



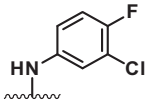
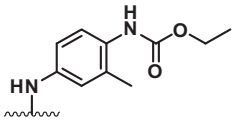
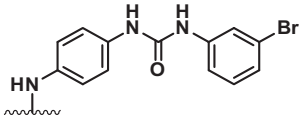
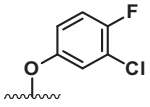
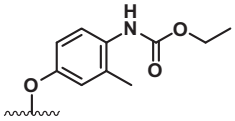
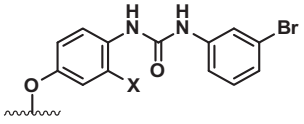
series B



series C

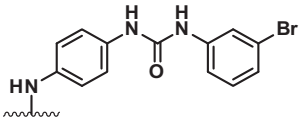
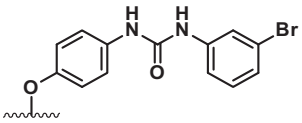


series D

	Compounds	Series	X	Enzymatic inhibition (IC_{50}, nM) EGFR[a]	VEGFR-2[b]
	P1	A	–	400	5300
	1	B	–	30	>10,000
	7	C	–	2980	>10,000
	15	D	–	1330	>10,000
	P2	A	–	900	500
	2	B	–	>10,000	3820
	8	C	–	>10,000	>10,000
	16	D	–	>10,000	3140
	P3	A	–	7710	12
	3	B	–	>10,000	91
	9	C	–	>10,000	1160
	17	D	–	3950	310
	P4	A	–	>10,000	9500
	4	B	–	590	>10,000
	10	C	–	>10,000	>10,000
	18	D	–	>10,000	3500
	P5	A	–	>10,000	600
	5	B	–	>10,000	710
	11	C	–	>10,000	1140
	P6	A	H	>10,000	6
	P7		CH_3	>10,000	4
	P8		Cl	>10,000	8
	6	B	H	>10,000	8
	12	C	H	>10,000	18
	13		CH_3	>10,000	16
	14		Cl	>10,000	9
	19	D	H	>10,000	11
	20		CH_3	3290	34
	21		Cl	1260	15

[a] Inhibition of EGFR (purified from human carcinoma A431 cells) tyrosine kinase activity.
[b] Inhibition of VEGFR-2 (recombinant protein) tyrosine kinase activity.

Table 2
Enzymatic inhibitions (EGFR, VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-ß, wt c-Kit, c-Met, Src and raf) of urea analogs

	#	Series	EGFR[a]	C-Met[c]	Raf[c]	Src[c]	VEGFR-1[c]	VEGFR-2[b]	VEGFR-3[c]	PDGFR-β[c]	wt c-Kit[c]
	P3	A	7720	N.D.	N.D.	N.D.	N.D.	12	N.D.	N.D.	3
	3	B	>10,000	>10,000	>10,000	>10,000	5750	91	47	850	1
	9	C	>10,000	>10,000	4020	>10,000	>10,000	1160	1980	2120	1
	17	D	3950	>10,000	>10,000	7640	7150	310	420	2820	6
	P6	A	>10,000	2310	930	5470	46	6	9	5	16
	6	B	>10,000	>10,000	6220	>10,000	650	8	7	320	14
	12	C	>10,000	>10,000	>10,000	>10,000	1080	18	6	570	11
	19	D	>10,000	>10,000	5840	8460	1700	11	1020	2300	17

N.D. Not determined.
[a] Inhibition of EGFR (purified from human carcinoma A431 cells) tyrosine kinase activity.
[b] Inhibition of VEGFR-2 (recombinant protein) tyrosine kinase activity.
[c] Inhibition of VEGFR-1, VEGFR-3, PDGFR-ß, wt c-Kit, c-Met, Src and Raf was performed at ProQinase GmbH (Freiburg, Germany).

9. The results of this study allow us to conclude that the thienopyrimidine heterocycle is a promising scaffold to design novel kinase inhibitors and especially potent and selective c-Kit inhibitors.

Recently, it was suggested that targeting multiple RTKs on GIST is more effective than single agent RTK therapy targeting predominantly c-Kit. Nevertheless, it was demonstrated that targeting c-Kit and EGFR (imatinib and erlotinib or afatinib) or c-Kit and C-Met (imatinib and amuvatinib) with novel combinations of RTK inhibitors abrogates imatinib resistance in GIST.[13] Hence, induction of degradation of c-Kit by chemo-agents may provide a new insight for treatment of GISTs. That is why the design of potent and selective c-Kit inhibitors, such as compound **9**, is a potential and promising therapeutic strategy for the treatment of this disease.

2.3. Docking study

In order to predict the possible binding mode of compounds inside the binding site of c-Kit kinase, compounds were docked into the ATP binding site of c-Kit kinase (PDB ID:4U0I) using GOLD® software (Fig. 3).

