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Discovery of 4-aminoquinazoline—urea derivatives as Aurora kinase inhibitors with antiproliferative activity

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ABSTRACT

Two series of 20 novel 4-aminoquinazoline—urea derivatives have been designed and synthesized. The entire target compounds were investigated for their in vitro antiproliferative activity against six human cancer cell lines (K562, U937, A549, NCI-H661, HT29 and LoVo) using the MTT-based assay. Most compounds showed significant antiproliferative activities against four solid tumor cell lines, but no or poor activities against two leukemia cell lines. Furthermore, the target compounds were screened for Aurora A/B kinases inhibitory activity. Among them, **7c**, **7d**, **8c**, and **8d** are more potent against Aurora A kinase than ZM447439. Docking study of compounds **7d** and ZM447439 revealed that they bound strongly to the ATP-binding sites of Aurora A and B. Thus, they may be promising lead compounds for the development of novel anti-tumor drug potentially via inhibiting Aurora kinases.

1. Introduction

Aurora kinases are a family of three highly homologous serine/threonine kinases which play critical roles during the mitotic stage of the cell cycle.[1–3] They are overexpressed in various cancer types and implicated in many aspects of tumor development. They are expressed during the G2/M phase of the cell cycle and are critical for the proper regulation of mitosis.[4–6]

Aurora A and B have received a lot of attention to date as anticancer targets.[7] In mitosis, Aurora A associates with the spindle poles and is involved in both centrosomal and acentrosomal spindle assembly. Aurora A is highly expressed in many tumors; its upregulation may play an important role in tumor progression.[8] Aurora B regulates chromatin remodeling and phosphorylates histone H3 at Ser-10.[9] Aurora C has similar functions as Aurora B; it is highly expressed in the testis but is also present at a low level in other tissues.[10]

In recent years, the Aurora kinases have been actively pursued as anticancer targets for the discovery of new cancer chemotherapeutic drugs.[11] Considerable research efforts from both the pharmaceutical industry and academic laboratories have led to many reports of small molecule Aurora kinase inhibitors.[12–14] VX-680 (Fig. 1), the first Aurora inhibitor to enter clinical trials, is a potent inhibitor of all three Aurora kinases and generally has low affinities to other kinases.[15,16] AZD1152 (Fig. 1) is the first Aurora-B selective inhibitor to enter clinical trials. It is a dihydrogen phosphate prodrug of a pyrazoloquinazoline Aurora kinase inhibitor, and is converted rapidly to the active form in plasma.[17]

Researchers from Sunesis Pharmaceuticals reported the discovery of potent Aurora kinase inhibitor SNS-314 (Fig. 1), which exhibited a compelling preclinical profile and entered clinical trials in patients with advanced solid tumors.[18–20] SNS-314 is a pan-Aurora inhibitor with good affinity against all three isoforms.

ZM447439 (Fig. 1) was the first small molecule Aurora kinase inhibitor disclosed by AstraZeneca in 2003. It has a 4-aminoquinazoline scaffold and inhibits both Aurora A and B (IC_{50} of 110 and 130 nM, respectively) with good selectivity over other unrelated kinases (affinity for Aurora C is not reported).[21,5] In addition, ZM447439 impairs certain mitosis events, including chromosome alignment, chromosome segregation, and cytokinesis.[5,22]

The crystal structure of SNS-314 demonstrated the importance of the urea moiety, which extends deep into the selectivity pocket of the protein.[23] The urea oxygen is within close proximity of the catalytic lysine (Lys175), while the two NH groups form hydrogen bonding interactions with the catalytic glutamic acid residue

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0968-0896/

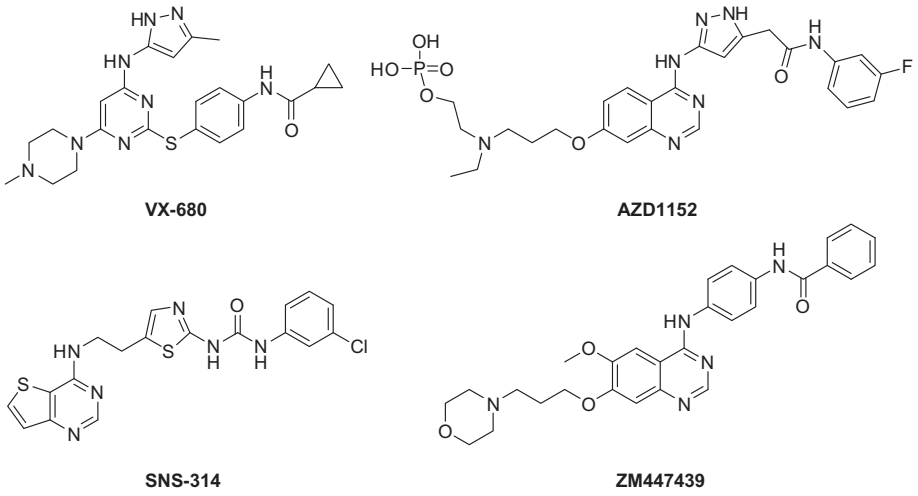


Figure 1. Chemical structures of VX-680, AZD1152, SNS-314 and ZM447439.

(Glu194). The SAR of SNS-314 and its analogues also explain the importance of the urea moiety.[24] Disruption of these hydrogen bonding interactions by replacing the urea moiety of SNS-314 with an amide linkage results in significant reduction of the inhibitory activity at enzyme as well as cellular level.[18] This indicated that the urea moiety of SNS-314 (in a specific location) is essential for its potent Aurora kinase activity.

We used ZM447439 as a lead compound for our work to develop Aurora kinase inhibitors as anticancer agents according to the SAR analysis of ZM447439 and SNS-314. On the basis of our previous work,[25,26] we have devised and synthesized novel 4-aminoquinazoline—urea derivatives (Fig. 2): (1) replacement of the amide linkage with urea moiety as an essential binding element in the DFG pocket. (2) Various secondary amino-substituted propoxy side chains at position 7 of the quinazoline nucleus. (3) Both benzyl group substituted series and cyclohexyl group substituted series were investigated. Our objective was to determine whether these compounds would manifest antiproliferative activities against cancer cells and be highly potent in inhibiting both Aurora A and B kinases.

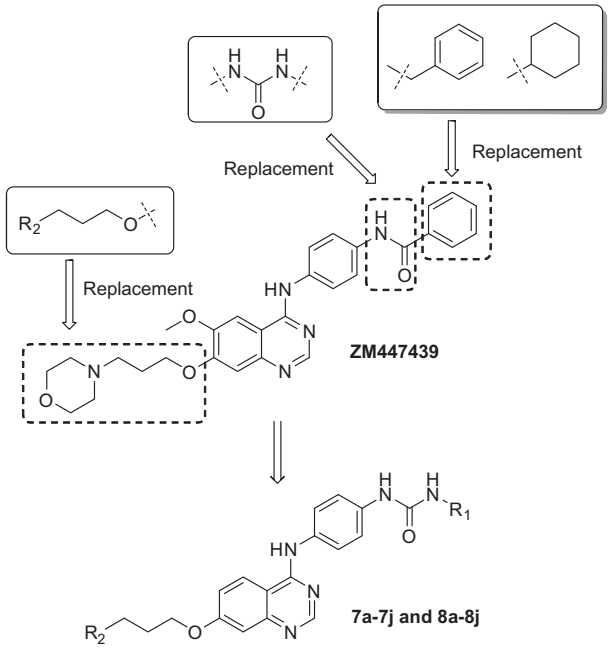


Figure 2. A design for ZM447439 derivatives.

2. Results and discussion

2.1. Chemistry

As summarized in Table 1, up to twenty compounds (**7a**–**7j** and **8a**–**8j**) were synthesized. The synthetic route was illustrated in Scheme 1.