As depicted in Fig. 3, the 4-anilinothienopyrimidine scaffold is inserted in the adenine pocket of c-Kit ATP-binding site. The docking demonstrates that the nitrogen (N1) of the thienopyrimidine forms a hydrogen bond with the NH of the peptide bond between the Y672 and the C673. About the urea group, NH interacts with the side chain of E640. The oxygen atom forms a hydrogen bond with NH of the peptide bond between the C809 and D810. The docking structure reveals that the bis-aryl urea group is essential for the inhibition of c-Kit.

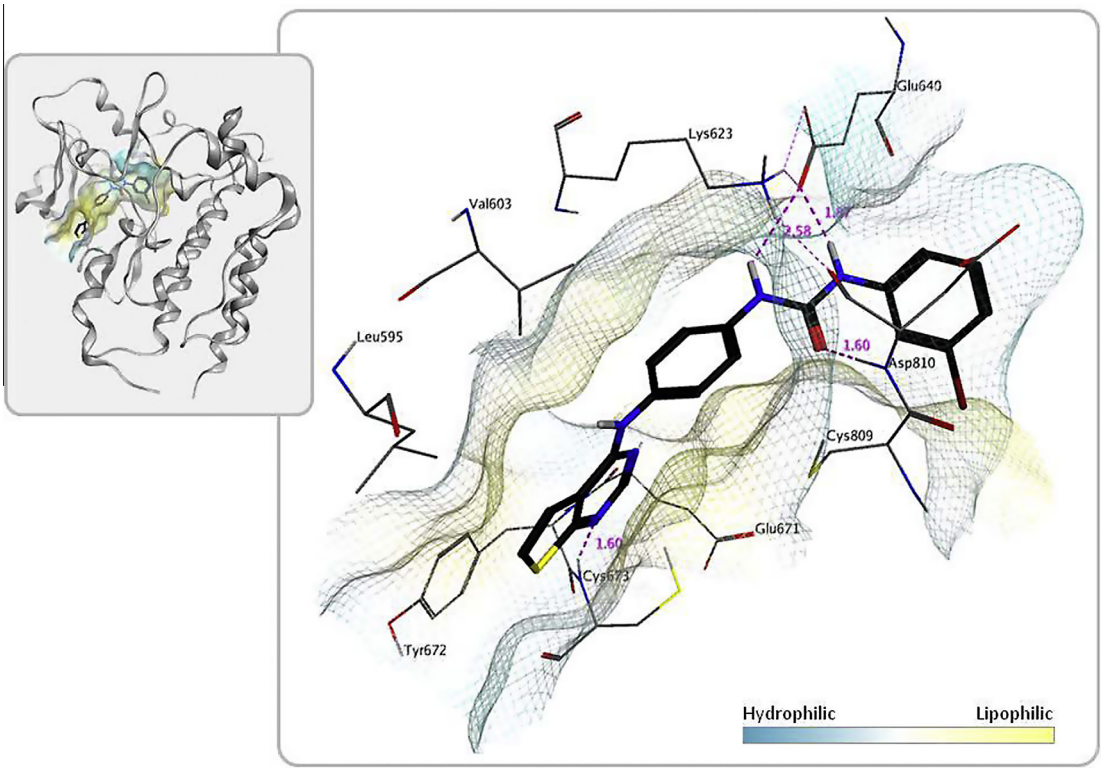


Figure 3. Docking of compound **9** in the binding site of c-Kit kinase (PDB ID: 4U0I). Pictures made using MOE software.

3. Conclusions

In summary, we have described the discovery, SAR study and preliminary biological evaluation of selective and potent c-Kit inhibitors. A small-sized library of novel thieno and tetrahydrobenzothieno-pyrimidines was synthesized and a dozen of urea compounds were identified as kinase inhibitors. More precisely, the 4-aryloxy compounds show multi-kinase inhibitions of VEGFR (1, 2 and 3), PDGFR-β and c-Kit while their anilino analogs exhibit potent and selective inhibition of c-Kit with IC_{50} values in the nanomolar range. Indeed, the most active and selective compound **9** inhibits c-Kit at nanomolar concentration (IC_{50} = 1 nM), while exhibits no significant inhibition against other tested kinase (IC_{50} >1 160 nM). This 4-anilino(urea)thienopyrimidine can be used as lead candidate for future c-Kit inhibitor development. First results on cancer cell lines (prostate PC3, breast MCF7 and colon HT29) show no or low inhibition of cellular proliferation at 10 μM. Other investigations on several cancer cell lines must be led. Thus, some modifications will be undertaken to increase DMPK properties and further in vivo pharmacological evaluations with the best candidate will be performed.

4. Experimental section

4.1. General chemistry

Melting points were determined with a Büchi 535 capillary melting point apparatus and are uncorrected. Macherey Nagel Polygram® sil G/UV254 commercial plates were used for analytical TLC as well as UV light and/or with iodine to follow the course of the reaction. Flash chromatography (FC) was performed with silica gel Macherey Nagel Si 60, 0.015–0.040 mm (Merck). The structure of each compound was confirmed by IR (Bruker VECTOR 22 instrument) and ^{1}H NMR (300 MHz, Bruker AC300P spectrometer). Chemicals shifts (δ) are reported in parts per million downfield from TMS. J values are in hertz, and the splitting patterns are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The purity of the compounds was tested by HPLC separation followed by APCI$^+$ (atmospheric pressure chemical ionization) mass spectral detection on an LC–MS system, ThermoElecton Surveyor MSQ, and was >95%. Purity of all tested compounds is superior of 98%. HRMS experiments were performed on Q Exactive Benchtop LC–MS/MS (Thermo Scientific).

4.1.1. General procedure for 4-anilino-quinazolines/thienopyrimidines

To a stirred solution of commercial chloro derivative (1 equiv) in isopropanol was added aniline (1.2 equiv). After 2 h at reflux, the precipitate was filtered, washed with isopropanol. The obtained residue was dissolved in a 10% K_2CO_3 solution and the mixture was stirred 30 min at room temperature. Then, the aqueous layer was extracted with EtOAc and the organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure.

4.1.1.1. 4-(3-Chloro-4-fluoroanilino)quinazoline (1). Compound **1** was obtained by crystallization from acetonitrile as a white solid (99%). Mp: 216–218 °C. IR (cm^{-1}): 3122 (NH).^{1}H NMR (DMSO-d_6): δ (ppm) 7.41–7.46 (ddd, 1H, J = 9.0 and 3.1 Hz, ArH), 7.61–7.65 (m, 1H, ArH), 7.77–7.85 (m, 3H, 3 ArH), 8.19–8.22 (m, 1H, ArH), 8.53–8.55 (dd, 1H, J = 8.5 and 1.3 Hz, ArH), 8.60 (d, 1H, J = 3.4 Hz, ArH), 9.95 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm). 115.60, 116.34 (d, J = 21.7 Hz), 118.58 (d, J = 18.3 Hz), 122.54 (d, J = 6.7 Hz), 123.09, 123.63, 126.13, 127.64, 132.92, 137.57, 149.65 (D, J = 224.0 Hz), 154.38, 157.60. LC–MS (APCI$^+$): m/z calcd for $C_{14}H_9ClFN_3$ 274 [(M+H)$^+$ for ^{35}Cl] and 276 [(M+H)$^+$ for ^{37}Cl]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{14}H_9ClFN_3$ 274.0541. Found 274.0535.

4.1.1.2. *N*-[2-Methyl-4-(quinazolin-4-ylamino)phenyl]carbamic acid ethyl ester (2). Compound 2 was obtained by crystallization from ethanol as a white solid (82%). Mp: 210–212 °C. IR (cm^{-1}): 3484 (NH), 1706 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.24–1.27 (t, 3H, J = 7.1 Hz, CH_3), 2.24 (s, 3H, CH_3), 4.09–4.15 (q, 2H, J = 7.1 Hz, CH_2), 7.32–7.34 (m, 1H, ArH), 7.60–7.64 (m, 3H, 3 ArH), 7.76–7.78 (m, 1H, ArH), 7.83–7.87 (m, 1H, ArH), 8.54–8.56 (m, 2H, 2 ArH), 8.81 (br s, 1H, NH), 9.77 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 14.60, 18.01, 60.08, 115.26, 120.54, 123.00, 124.37, 126.07, 127.68, 129.15, 129.38, 132.25, 132.85, 149.62, 154.60, 157.80 (C=O). LC–MS (APCI$^+$): m/z calcd for $C_{18}H_{18}N_4O_2$ 323 [(M+H)$^+$]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{18}H_{18}N_4O_2$ 323.1502. Found 323.1496.

4.1.1.3. *N*-(3-Bromophenyl)-*N'*-[4-(quinazolin-4-ylamino)phenyl]urea (3). Compound 3 was obtained by crystallization from methanol as a white solid (99%). Mp: 232–234 °C. IR (cm^{-1}): 3171 (NH), 1682 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.17–7.35 (m, 3H, 3 ArH), 7.58–7.98 (m, 8H, 8 ArH), 8.73 (m, 2H, 2 ArH), 9.38 (br s, 1H, NH), 9.52 (br s, 1H, NH), 10.64 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 114.37, 116.64, 118.09, 118.85, 120.00, 121.72, 123.72, 124.09, 124.29, 127.15, 130.70, 131.98, 134.28, 136.81, 141.60, 152.54, 153.02, 158.48 (C=O). LC–MS (APCI$^+$): m/z calcd for $C_{21}H_{16}BrN_5O$434 [(M+H)$^+$ for ^{79}Br] and 436 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{21}H_{16}BrN_5O$ 434.0611. Found 434.0598.