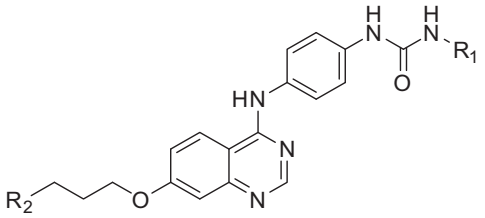
Cyclization of 2-amino-4-fluorobenzoic acid **1** with formamidine acetate in 2-methoxyethanol produced **2** in satisfactory yield. Compound **2** was then treated with propylene glycol in the presence of sodium hydride to afford **3** in 91% yield, which was chlorinated with thionyl chloride to give chlorinated compound **4** in 77% yield. Aminolysis of **4** was performed using *p*-phenylenediamine to generate 4-aminoquinazoline **5**, which was condensed with various isocyanates to yield the corresponding compounds **6a**–**6b**. Nucleophilic substitution reaction of compounds **6a**–**6b** with various secondary amines yielded the target compounds **7a**–**7j** and **8a**–**8j**.

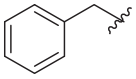
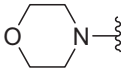
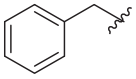
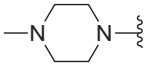
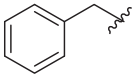
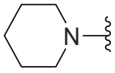
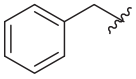
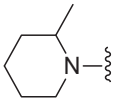
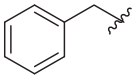
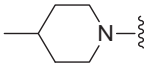
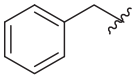
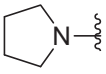
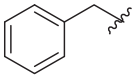
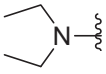
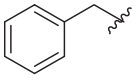
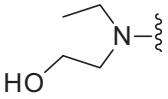
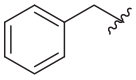
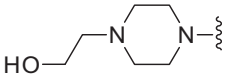
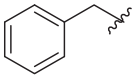
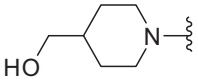
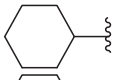
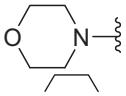
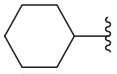
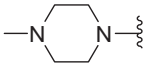
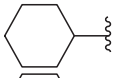
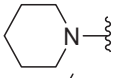
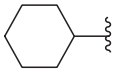
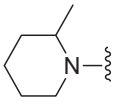
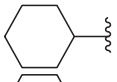
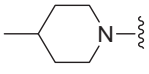
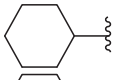
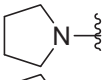
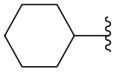
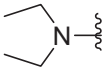
2.2. In vitro antiproliferative activity of the target compounds

Twenty newly synthesized ZM447439 derivatives (**7a**–**7j** and **8a**–**8j**) were investigated for their antiproliferative activity employing the MTT-based assay using ZM447439 as a positive control against six human cancer cell lines, representing different tumor types: human chronic myeloid leukemia cell line (K562), human acute monocytic myeloid leukemia cell line (U937), human lung cancer cell lines (A549 and NCI-H661) and human colon cancer cell lines (HT29 and LoVo). Antiproliferative activities of the compounds indicated by IC_{50} values were calculated by linear regression analysis of the concentration–response curves obtained for each compound.

The results from the antiproliferation assay are summarized in Table 2. Most compounds manifest evident antiproliferative activities against solid tumor cell lines (A549, NCI-H661, HT29 and LoVo), but poor antiproliferative activities against leukemia cell lines (K562 and U937) except for **8c** and **8d**. These results accord with our previous expectation, because Aurora kinases are known to be overexpressed in solid tumors and also may be inappropriately activated in certain cell types.[1,27–34]

Table 1
Structure of the target compounds **7a**–**7j** and **8a**–**8j**



Compd	R_1	R_2	Mp (°C)	Yields[a] (%)
7a			219–220	79
7b			104–105	64
7c			210–211	73
7d			218–219	69
7e			215–216	78
7f			209–210	61
7g			99–100	72
7h			Oil	67
7i			95–96	63
7j			119–120	70
8a			276–277	78
8b			115–116	60
8c			172–173	72
8d			179–180	66
8e			174–175	75
8f			131–132	67
8g			115–116	68

(*continued on next page*)

Table 1 (*continued*)

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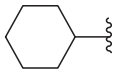
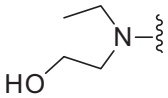
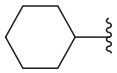
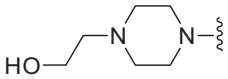
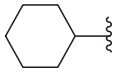
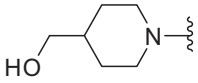
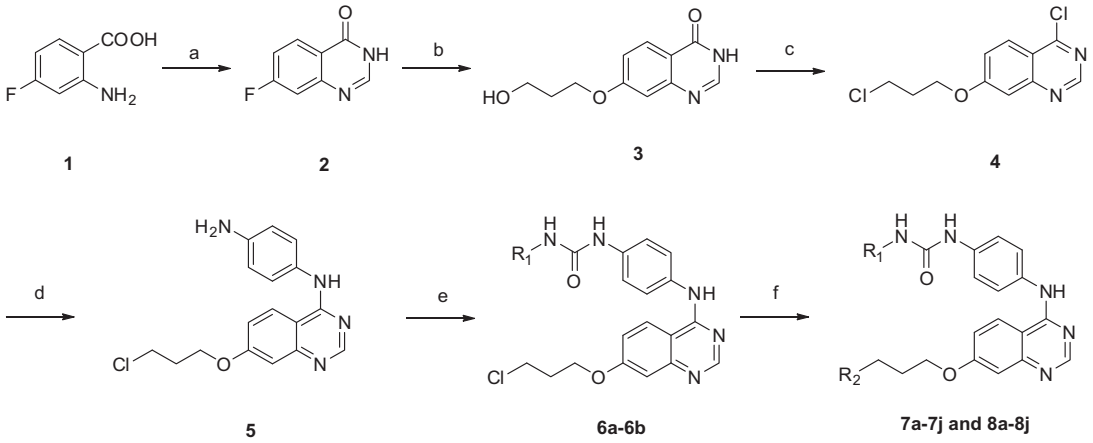
8h			102–103	63
8i			100–101	68
8j			120–121	65

[a] Yield for the last step.



Scheme 1. Synthetic route for the preparation of the target compounds **7a**–**7j** and **8a**–**8j**. Reagents and conditions: (a) formamidine acetate, 2-methoxyethanol, 10 h, reflux, 69%; (b) 1,3-propanediol, NaH, DMF, 24 h, 110 °C, 91%; (c) $SOCl_2$, DMF, 5 h, reflux, 77%; (d) *p*-phenylenediamine, isopropanol, 4 h, reflux, 82%; (e) isocyanatomethyl-benzene or isocyanatocyclohexane, 5 h, reflux, 87%/81%; (f) HNR_2, KI, 6 h, 60 °C, 60–79%.

Compared with *N*-benzyl (**7**), *N*-cyclohexyl series (**8**) compounds displayed slightly lower inhibitory activities. The terminal phenyl ring of the urea functional group enters the region commonly referred to as the 'back pocket' in kinase literature, where it forms hydrophobic interactions with the surrounding amino acid residues.[35] Removal of the terminal benzyl group away from the urea function and replacement with cyclohexyl group (**8**) led to decreased activity. These results demonstrate the importance of aromatic hydrophobic interactions in the back pocket for their inhibitory activities against cancer cells.