4.1.1.4. 4-(3-Chloro-4-fluoroanilino)thieno[2,3-d]pyrimidine (7). Compound 7 was obtained by crystallization from acetonitrile as a white solid (55%). Mp: 228–229 °C. IR (cm^{-1}): 3348 (NH). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.34–7.36 (dd, 1H, J = 9.0 and 9.0 Hz, ArH), 7.69–7.72 (m, 2H, 2 ArH), 7.77–7.79 (d, 1H, J = 6.0 Hz, ArH), 8.11–8.14 (dd, 1H, J = 6.7 and 2.4 Hz, ArH), 8.47 (s, 1H, ArH), 9.71 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 116.61, 116.82, 116.93 (J = 5.0 Hz), 118.80 (J = 20.1 Hz), 119.28, 121.38 (J = 6.9 Hz), 122.54, 123.55 (J = 5.7 Hz), 124.36, 136.57, 152.99 (J = 143.0 Hz), 166.62. LC–MS (APCI$^+$): m/z calcd for $C_{12}H_7ClFN_3S$280 [(M+H)$^+$ for ^{35}Cl] and 282 [(M+H)$^+$ for ^{37}Cl]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{12}H_7ClFN_3S$ 280.0106. Found 280.0100.

4.1.1.5. *N*-[2-Methyl-4-(thieno[2,3-d]pyrimidin-4-ylamino) phenyl]carbamic acid ethyl ester (8). Compound 8 was obtained by crystallization from ethanol as a white solid (74%). Mp: 224–226 °C. IR (cm^{-1}): 3250 (NH), 1681 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.24–1.27 (t, 3H, J = 7.0 Hz, CH_3), 2.24 (s, 3H, CH_3), 4.10–4.15 (q, 2H, J = 7.1 Hz, CH_2), 7.38–7.41(m, 1H, ArH), 7.57–7.58 (m, 2H, 2 ArH), 7.83–7.84 (d, 1H, J = 5.7 Hz, ArH), 8.10 (d, 1H, J = 5.7 Hz, ArH), 8.64 (s, 1H, ArH), 8.89 (br s, 1H, NH), 10.67 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 14.58, 17.99, 60.18, 117.47, 120.44, 121.14, 124.97, 125.50, 132.10, 133.81, 149.63, 154.45, 154.57. LC–MS (APCI$^+$): m/z calcd for $C_{16}H_{16}N_4O_2S$ 329 [(M+H)$^+$]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{16}H_{16}N_4O_2S$ 329.1066. Found 329.1057.

4.1.1.6. *N*-(3-Bromophenyl)-*N'*-[4-(thieno[2,3-d]pyrimidin-4-ylamino)phenyl]urea (9). Compound 9 was obtained by crystallization from methanol as a white solid (48%). Mp: 240–242 °C. IR (cm^{-1}): 3251 (NH), 1671 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.13–7.15 (m, 1H, ArH), 7.22–7.26 (ddd, 2H, J = 7.9 Hz, 2 ArH), 7.33–7.35 (m, 1H, ArH), 7.48–7.50 (d, 2H,

J = 8.9 Hz, 2 ArH), 7.70–7.72 (m, 3H, 3 ArH), 7.85–7.87 (d, 1H, J = 6.0 Hz, ArH), 7.89–7.90 (dd, 1H, J = 1.9 Hz, ArH), 8.46 (s, 1H, ArH), 9.01 (br s, 1H, NH), 9.14 (br s, 1H, NH), 9.62 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 116.64, 116.94, 118.66, 119.52, 120.26, 121.73, 122.34, 123.57, 124.12, 130.69, 133.45, 153.27, 154.86. LC–MS (APCI$^+$): m/z calcd for $C_{19}H_{14}BrN_5OS$440 [(M+H)$^+$ for ^{79}Br] and 442 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{19}H_{14}BrN_5OS$ 440.0175. Found 440.0163.

4.1.1.7. 4-(3-Chloro-4-fluoroanilino)-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidine (15). Compound 15 was obtained by crystallization from acetonitrile as a white solid (33%). Mp: 128–130 °C. IR (cm^{-1}): 3457 (NH). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.85 (m, 4H, 2 CH_2), 2.83 (m, 2H, 2 CH_2), 3.12 (m, 2H, CH_2), 7.40 (dd, 1H, J = 9.1 and 9.1 Hz, ArH), 7.60–7.67 (m, 1H, ArH), 7.92 (dd, 1H, J = 6.9 and 2.5 Hz, ArH), 8.22 (s, 1H, ArH), 8.41 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 21.92, 22.12, 25.05, 25.28, 116.28 (J = 21.5 Hz), 116.76, 118.61 (J = 18.6 Hz), 122.63 (J = 6.8 Hz), 123.70, 126.49, 133.28, 136.42, 151.90 (J = 257 Hz), 152.07, 165.94. LC–MS (APCI$^+$): m/z calcd for $C_{16}H_{13}ClFN_3S$ 334 [(M+H)$^+$ for ^{35}Cl] and 336 [(M+H)$^+$ for ^{37}Cl]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{16}H_{13}ClFN_3S$ 334.0575. Found 334.0566.