It is worth pointing out that the most significant inhibition was achieved for compounds **7c**, **7d**, **8c** and **8d** with IC_{50} values ranging from 0.9 to 3.4 μM toward solid tumor cells, which are similar to or better than that of ZM447439 (IC_{50} = 1.9–3.3 μM). In addition, compounds **7d**, **8c** and **8d** also showed increased potency against leukemia cell lines as compared to ZM447439. It is noticeable that compounds **7d** and **8d** with 2-methylpiperidine substitution displayed the relatively higher antiproliferative activities than other compounds in their respective series, while the piperidine substituted **7c** and **8c** showed the lower antiproliferative activities. We speculated that conformational restriction of methyl group in piperidine moiety would help to form appropriate angle by amino quinazolines ring, this was more conducive for aniline ring to reside in the ligand binding site, meanwhile the nitrogen of 2-methylpiperidine form a hydrogen bond to the hinge region, all of which would help to improve the inhibitory activity. Meanwhile, compounds **7i**, **7j** and **8i**, **8j**, which have hydroxyethyl-piperazine and hydroxymethyl-piperidine group at R2, exhibited significant decrease in the antiproliferative activities (IC_{50} values ranging from 13.5 to 53.6 μM toward solid tumor cells), which might be induced by the electro-negativity of the oxygen atoms by our conjecture. As the antiproliferation assay was carried out in vitro, the inhibitory effect variance among these compounds could be attributed to the hydrophobicity and permeability changes resulting from different basic side chains. Besides these factors, the structural difference among these compounds in each series was the basic side chain at position 7 of quinazoline scaffold, which was reported to affect the pharmacokinetic properties and solubility of compound in vivo.[36,37] The introduction of the basic side chains was to increase the volume of distribution at steady state (V_{dss}), and thus increased the observed half-life and a long terminal half-life was desirable in terms of increasing drug exposure and ultimately efficacy.[38]

2.3. Kinase inhibitory activity of the target compounds

ZM447439 was initially designed and synthesized for Aurora A/B kinases inhibition. In order to study the mechanism of action of the new synthesized compounds, all compounds were assayed for their inhibitory activities against Aurora A/B kinases using ZM447439 as a reference compound. As shown in Table 2, the compounds manifest Aurora kinase inhibitory activities at submicromolar concentrations, and most derivatives exhibit a higher selectivity for Aurora A over Aurora B. For example, compound **7d** has a lower IC_{50} value (61 nM) for Aurora A than that for Aurora B (172 nM). In addition, the kinase inhibitory activities are

Table 2
In vitro antiproliferative activity in different cell lines and enzyme inhibition activity of target compounds **7a–7j** and **8a–8j**

Compd	Antiproliferation in different cell lines[a,b] (IC_{50} μM)						Enzyme inhibition		
	K562	U937	A549	NCI-H661	HT29	LoVo	Aurora A (IC_{50} nM)[c]	Aurora B	EGFR (%)[c,d]
7a	>100	>100	18.3	12.5	6.3	9.1	212	367	45.3
7b	40.3	>100	7.4	6.8	7.1	6.9	195	289	35.8
7c	>100	>100	2.9	2.1	1.4	1.5	78	193	38.1
7d	42.8	15.6	3.1	1.5	1.2	0.9	61	172	55.6
7e	>100	>100	6.8	5.2	2.5	3.8	219	292	35.8
7f	>100	61.3	4.3	4.2	3.7	1.8	143	278	46.7
7g	37.8	>100	5.6	4.1	2.8	3.7	128	196	29.6
7h	>100	>100	29.5	18.2	19.8	12.1	468	573	33.5
7i	>100	>100	36.9	28.2	26.7	16.3	496	621	43.6
7j	>100	>100	47.8	32.9	13.5	26.1	564	783	21.9
8a	>100	>100	32.5	24.3	7.6	8.2	193	264	37.1
8b	33.8	>100	6.3	8.2	5.9	7.1	202	318	38.3
8c	58.9	4.3	3.4	2.5	2.6	2.7	103	221	29.4
8d	23.8	3.9	2.9	3.3	1.8	1.5	82	197	36.3
8e	>100	10.6	5.5	3.9	4.1	1.8	157	232	50.2
8f	18.1	>100	3.9	1.8	2.6	5.0	115	181	61.8
8g	55.7	>100	4.1	2.9	6.3	4.8	123	176	31.9
8h	>100	>100	29.6	16.6	21.5	17.8	312	387	36.5
8i	>100	>100	53.6	28.9	31.1	26.8	436	589	32.7
8j	>100	>100	45.3	51.6	29.8	31.6	796	845	27.5
ZM447439	>100	>100	3.3	2.9	2.2	1.9	138	156	39.8
Gefitinib	NT[e]	NT	NT	NT	NT	NT	NT	NT	98.5

[a] Values are averages of three independent experiments, SD <10%.
[b] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.
[c] Values are averages of at least two independent experiments, SD <10%.
[d] Compounds tested at a concentration of 10 μM.
[e] Not tested.

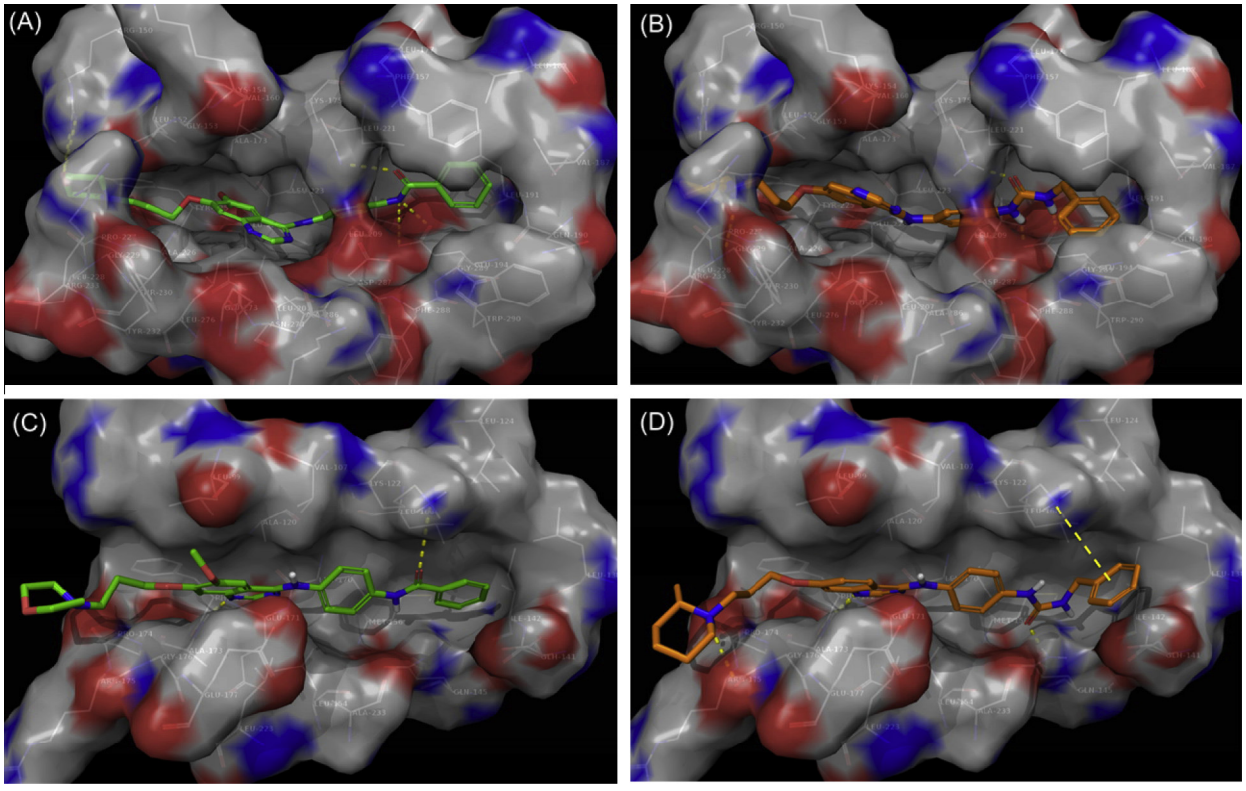


Figure 3. Docked binding modes of compounds in the ATP binding site of Aurora A and B. Interactions between the protein and the ligand are shown as yellow dotted lines. The ligands are shown in stick model, while the proteins are shown in surface model for better visualization. (A) Binding mode of ZM447439 with Aurora A (PDB ID: 3D15). (B) Binding mode of compounds **7d** with Aurora A. (C) X-ray co-crystal structure of Aurora B in complex with ZM447439 (PDB ID: 2VRX). (D) Binding mode of compounds **7d** with Aurora B.

consistent with their antiproliferative activities. Compounds **7c**, **7d**, **8c** and **8d** with stronger antiproliferative activity also show better Aurora A inhibitory activity than ZM447439. Since Gefitinib, a selective EGFR inhibitor, also contains quinazoline moiety, it was thought that the quinazoline moiety might play a role in inhibiting EGFR and it would be valuable to evaluate the EGFR inhibitory activity of our compounds. As shown in Table 2, the new compounds exhibit much lower EGFR inhibitory activities than Gefitinib, which indicates that these compounds are not specific EGFR tyrosine kinase inhibitors. Taken the biological data together, we could preliminarily arrive at the conclusion that some target compounds are potent Aurora kinase inhibitors. Thus they may be promising lead compounds for the development of novel anti-tumor drug potentially via inhibiting Aurora kinases.

2.4. Docking simulation study

To understand the structural basis of compounds **7d** and ZM447439 for the differential selectivity profiles, we performed docking simulations to model the possible binding modes using Glide in Schrodinger Suite from Schrodinger LLC. Following ligand preparation, the compounds were docked to the ATP binding pocket of Aurora A and Aurora B as shown in Figure 3. The docking simulation suggested that **7d** and ZM447439 might have a similar binding mode in Aurora A (Fig. 3A and B) (PDB ID: 3D15).[18] Compound **7d** binds to the Aurora A ATP binding pocket with the bi-aryl urea core forming several essential hydrogen bonds. The urea carbonyl oxygen forms a hydrogen bonding interaction with Lys175 and the two NH groups form a bidentate hydrogen bonding interaction with Glu194. In addition to these hydrogen bonds, the nitrogen of the piperidine moiety is within close proximity to Gly229 for a coulombic interaction. The terminal benzyl group of the urea functional group occupies back pocket, where it forms hydrophobic interactions with Phe157, Leu182, Val187, Leu191, Gly289, and Trp290.

The X-ray crystal structure of the complex of Aurora B with ZM447439 was shown in Figure 3C (PDB ID: 2VRX).[39] 4-Aminoaniline-quinazoline moiety occupies the pocket made up of Leu99, Val107, Ala120, Lys122, Gln145, Leu154, Leu170, Glu171, Phe172, Ala173, Gly176, Glu177, Leu223, and Ala233 and a hydrogen bonding interaction is formed between Ala173 and N1 of this moiety (underlined amino acid residues interact with the 4-aminoaniline moiety). Benzoylamide forms a hydrogen bonding interaction with Lys122, a coulombic interaction with Gln145, and hydrophobic interaction with Leu124, Leu138, Ile142, Glu141, Met156, Leu168, and Leu170. Methoxy of the quinazoline moiety forms a coulombic interaction with Glu177. The mopholinopropoxy moiety is exposed to the solvent and Ala173, Pro174, Arg175, and Gly176 are in proximity (4 Å) to this moiety. When compared to the crystal structure of ZM447439 with Aurora B, **7d** forms a slightly different interaction with the binding site in the docking simulation (Fig. 3D). Compound **7d** binds to the Aurora B in the catalytic cleft and forms a hydrogen bonding interaction between the quinazoline moiety N1 and the NH group of Ala173 in the hinge region. The bi-aryl urea moiety extends into the pocket and the oxygen of the urea moiety forms a hydrogen bond with Gln145. An additional hydrogen bonding interaction is found for the nitrogen of piperidine with Arg175. Significantly, besides hydrophobic interaction, **7d** forms a pi–cation interaction between Lys122 and the aromatic moiety of the benzyl group.

3. Conclusion

In our study, using ZM447439 as a lead compound, two series of 20 novel 4-aminoquinazoline—urea derivatives have been designed and synthesized. The entire target compounds were investigated for their in vitro antiproliferative activity using the MTT-based assay against six human cancer cell lines (K562, U937, A549, NCI-H661, HT29 and LoVo). Most compounds showed significant antiproliferative activities against four solid tumor cell lines, but no or poor activities against two leukemia cell lines. Furthermore, the target compounds were screened for Aurora A/B kinases inhibitory activity. Among them, **7c**, **7d**, **8c**, and **8d** are more potent against Aurora A kinase than ZM447439. Docking study performed with selected compounds **7d** and ZM447439 revealed that the compounds bound strongly to the ATP-binding sites of Aurora A and B. The results suggest that they may be promising lead compounds for the development of novel anti-tumor drug potentially via inhibiting Aurora kinases.

4. Experimental protocols

4.1. Synthesis

All reagents were purchased from commercial sources and used without further purification. Melting points were measured on an RY-1 hot-stage microscope, and the thermometer was uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker-ACF 300/500 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), used as an internal standard. Mass spectra (MS) were obtained from Agilent 1100 LC/MS Spectrometry Services. IR spectra were obtained using on FT-IR Spectrometer (Perkin–Elmer). Elementary analyses were performed on Elementar Vario EL III instrument. All compounds were routinely checked by TLC with silica gel GF-254 glass plates and viewed under UV light at 254 nm.

4.1.1. 7-Fluoroquinazolin-4(3*H*)-one (2)

A solution of 2-amino-4-fluorobenzoic acid **1** (31.5 g, 0.20 mol) and formamidine acetate (42.5 g, 0.36 mol) in 2-methoxyethanol (150 mL) was heated and refluxed for 10 h. The solvent was then removed under reduced pressure and the residue washed with ammonia water (0.01 M), and then dried to afford **2** (23.1 g, 69%) as beige solid, mp: 237–239 °C (lit.[40] mp: 234 °C).

4.1.2. 7-(3-Hydroxypropoxy)quinazolin-4(3*H*)-one (3)

1,3-Propanediol (55.6 g, 0.73 mol) was added dropwise at 0–5 °C to a solution of NaH (29.2 g, 1.22 mol) in DMF (100 mL), the solution was stirred and heated at 55 °C for 1 h. Then to this solution **2** (20 g, 0.122 mol) was added and the mixture was heated at 110 °C for 24 h. After DMF was evaporated under reduced pressure, ice water (300 mL) was added and the pH adjusted to 6 with acetic acid. The precipitate obtained was filtered, washed with water and dried to yield **3** (24.4 g, 91%) as beige solid, mp: 190–191 °C. ^{1}H NMR ($CDCl_3$-d_6, 300 MHz) δ (ppm): 2.33–2.40 (m, 2H, OH–CH_2–CH_2–CH_2–O–), 3.61 (m, 2H, OH–CH_2–CH_2–CH_2–O–), 3.68 (br, 1H, –OH), 4.47 (t, J = 6.0 Hz, 2H, OH–CH_2–CH_2–CH_2–O–), 7.29 (d, J = 2.5 Hz, 1H, Ar-H), 7.51 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H, Ar-H), 7.82 (br, 1H, –NH), 8.38 (d, J = 9.0 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H); ESI-MS m/z: 221.1 $[M+H]^+$.