4.1.1.8. *N*-[2-Methyl-4-(5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-d]pyrimidin-4-ylamino)phenyl]carbamic acid ethyl ester (16). Compound 16 was obtained by crystallization from ethanol as a white solid (18%). Mp: 184–185 °C. IR (cm^{-1}): 3237 (NH), 1681 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.23–1.25 (t, 3H, J = 7.7 Hz, CH_3), 1.86 (m, 4H, 2 CH_2), 2.22 (s, 3H, CH_3), 2.85 (m, 2H, CH_2), 3.14 (m, 2H, CH_2), 4.09–4.13 (q, 2H, J = 7.7 Hz, CH_2), 7.31–7.50 (m, 3H, 3 ArH), 8.52 (s, 1H, ArH), 8.72 (br s, 1H, NH), 8.88 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 14.59, 17.90, 21.74, 21.98, 24.96, 25.23, 60.15, 116.68, 121.70, 124.89, 125.58, 127.12, 132.10, 133.91, 134.12, 134.36, 149.35, 154.48, 154.92. LC–MS (APCI$^+$): m/z calcd for $C_{20}H_{22}N_4O_2S$ 383 [(M+H)$^+$]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{20}H_{22}N_4O_2S$ 383.1536. Found 383.1515.

4.1.1.9. *N*-(3-Bromophenyl)-*N′*-[4-(5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin-4-ylamino)phenyl]urea (17). Compound 17 was obtained by crystallization from methanol as a white solid (65%). Mp: 241–243 °C. IR (cm^{-1}): 3282 (NH), 1633 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.86 (m, 4H, 2 CH_2), 2.82 (m, 4H, 2 CH_2), 3.13 (m, 2H, CH_2), 7.15 (m, 1H, ArH), 7.23 (dd, 1H, J = 7.9 and 7.9 Hz, ArH), 7.30 (m, 1H, ArH), 7.45 (d, 2H, J = 8.9 Hz, ArH), 7.58 (d, 2H, J = 8.9 Hz, ArH), 7.88 (dd, 1H, J = 1.5 and 1.5 Hz, ArH), 8.05 (s, 1H, NH), 8.33 (s, 1H, ArH), 8.72 (s, 1H, NH), 8.88(s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 21.98, 22.13, 25.03, 25.39, 116.37, 116.92, 118.54, 120.29, 121.71, 123.32, 124.20, 126.59, 130.66, 132.53, 133.48, 135.24, 141.48, 152.16, 152.37, 155.05, 165.51. LC–MS (APCI$^+$): m/z calcd for $C_{23}H_{20}BrN_5$-OS494 [(M+H)$^+$ for ^{79}Br] and 496 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{23}H_{20}BrN_5OS$ 494.0644. Found 494.0629.

4.1.2. General procedure for 4-aryloxy-quinazolines/thienopyrimidines substituted by halogen or urea

To a stirred solution of commercial chloro derivative (1 equiv) and tetrabutylammonium bromide in 10 mL of a mixture of 20% NaOH and 2-butanone (1:2) were added phenol (1 equiv). After 1 h at room temperature, the reaction was quenched by water, and then the aqueous solution was extracted with EtOAc (3 × 10 mL), washed with a solution of NaOH 1 N, and dried over $MgSO_4$. The solvent was removed under reduced pressure.

4.1.2.1. 4-(3-Chloro-4-fluoroaryloxy)quinazoline (4). Compound 4 was purified in heptane and filtered while hot as a white solid (4%). Mp: 122–124 °C.^{1}H NMR (DMSO-d_6): δ (ppm) 7.41–7.48 (m, 1H, ArH), 7.57 (dd, 1H, J = 9.0 and 9.0 Hz, ArH), 7.75–7.84 (m, 2H, ArH), 7.98–8.10 (m, 2H, 2 ArH), 8.34–8.40 (m, 1H, ArH), 8.65 (s, 1H, ArH). LC–MS (APCI$^+$): m/z calcd for $C_{14}H_8ClFN_2O$275 [(M+H)$^+$ for ^{35}Cl] and 277 [(M+H)$^+$ for ^{37}Cl]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{14}H_8ClFN_2O$275.0382. Found 275.0377.

4.1.2.2. *N*-[2-Methyl-4-(quinazolin-4-yloxy)phenyl]carbamic acid ethyl ester (5). Compound 5 was purified in methanol and filtered while hot as a white solid (31%). Mp: 198–200 °C. IR (cm^{-1}): 3171 (NH), 1713 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.25–1.28 (t, 3H, J = 6.0 Hz, CH_3), 2.25 (s, 3H, CH_3), 4.11–4.17 (q, 2H, J = 6.0 Hz, CH_2), 7.12–7.15 (dd, 1H, J = 8.7 and 2.7 Hz, ArH), 7.19 (d, 1H, J = 2.7 Hz, ArH), 7.43–7.45 (d, 1H, J = 8.7 Hz, ArH), 7.75–7.84 (m, 1H, ArH), 7.97–8.07 (m, 2H, 2 ArH), 8.34–8.40 (m, 1H, ArH), 8,74 (s, 1H, ArH), 8.93 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 14.57, 17.79, 60.22, 115.55, 119.58, 123.39, 123.52, 125.86, 127.51, 128.08, 133.63, 134.17, 134.62, 148.81, 151.08, 153.91, 154.60, 166.64 (C=O). LC–MS (APCI$^+$): m/z calcd for $C_{18}H_{17}N_3O_3$ 324 [(M+H)$^+$]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{18}H_{17}N_3O_3$ 324.1342. Found 324.1331.