4.1.3. 4-Chloro-7-(3-chloropropoxy)quinazoline (4)

Compound **3** (20 g, 0.091 mol) was added to thionyl chloride (150 mL) with magnetic stirring. DMF (15 mL) was then added dropwise and the mixture was heated to reflux for 5 h. Excess thionyl chloride was then removed under reduced pressure and the residue was dissolved in dichloromethane (200 mL), washed with a saturated solution of sodium carbonate (3 × 100 mL) and water (2 × 100 mL), and dried with Na_2SO_4. Dichloromethane was then removed under reduced pressure to give a crude product, which

was washed with ethyl ether and dried to give **4** (17.9 g, 77%) as light yellow powder, mp: 74–76 °C. ^{1}H NMR ($CDCl_3$-d_6, 300 MHz) δ (ppm): 2.31–2.39 (m, 2H, Cl–CH_2–CH_2–CH_2–O–), 3.79 (t, J = 6.3 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 4.34 (t, J = 5.8 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 7.35 (dd, J_1 = 9.1 Hz, J_2 = 2.4 Hz, 1H, Ar-H), 7.45 (d, J = 2.4 Hz, 1H, Ar-H), 8.18 (d, J = 9.2 Hz, 1H, Ar-H), 8.97 (s, 1H, Ar-H); ESI-MS m/z: 257.0 $[M+H]^+$.

4.1.4. N^1-(7-(3-Chloropropoxy)quinazolin-4-yl)benzene-1,4-diamine (5)

A mixture of compound **4** (18 g, 0.07 mol) and *p*-phenylenediamine (7.65 g, 0.07 mol) in isopropanol (200 mL) was stirred at reflux for 4 h. The mixture was cooled to room temperature, the precipitate obtained was filtered and dried to yield **5** (18.9 g, 82%) as orange-yellow solid, mp: 232–234 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.25–2.29 (m, 2H, Cl–CH_2–CH_2–CH_2–O–), 3.85 (t, J = 6.4 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 4.30 (t, J = 5.9 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 6.76 (d, J = 8.6 Hz, 2H, Ar-H), 7.31–7.46 (m, 4H, Ar-H), 8.69 (d, J = 9.2 Hz, 1H, Ar-H), 8.74 (s, 1H, Ar-H), 11.10 (s, 1H, –NH–); ESI-MS m/z: 329.2 $[M+H]^+$.

4.1.5. 1-Benzyl-3-(4-((7-(3-chloropropoxy)quinazolin-4-yl)amino)phenyl)urea (6a)

Compound **5** (9 g, 0.0274 mol) was added to toluene (150 mL) at room temperature. The solution of isocyanatomethyl-benzene (3.6 g, 0.0274 mol) in toluene (40 mL) was the added dropwise and the mixture was heated at reflux for 5 h. When cooled to room temperature, the precipitate obtained was filtered and dried to yield **6a** (11 g, 87%) as bright yellow solid, mp: 246–248 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26–2.30 (m, 2H, Cl–CH_2–CH_2–CH_2–O–), 3.86 (t, J = 6.3 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 4.30–4.33 (m, 4H, Cl–CH_2–CH_2–CH_2–O– and Ar–CH_2–), 6.76 (t, J = 6.0 Hz, 1H, –NH–), 7.23–7.53 (m, 11H, Ar-H), 8.73 (d, J = 9.3 Hz, 1H, Ar-H), 8.83 (s, 1H, Ar-H), 9.06 (s, 1H, –NH), 11.32 (s, 1H, –NH–); ESI-MS m/z: 462.1 $[M+H]^+$.

4.1.6. 1-(4-((7-(3-Chloropropoxy)quinazolin-4-yl)amino)-phenyl)-3-cyclohexylurea (6b)

Preparation of **6b** is followed the procedure for **6a** started from isocyanatocyclohexane. Bright yellow solid, yield: 81%, mp: 232–234 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.23–1.82 (m, 10H, cyclohexane–H), 2.25–2.30 (m, 2H, Cl–CH_2–CH_2–CH_2–O–), 3.47–3.48 (m, 1H, cyclohexane–H), 3.85 (t, J = 6.4 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 4.32 (t, J = 5.8 Hz, 2H, Cl–CH_2–CH_2–CH_2–O–), 6.21 (d, J = 7.8 Hz, 1H, –NH–), 7.15 (s, 1H, Ar-H), 7.26 (d, J = 9.3 Hz, 1H, Ar-H), 7.46–7.52 (m, 4H, Ar-H), 8.60 (s, 1H, Ar-H), 8.66 (d, J = 9.3 Hz, 1H, Ar-H), 8.80 (s, 1H, –NH–), 11.14 (s, 1H, –NH–); ESI-MS m/z: 454.3 $[M+H]^+$.

4.1.7. 1-Benzyl-3-(4-((7-(3-morpholinopropoxy)quinazolin-4-yl)amino)phenyl)urea (7a)

Compound **6a** (2 g, 4.34 mmol) and KI (0.1 g, 0.6 mmol) were added to morpholine (30 mL). The solution was stirred at 60 °C for 6 h; then slowly poured into ice water, the precipitate was collected by filtration. Flash chromatography was performed with CH_2Cl_2/CH_3OH (40:1, v/v) to afford **7a** (1.75 g, 79%) as deep yellow solid, mp: 219–220 °C. IR (KBr, cm^{-1}): 3311, 2954, 1620, 1578, 1514, 1458, 1417, 1333, 1309, 1228, 1117, 862, 700; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.91–1.97 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.38–2.50 (m, 6H, N–CH_2–), 3.57–3.61 (m, 4H, O–CH_2–), 4.18 (t, J = 6.5 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31 (d, J = 5.8 Hz, 2H, Ar–CH_2–), 6.57–6.59 (m, 1H, –NH–), 7.14–7.42 (m, 9H, Ar-H), 7.63 (d, J = 8.9 Hz, 2H, Ar-H), 8.41 (d, J = 9.3 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.54 (s, 1H, –NH–), 9.55 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 26.32, 43.73, 56.02, 58.15, 68.38, 69.05, 105.14, 110.25, 117.39, 118.86, 120.35, 123.21, 126.84, 126.98, 127.15, 128.26, 128.38, 130.22, 136.45, 139.40, 151.47, 153.65, 155.80, 157.19, 163.79; ESI-MS m/z: 513.2 $[M+H]^+$; Anal. Calcd for $C_{29}H_{32}N_6O_3$ (%): C, 67.95; H, 6.29; N, 16.39. Found: C, 67.69; H, 6.32; N, 16.18.

4.1.8. 1-Benzyl-3-(4-((7-(3-(4-methylpiperazin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (7b)

Preparation of **7b** is followed the procedure for **7a**. Beige solid, yield: 64%, mp: 104–105 °C. IR (KBr, cm^{-1}): 3331, 2943, 2808, 1681, 1620, 1587, 1558, 1513, 1457, 1419, 1336, 1313, 1224, 1133, 1003, 843; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.90–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.16 (s, 3H, N–CH_3), 2.33–2.50 (m, 10H, N–CH_2–), 4.17 (t, J = 6.5 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31 (d, J = 5.5 Hz, 2H, Ar–CH_2–), 6.58 (t, J = 6.0 Hz, 1H, –NH–), 7.12–7.41 (m, 9H, Ar-H), 7.64 (d, J = 8.5 Hz, 2H, Ar-H), 8.41 (d, J = 9.0 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.52 (s, 1H, –NH–), 9.53 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 26.29, 43.71, 46.63, 55.98, 57.23, 58.21, 69.10, 105.08, 110.19, 117.38, 118.71, 120.32, 123.18, 126.82, 127.02, 127.09, 128.25, 128.34, 130.26, 136.44, 139.38, 151.50, 153.63, 155.82, 157.21, 163.80; ESI-MS m/z: 526.3 $[M+H]^+$; Anal. Calcd for $C_{30}H_{35}N_7O_2$ (%): C, 68.55; H, 6.71; N, 18.65. Found: C, 68.63; H, 6.49; N, 18.48.

4.1.9. 1-Benzyl-3-(4-((7-(3-(piperidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (7c)

Preparation of **7c** is followed the procedure for **7a**. Beige solid, yield: 73%, mp: 210–211 °C. IR (KBr, cm^{-1}): 3331, 2943, 2808, 1682, 1620, 1558, 1511, 1458, 1419, 1335, 1227, 1132, 1047, 918, 841; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.38–1.40 (m, 2H, piperidine–H), 1.49–1.51 (m, 4H, piperidine–H), 1.90–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.35–2.50 (m, 6H, N–CH_2–), 4.17 (t, J = 6.4 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31 (d, J = 5.9 Hz, 2H, Ar–CH_2–), 6.56 (t, J = 6.0 Hz, 1H, –NH–), 7.12–7.42 (m, 9H, Ar-H), 7.64 (d, J = 8.8 Hz, 2H, Ar-H), 8.41 (d, J = 9.3 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.52 (s, 1H, –NH–), 9.53 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.19, 25.53, 26.20, 43.73, 54.07, 54.15, 57.18, 69.12, 105.06, 110.42, 117.48, 118.76, 120.33, 123.29, 126.76, 126.93, 127.18, 128.25, 128.40, 130.28, 136.51, 139.42, 151.46, 153.68, 155.81, 157.23, 163.83; ESI-MS m/z: 511.3 $[M+H]^+$; Anal. Calcd for $C_{30}H_{34}N_6O_2$ (%): C, 70.56; H, 6.71; N, 16.46. Found: C, 70.58; H, 6.67; N, 16.35.

4.1.10. 1-Benzyl-3-(4-((7-(3-(2-methylpiperidin-1-yl)propoxy)-quinazolin-4-yl)amino)phenyl)urea (7d)

Preparation of **7d** is followed the procedure for **7a**. Beige solid, yield: 69%, mp: 218–219 °C. IR (KBr, cm^{-1}): 3329, 2938, 2807, 1685, 1621, 1559, 1456, 1423, 1336, 1228, 1130, 1047, 915, 836; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.01 (d, J = 6.1 Hz, 3H, piperidine–CH_3), 1.19–1.25 (m, 2H, piperidine–H), 1.43–1.58 (m, 4H, piperidine–H), 1.91–1.96 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.29–2.51 (m, 5H, N–CH_2– and N–CH–), 4.19 (t, J = 6.5 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.30 (d, J = 6.0 Hz, 2H, Ar–CH_2–), 6.55 (t, J = 6.0 Hz, 1H, –NH–), 7.11–7.43 (m, 9H, Ar-H), 7.65 (d, J = 8.7 Hz, 2H, Ar-H), 8.42 (d, J = 9.2 Hz, 1H, Ar-H), 8.46 (s, 1H, Ar-H), 8.51 (s, 1H, –NH–), 9.55 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 17.87, 23.25, 25.69, 26.31, 34.36, 43.68, 50.92, 56.16, 58.15, 69.01, 105.23, 110.34, 117.65, 118.93, 120.26, 123.12, 126.78, 127.04, 127.10, 128.36, 128.43, 130.23, 136.47, 139.34, 151.49, 153.65, 155.78, 157.18, 163.82; ESI-MS m/z: 525.2 $[M+H]^+$; Anal. Calcd for $C_{31}H_{36}N_6O_2$ (%): C, 70.97; H, 6.92; N, 16.02. Found: C, 70.61; H, 6.82; N, 16.08.

4.1.11. 1-Benzyl-3-(4-((7-(3-(4-methylpiperidin-1-yl)propoxy)-quinazolin-4-yl)amino)phenyl)urea (7e)

Preparation of **7e** is followed the procedure for **7a**. Beige solid, yield: 78%, mp: 215–216 °C. IR (KBr, cm^{-1}): 3335, 2928, 2809,

1688, 1625, 1561, 1457, 1420, 1334, 1226, 1128, 1045, 916, 832; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 0.90 (d, J = 6.3 Hz, 3H, piperidine–CH_3), 1.13–1.22 (m, 2H, piperidine–H), 1.27–1.35 (m, 1H, piperidine–H), 1.52–1.63 (m, 2H, piperidine–H), 1.91–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.19–2.63 (m, 6H, N–CH_2–), 4.25 (t, J = 6.4 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31 (d, J = 6.0 Hz, 2H, Ar–CH_2–), 6.55 (t, J = 6.0 Hz, 1H, –NH–), 7.11–7.43 (m, 9H, Ar-H), 7.65 (d, J = 8.8 Hz, 2H, Ar-H), 8.43 (d, J = 9.1 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.52 (s, 1H, –NH–), 9.56 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.18, 25.02, 30.33, 33.96, 43.76, 53.47, 54.51, 56.12, 68.92, 105.32, 110.29, 117.58, 118.87, 120.19, 123.08, 126.65, 127.03, 127.21, 128.35, 128.49, 130.19, 136.44, 139.41, 151.52, 153.67, 155.74, 157.16, 163.86; ESI-MS m/z: 525.2 $[M+H]^+$; Anal. Calcd for $C_{31}H_{36}N_6O_2$ (%): C, 70.97; H, 6.92; N, 16.02. Found: C, 70.86; H, 6.85; N, 15.91.

4.1.12. 1-Benzyl-3-(4-((7-(3-(pyrrolidin-1-yl)propoxy)-quinazolin-4-yl)amino)phenyl)urea (7f)

Preparation of **7f** is followed the procedure for **7a**. Gray solid, yield: 61%, mp: 209–210 °C. IR (KBr, cm^{-1}): 3377, 2956, 2818, 1676, 1616, 1558, 1513, 1456, 1419, 1335, 1301, 1226, 1132, 845; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.69 (quint, J = 3.0 Hz, 4H, pyrrolidine–H), 1.96 (q, J = 6.5 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 2.47–2.51 (m, 4H, N–CH_2–), 2.58 (t, J = 7.0 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.18 (t, J = 6.5 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31 (d, J = 6.0 Hz, 2H, Ar–CH_2–), 6.57 (t, J = 6.0 Hz, 1H, –NH–), 7.12–7.42 (m, 9H, Ar-H), 7.63 (d, J = 8.6 Hz, 2H, Ar-H), 8.41 (d, J = 9.0 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.52 (s, 1H, –NH–), 9.52 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 19.02, 23.56, 27.10, 43.83, 54.05, 54.16, 57.29, 69.18, 105.07, 110.43, 117.50, 118.73, 120.32, 123.26, 126.74, 126.91, 127.15, 128.26, 128.41, 130.26, 136.53, 139.44, 151.43, 153.69, 155.82, 157.25, 163.85; ESI-MS m/z: 497.3 $[M+H]^+$; Anal. Calcd for $C_{29}H_{32}N_6O_2$ (%): C, 70.14; H, 6.49; N, 16.92. Found: C, 70.16; H, 6.35; N, 16.81.