4.1.2.3. *N*-(3-Bromophenyl)-*N′*-{4-[(quinazolin-4-yl)oxy]phenyl} urea (6). Compound 6 was purified in acetonitrile and filtered while hot as a white solid (12%). Mp: 248–250 °C. IR (cm^{-1}): 3266 (NH), 1645 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.23–7.25 (m, 1H, ArH), 7.33–7.39 (m, 4H, 4 ArH), 7.61–7.64 (d, 2H, J = 8.9 Hz, ArH), 7.85–7.87 (m, 1H, ArH), 7.94–7.95 (m, 1H, ArH), 7.98–8.08 (m, 2H, 2 ArH), 8.44–8.46 (m, 1H, ArH), 8.79 (s, 1H, ArH), 8.94 (br s, 1H, NH), 8.99 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 115.60, 117.05, 119.54, 120.40, 121.72, 122.35, 123.42, 124.34, 127.50, 128.06, 130.71, 134.60, 137.11, 141.40, 146.72, 151.07, 152.43, 153.93, 166.60 (C=O). LC–MS (APCI$^+$): m/z calcd for $C_{21}H_{15}BrN_4O_2$ 435 [(M+H)$^+$ for ^{79}Br] and 437 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{21}H_{15}BrN_4O_2$ 435.0451. Found 435.0432.

4.1.2.4. 4-(3-Chloro-4-fluoroaryloxy)-thieno[2,3-d]pyrimidine (10). Compound 10 was obtained by crystallization from acetonitrile as a white solid (40%). Mp: 128–130 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.40–7.44 (m, 1H, ArH), 7.54–7.59 (dd, 1H, J = 9.0 Hz, ArH), 7.67–7.68 (d, 1H, J = 6.0 Hz, ArH), 7.74–7.76 (dd, 1H, J = 6.3 and 3.0 Hz), 7.99 (d, 1H, J = 6.0 Hz, ArH), 8.66 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ (ppm) 117.39 (d, J = 23 Hz), 118.40, 118.48, 119.86 (d, J = 19 Hz), 122.90 (d, J = 7 Hz), 124.58, 127.66, 148.14, 152.81, 153.89 (D, J = 244 Hz), 162.85, 169.11. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{12}H_7ON_2ClFS$ 280.9946. Found 280.9939.

4.1.2.5. *N*-[2-Methyl-4-(thieno[2,3-d]pyrimidin-4-yloxy) phenyl]carbamic acid ethyl ester (11). Compound **11** was purified in methanol and filtered while hot as a white solid (29%). Mp: 207–209 °C. IR (cm^{-1}): 3219 (NH), 1711 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.25 (t, 3H, J = 6.0 Hz, CH_3), 2.22 (s, 3H, CH_3), 4.12 (q, 2H, J = 6.0 Hz, CH_2), 7.08 (dd, 1H, J = 8.6 and 2.5 Hz, ArH), 7.14 (d, 1H, J = 2.5 Hz, ArH), 7.39 (d, 1H, J = 8.6 Hz, ArH), 7.65 (d, 1H, J = 5.9 Hz, ArH), 7.96 (d, 1H, J = 5.9 Hz, ArH), 8,61 (s, 1H, ArH), 8.88 (s, 1H, NH). LC–MS (APCI$^+$): m/z calcd for $C_{16}H_{15}N_3O_3S$ 330 [(M+H)$^+$]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{16}H_{15}N_3O_3S$ 330.0906. Found 330.0897.

4.1.2.6. *N*-(3-Bromophenyl)-*N′*-{4-[(thieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}urea (12). Compound 12 was purified in acetonitrile and filtered while hot as a white solid (36%). Mp: 210–212 °C. IR (cm^{-1}): 3280 (NH), 1629 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.11–7.13 (m, 1H, ArH), 7.19–7.23 (m, 3H, 3 ArH), 7.38–7.40 (m, 1H, ArH), 7.58–7.60 (m, 2H, 2 ArH), 7.65–7.66

(d, 1H, J = 5.9 Hz, ArH), 7.90–7.91 (dd, 1H, J = 1.8 Hz, ArH), 7.96–7.97 (d, 1H, J = 5.9 Hz, ArH), 8.62 (s, 1H, ArH), 9.80 (br s, 2H, 2 NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 116.96, 118.51, 119.44, 120.33, 121.66, 122.13, 123.99, 127.23, 130.57, 137.53, 141.87, 146.32, 149.00, 152.75, 152.98, 163.39, 168.89 (C=O). LC–MS (APCI$^+$): m/z calcd for $C_{19}H_{13}BrN_4O_2S$441 [(M+H)$^+$ for ^{79}Br] and 443 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{19}H_{13}BrN_4O_2S$ 441.0015. Found 441.0008.