4.1.13. 1-Benzyl-3-(4-((7-(3-(diethylamino)propoxy)quinazolin-4-yl)amino)phenyl)urea (7g)

Preparation of **7g** is followed the procedure for **7a**. Beige solid, yield: 72%, mp: 99–100 °C. IR (KBr, cm^{-1}): 3313, 2968, 1659, 1618, 1579, 1560, 1513, 1456, 1417, 1335, 1227, 1130, 1076, 984; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 0.96 (t, J = 7.2 Hz, 6H, N–CH_2CH_3), 1.87 (q, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 2.44–2.57 (m, 6H, N–CH_2–), 4.18 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31 (d, J = 5.9 Hz, 2H, Ar–CH_2–), 6.62–6.66 (m, 1H, –NH–), 7.11–7.42 (m, 9H, Ar-H), 7.63 (d, J = 9.0 Hz, 2H, Ar-H), 8.41 (d, J = 9.2 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 8.58 (s, 1H, –NH–), 9.53 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 11.78, 17.95, 27.12, 43.87, 46.38, 48.25, 57.25, 69.20, 105.06, 110.39, 117.47, 118.78, 120.33, 123.26, 126.75, 126.93, 127.12, 128.21, 128.43, 130.28, 136.55, 139.41, 151.49, 153.72, 155.83, 157.26, 163.89; ESI-MS m/z: 499.2 $[M+H]^+$; Anal. Calcd for $C_{29}H_{34}N_6O_2$ (%): C, 69.85; H, 6.87; N, 16.85. Found: C, 70.01; H, 6.52; N, 16.73.

4.1.14. 1-Benzyl-3-(4-((7-(3-(ethyl(2-hydroxyethyl)amino)-propoxy)quinazolin-4-yl)amino)phenyl)urea (7h)

Preparation of **7h** is followed the procedure for **7a**. Beige solid, yield: 67%, oil. IR (KBr, cm^{-1}): 3311, 2963, 1655, 1617, 1573, 1561, 1515, 1454, 1412, 1336, 1227, 1133, 1074, 985; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 0.97 (t, J = 7.1 Hz, 3H, N–CH_2CH_3), 1.89 (q, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 2.45–2.61 (m, 6H, N–CH_2–), 3.46 (t, J = 6.5 Hz, 2H, –CH_2OH), 4.17 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.32 (d, J = 6.0 Hz, 2H, Ar–CH_2–), 6.63–6.67 (m, 1H, –NH–), 7.12–7.44 (m, 9H, Ar-H), 7.63 (d, J = 8.9 Hz, 2H, Ar-H), 8.42 (d, J = 9.1 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.59 (s, 1H, –NH–), 9.54 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 11.98, 27.06, 43.85, 47.66, 49.73, 57.28, 59.30, 69.28, 105.12, 110.35, 117.50, 118.73, 120.35, 123.29, 126.71, 126.95, 127.10, 128.26, 128.39, 130.28, 136.57, 139.43, 151.55, 153.76, 155.81, 157.27, 163.92; ESI-MS m/z: 515.3 $[M+H]^+$; Anal. Calcd for $C_{29}H_{34}N_6O_3$ (%): C, 67.68; H, 6.66; N, 16.33. Found: C, 67.55; H, 6.78; N, 16.59.

4.1.15. 1-Benzyl-3-(4-((7-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (7i)

Preparation of **7i** is followed the procedure for **7a**. Beige solid, yield: 63%, mp: 95–96 °C. IR (KBr, cm^{-1}): 3329, 2945, 2807, 1683, 1618, 1585, 1559, 1512, 1456, 1420, 1332, 1314, 1225, 1136, 1001, 841; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.90–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.33–2.56 (m, 12H, N–CH_2–), 3.52 (t, J = 6.4 Hz, 2H, –CH_2OH), 4.18 (t, J = 6.4 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.32 (d, J = 5.6 Hz, 2H, Ar–CH_2–), 4.40 (br, 1H, –OH), 6.57 (t, J = 6.1 Hz, 1H, –NH–), 7.11–7.42 (m, 9H, Ar-H), 7.63 (d, J = 8.5 Hz, 2H, Ar-H), 8.42 (d, J = 9.1 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 8.53 (s, 1H, –NH–), 9.52 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 26.24, 43.59, 43.75, 52.78, 58.49, 60.12, 70.03, 105.02, 110.21, 117.39, 118.74, 120.35, 123.19, 126.83, 127.05, 127.11, 128.26, 128.35, 130.23, 136.47, 139.40, 151.52, 153.63, 155.81, 157.23, 163.85; ESI-MS m/z: 556.3 $[M+H]^+$; Anal. Calcd for $C_{31}H_{37}N_7O_3$ (%): C, 67.01; H, 6.71; N, 17.64. Found: C, 67.23; H, 6.59; N, 17.39.

4.1.16. 1-Benzyl-3-(4-((7-(3-(4-(hydroxymethyl)piperidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (7j)

Preparation of **7j** is followed the procedure for **7a**. Beige solid, yield: 70%, mp: 119–120 °C. IR (KBr, cm^{-1}): 3381, 2924, 2787, 1679, 1618, 1579, 1554, 1514, 1458, 1416, 1336, 1309, 1230, 1128, 1041, 829; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.10–1.18 (m, 3H, piperidine–H), 1.62–1.64 (m, 2H, piperidine–H), 1.84–1.98 (m, 4H, –CH_2–), 2.44 (t, J = 7.2 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 2.86–2.89 (m, 2H, N–CH_2–), 3.24 (t, J = 5.7 Hz, 2H, –CH_2–OH), 4.16 (t, J = 6.4 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.31–4.37 (m, 3H, Ar–CH_2– and –OH), 6.55–6.59 (m, 1H, –NH–), 7.12–7.41 (m, 9H, Ar-H), 7.63 (d, J = 9.0 Hz, 2H, Ar-H), 8.41 (d, J = 9.3 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 8.51 (s, 1H, –NH–), 9.52 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 25.05, 26.18, 30.31, 33.92, 43.78, 53.42, 54.53, 56.15, 68.93, 105.34, 110.28, 117.57, 118.88, 120.16, 123.09, 126.68, 127.01, 127.22, 128.35, 128.50, 130.18, 136.39, 139.40, 151.53, 153.67, 155.75, 157.19, 163.83; ESI-MS m/z: 541.49 $[M+H]^+$; Anal. Calcd for $C_{31}H_{36}N_6O_3$ (%): C, 68.87; H, 6.71; N, 15.54. Found: C, 68.66; H, 6.83; N, 15.61.

4.1.17. 1-Cyclohexyl-3-(4-((7-(3-morpholinopropoxy)-quinazolin-4-yl)amino)phenyl)urea (8a)

Preparation of **8a** is followed the procedure for **7a**. Beige solid, yield: 78%, mp: 276–277 °C. IR (KBr, cm^{-1}): 3338, 2929, 2854, 1649, 1620, 1578, 1556, 1514, 1458, 1417, 1333, 1225, 1117, 1041, 862; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.16–1.83 (m, 10H, cyclohexane–H), 1.93–1.96 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.39–2.51 (m, 6H, N–CH_2–), 3.47 (t, J = 4.0 Hz, 1H, cyclohexane–H), 3.59 (t, J = 4.5 Hz, 4H, O–CH_2–), 4.18 (t, J = 6.5 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.01 (d, J = 8.0 Hz, 1H, –NH–), 7.13 (d, J = 2.5 Hz, 1H, Ar-H), 7.20 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H, Ar-H), 7.36 (dd, J_1 = 7.0 Hz, J_2 = 2.0 Hz, 2H, Ar-H), 7.61 (dd, J_1 = 7.0 Hz, J_2 = 2.0 Hz, 2H, Ar-H), 8.25 (s, 1H, –NH–), 8.41 (d, J = 9.5 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 9.51 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.36, 24.63, 26.32, 30.15, 51.36, 56.08, 58.19, 68.35, 69.13, 105.20, 110.27, 117.35, 118.56, 120.15, 123.06, 124.56, 124.63, 130.21, 136.50, 151.45, 153.65, 155.63, 157.23, 163.81; ESI-MS m/z: 505.3 $[M+H]^+$; Anal. Calcd for $C_{28}H_{36}N_6O_3$ (%): C, 66.64; H, 7.19; N, 16.65. Found: C, 66.69; H, 6.98; N, 16.38.