4.1.2.7. *N*-(3-Bromophenyl)-*N′*-{3-methyl-4-[(thieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}urea (13). Compound 13 was purified in methanol and filtered while hot as a white solid (57%). Mp: 211–213 °C. IR (cm^{-1}): 3280 (NH), 1630 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 2.29 (s, 3H, CH_3), 7.09–7.18 (m, 3H, 3 ArH), 7.22 (m, 1H, 2 ArH), 7.32–7.38 (m, 1H, ArH), 7.68 (m, 1H, ArH), 7.82 (d, 1H, J = 5.8 Hz, ArH), 7.90 (m, 1H, ArH), 7.98 (d, 1H, J = 5.8 Hz, ArH), 8.53 (s, 1H, ArH), 8.63 (s, 1H, NH), 9.77 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 17.98, 116.70, 118.49, 118.54, 119.40, 120.09, 121.73, 122.93, 123.37, 124.01, 127.26, 130.42, 130.68, 134.90, 141.82, 147.28, 152.82, 153.01, 163.40, 168.91. LC–MS (APCI$^+$): m/z calcd for $C_{20}H_{15}BrN_4O_2S$455 [(M+H)$^+$ for ^{79}Br] and 457 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{20}H_{15}BrN_4O_2S$ 455.0171. Found 455.0166.

4.1.2.8. *N*-(3-Bromophenyl)-*N′*-{3-chloro-4-[(thieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}urea (14). Compound 14 was purified in acetonitrile and filtered while hot as a white solid (11%). Mp: 227–229 °C. IR (cm^{-1}): 3297 (NH), 1648 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 7.15–7.22 (m, 1H, ArH), 7.22–7.27 (m, 2H, 2 ArH), 7.31 (dd, 1H, J = 9.0 and 2.5 Hz, ArH), 7.57–7.59 (d, 1H, J = 2.5 Hz, ArH), 7.67–7.70 (d, 1H, J = 5.9 Hz, ArH), 7.86–7.93 (m, 1H, ArH), 7.98 (d, 1H, J = 5.9 Hz, ArH), 8.20 (d, 1H, J = 9.0 Hz, ArH), 8.66 (s, 1H, ArH), 9.10 (br s, 2H, 2 NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 116.97, 118.45, 118.51, 120.36, 121.37, 121.80, 122.50, 122.96, 123.03, 124.57, 127.50, 130.79, 133.70, 141.20, 146.83, 152.19, 152.88, 163.07, 169.04. LC–MS (APCI$^+$): m/z calcd for $C_{19}H_{12}BrClN_4O_2S$474 [(M+H)$^+$ for ^{35}Cl/^{79}Br], 476 [(M+H)$^+$ for ^{35}Cl/^{81}Br], 476 [(M+H)$^+$ for ^{37}Cl/^{79}Br], 478 [(M+H)$^+$ for ^{37}Cl/^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{19}H_{12}BrClN_4O_2S$ 474.9625. Found 474.962.

4.1.2.9. 4-(3-Chloro-4-fluoroaryloxy)-5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidine (18). Compound 18 was obtained by crystallization from cyclohexane as a white solid (29%). Mp: 145–147 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 1.85 (m, 4H, 2 CH_2), 2.85 (m, 2H, CH_2), 3.00 (m, 2H, CH_2), 7.35–7.40 (m, 1H, ArH), 7.53 (dd, 1H, J = 9.0 and 9.0 Hz, ArH), 7.69 (dd, 1H, J = 6.1 and 2.9 Hz), 8.51 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ (ppm) 21.73, 22.34, 24.96, 25.31, 117.25, 117.48, 118.25, 119.76, 119.96, 122.84, 122.92, 124.46, 126.82, 135.94, 148.19, 148.22, 151.85, 153.75, 156.18, 162.41, 167.75. LC–MS (APCI$^+$): m/z calcd for $C_{16}H_{12}ClFN_2OS$335 [(M+H)$^+$ for ^{35}Cl] and 337 [(M+H)$^+$ for ^{37}Cl]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{16}H_{12}ClFN_2OS$ 335.0415. Found 335.0405.

4.1.2.10. *N*-(3-Bromophenyl)-*N′*-{4-[(5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]-pyrimidin-4-yl)oxy]phenyl}urea (19). Compound 19 was purified in acetonitrile and filtered while hot as a white solid (69%). Mp: 231–233 °C. IR (cm^{-1}): 3269 (NH), 1649 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.86 (m, 4H, 2 CH_2), 2.87 (m, 2H, CH_2), 3.02 (m, 2H, CH_2), 7.10–7.28 (m, 4H, 4 ArH), 7.30–7.39 (m, 1H, ArH), 7.53 (d, 2H, J = 8.8 Hz, ArH), 7.83–7.90 (m, 1H, ArH), 8.47 (s, 1H, ArH), 9.67 (s, 1H, NH), 9.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 21.78, 22.35, 24.96, 25.46, 116.88, 118.32, 119.30, 120.26, 121.65, 122.14, 123.98, 126.93, 130.58, 135.53, 137.34, 141.87, 146.44, 152.05, 152.69, 163.05, 167.51. LC–MS (APCI$^+$): m/z calcd for $C_{23}H_{19}BrN_4O_2S$495 [(M+H)$^+$ for ^{79}Br] and 497 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{23}H_{19}BrN_4O_2S$ 495.0484. Found 495.0483.

4.1.2.11. *N*-(3-Bromophenyl)-*N′*-{3-methyl-4-[(5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}urea (20). Compound 20 was purified in methanol and filtered while hot as white solid (64%). Mp: 208–210 °C. IR (cm^{-1}): 3278 (NH), 1641 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.85 (m, 4H, 2 CH_2), 2.80 (m, 2H, CH_2), 2.07 (s, 3H, CH_3), 2.99 (m, 2H, CH_2),7.11 (m, 1H, ArH), 7.14 (m, 1H, 1 ArH), 7.23 (m, 2H, 2 ArH), 7.35–7.46 (m, 1H, ArH), 7.62 (m, 1H, ArH), 7.91 (m, 1H, ArH), 8.54 (s, 1H, ArH), 8.80 (s, 1H, NH), 9.12 (s, 1H, NH). LC–MS (APCI$^+$): m/z calcd for $C_{24}H_{21}BrN_4O_2S$509 [(M+H)$^+$ for ^{79}Br] and 511 [(M+H)$^+$ for ^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{24}H_{21}BrN_4O_2S$ 509.0641. Found 509.0639.

4.1.2.12. *N*-(3-Bromophenyl)-*N′*-{3-chloro-4-[(5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}urea (21). Compound 21 was purified in methanol and filtered while hot as a white solid (13%). Mp: 239–241 °C. IR (cm^{-1}): 3289 (NH), 1617 (C=O). ^{1}H NMR (DMSO-d_6): δ (ppm) 1.85 (m, 4H, 4 ArH), 2.86 (m, 2H, 2 ArH), 3.00 (m, 2H, 2 ArH), 7.10–7.38 (m, 4H, 4 ArH), 7.45–7.62 (m, 1H, ArH), 7.80–7.97 (m, 1H, ArH), 8.09–8.12 (m, 1H, ArH), 8.42 (s, 1H, NH), 8.52 (s, 1H, ArH), 9.56 (s, 1H, NH). LC–MS (APCI$^+$): m/z calcd for $C_{23}H_{18}BrClN_4O_2S$ 528 [(M+H)$^+$ for ^{35}Cl/^{79}Br], 530 [(M+H)$^+$ for ^{35}Cl/^{81}Br], 530 [(M+H)$^+$ for ^{37}Cl/^{79}Br], 532 [(M+H)$^+$ for ^{37}Cl/^{81}Br]. HRMS (ESI (M+H)$^+$ m/z) calcd for $C_{23}H_{18}BrClN_4O_2S$ 529.0095. Found 529.0091.

4.2. In vitro kinase assays

Kinase assays were performed in 96-well plates (Multiscreen Durapore. Millipore) using [γ-^{32}P]ATP (Perkin Elmer) and the synthetic polymer poly(Glu4/Tyr) (Sigma Chemicals) as a phosphoacceptor substrate. Tested compounds were dissolved in DMSO. The final concentration of DMSO in assay solutions was 0.1%, which was shown to have no effect on kinase activity.

4.2.1. EGFR tyrosine kinase activity

20 ng of EGFR (purified from human carcinoma A431 cells, Sigma Chemicals) were incubated for 1 h at 28 °C using various concentrations of tested compounds in kinase buffer (HEPES 50 mM pH 7.5, BSA 0.1 mg/mL, $MnCl_2$ 10 mM. $MgCl_2$ 5 mM, Na_3VO_4 100 μM, DTT 0.5 mM, poly(Glu4/Tyr) 250 μg/mL, ATP 5 μM. [γ-^{32}P]ATP 0.5 μCi).

4.2.2. VEGFR-2. Tyrosine kinase activity

10 ng of VEGFR-2 (Recombinant Human Protein, Invitrogen) were incubated for 1 h at 28 °C using various concentrations of tested compounds in kinase buffer (Tris 50 mM 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, ß-glycerophosphate 5 mM, poly(Glu4/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 washed 10 times with trichloroacetic acid, 10%. Plates were counted in a Top Count (Perkin Elmer) for 1 min per well.

4.3. Molecular modeling

The compound was built using the Chembiodraw 3D module and minimized. Docking simulation was then performed into c-Kit (RCSB Protein Data Bank 4U0I) with the automated GOLD program.[14] The active site was defined including all residues in a volume of 10 Å around ponatinib taken as reference.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.10.035.

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