4.1.18. 1-Cyclohexyl-3-(4-((7-(3-(4-methylpiperazin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8b)

Preparation of **8b** is followed the procedure for **7a**. Beige solid, yield: 60%, mp: 115–116 °C. IR (KBr, cm^{-1}): 3334, 2931, 2852, 2808, 1672, 1623, 1579, 1552, 1514, 1419, 1417, 1335, 1296, 1225, 1132, 839, 789, 679; ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.18–1.83 (m, 10H, cyclohexane–H), 1.93–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.15 (s, 3H, N–CH_3), 2.33–2.51 (m, 10H, N–CH_2–), 3.49–3.53 (m, 1H, cyclohexane–H), 4.17 (t, J = 6.2 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.01 (d, J = 7.7 Hz, 1H, –NH–), 7.12 (s, 1H, Ar-H), 7.19 (d, J = 9.3 Hz, 1H, Ar-H), 7.37 (d, J = 8.9 Hz, 2H, Ar-H), 7.62 (d, J = 8.8 Hz, 2H, Ar-H), 8.27 (s, 1H, –NH–), 8.41 (d, J = 9.3 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 9.52 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.35, 24.64, 26.33, 30.18, 46.67, 51.32, 56.01, 57.24, 58.23, 69.10, 105.22, 110.26, 117.38, 118.57, 120.12, 123.09, 124.55, 124.67, 130.19, 136.51, 151.43, 153.66, 155.63, 157.25, 163.83; ESI-MS m/z: 518.3 $[M+H]^+$; Anal. Calcd for $C_{29}H_{39}N_7O_2$ (%): C, 67.28; H, 7.59; N, 18.94. Found: C, 67.33; H, 7.43; N, 18.59.

4.1.19. 1-Cyclohexyl-3-(4-((7-(3-(piperidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8c)

Preparation of **8c** is followed the procedure for **7a**. Beige solid, yield: 72%, mp: 172–173 °C. IR (KBr, cm^{-1}): 3319, 2931, 2854, 1676, 1620, 1554, 1513, 1456, 1417, 1335, 1223, 1132, 1039, 987, 841; ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.18–1.79 (m, 16H, cyclohexane–H and piperidine–H), 1.90–1.94 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.35–2.51 (m, 6H, N–CH_2–), 3.44–3.48 (m, 1H, cyclohexane–H), 4.16 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.01 (d, J = 7.8 Hz, 1H, –NH–), 7.12 (d, J = 2.4 Hz, 1H, Ar-H), 7.19 (d, J = 9.1 Hz, 1H, Ar-H), 7.37 (d, J = 8.9 Hz, 2H, Ar-H), 7.62 (d, J = 8.9 Hz, 2H, Ar-H), 8.27 (s, 1H, –NH–), 8.41 (d, J = 9.2 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 9.53 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.33, 24.20, 24.65, 25.57, 26.31, 30.19, 51.34, 54.08, 54.16, 57.20, 69.16, 105.25, 110.23, 117.35, 118.59, 120.10, 123.13, 124.58, 124.65, 130.16, 136.54, 151.47, 153.67, 155.63, 157.28, 163.89; ESI-MS m/z: 503.3 $[M+H]^+$; Anal. Calcd for $C_{29}H_{38}N_6O_2$ (%): C, 69.29; H, 7.62; N, 16.72. Found: C, 69.53; H, 7.68; N, 16.45.

4.1.20. 1-Cyclohexyl-3-(4-((7-(3-(2-methylpiperidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8d)

Preparation of **8d** is followed the procedure for **7a**. Beige solid, yield: 66%, mp: 179–180 °C. IR (KBr, cm^{-1}): 3328, 2936, 2805, 1688, 1619, 1560, 1458, 1425, 1339, 1229, 1131, 1049, 916, 833; ^1H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.02 (d, J = 6.0 Hz, 3H, piperidine–CH_3), 1.17–1.78 (m, 16H, cyclohexane–H and piperidine–H), 1.91–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.30–2.52 (m, 5H, N–CH_2– and N–CH–), 3.45–3.49 (m, 1H, cyclohexane–H), 4.17 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.03 (d, J = 7.8 Hz, 1H, –NH–), 7.11 (d, J = 2.3 Hz, 1H, Ar-H), 7.20 (d, J = 9.0 Hz, 1H, Ar-H), 7.36 (d, J = 8.9 Hz, 2H, Ar-H), 7.61 (d, J = 8.9 Hz, 2H, Ar-H), 8.28 (s, 1H, –NH–), 8.42 (d, J = 9.1 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 9.52 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 17.53, 22.36, 23.23, 24.65, 25.71, 26.35, 30.16, 34.35, 50.93, 51.34, 56.18, 58.19, 69.15, 105.23, 110.27, 117.36, 118.59, 120.12, 123.15, 124.59, 124.63, 130.18, 136.57, 151.43, 153.65, 155.63, 157.29, 163.82; ESI-MS m/z: 517.2 $[M+H]^+$; Anal. Calcd for $C_{30}H_{40}N_6O_2$ (%): C, 69.74; H, 7.80; N, 16.27. Found: C, 69.65; H, 7.72; N, 16.05.

4.1.21. 1-Cyclohexyl-3-(4-((7-(3-(4-methylpiperidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8e)

Preparation of **7o** is followed the procedure for **7a**. Beige solid, yield: 75%, mp: 174–175 °C. IR (KBr, cm^{-1}): 3336, 2927, 2810, 1689, 1627, 1563, 1458, 1422, 1335, 1227, 1126, 1043, 917, 836; ^1H NMR (DMSO-d_6, 500 MHz) δ (ppm): 0.92 (d, J = 6.3 Hz, 3H, piperidine–CH_3), 1.12–1.75 (m, 15H, cyclohexane–H and piperidine–H), 1.91–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.21–2.60 (m, 6H, N–CH_2–), 3.43–3.47 (m, 1H, cyclohexane–H), 4.20 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.03 (d, J = 7.8 Hz, 1H, –NH–), 7.13 (d, J = 2.3 Hz, 1H, Ar-H), 7.20 (d, J = 9.0 Hz, 1H, Ar-H), 7.38 (d, J = 8.9 Hz, 2H, Ar-H), 7.61 (d, J = 8.9 Hz, 2H, Ar-H), 8.26 (s, 1H, –NH–), 8.42 (d, J = 9.1 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 9.53 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.16, 22.35, 24.65, 26.37, 30.17, 30.35, 33.92, 51.37, 53.49, 54.52, 56.15, 68.99, 105.22, 110.26, 117.37, 118.55, 120.13, 123.16, 124.61, 124.65, 130.19, 136.57, 151.46, 153.63, 155.69, 157.28, 163.85; ESI-MS m/z: 517.2 $[M+H]^+$; Anal. Calcd for $C_{30}H_{40}N_6O_2$ (%): C, 69.74; H, 7.80; N, 16.27. Found: C, 69.55; H, 7.76; N, 16.23.

4.1.22. 1-Cyclohexyl-3-(4-((7-(3-(pyrrolidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8f)

Preparation of **8f** is followed the procedure for **7a**. Gray solid, yield: 67%, mp: 131–132 °C. IR (KBr, cm^{-1}): 3315, 2931, 2854, 2812, 1672, 1618, 1578, 1552, 1514, 1458, 1415, 1336, 1223, 1132, 1041, 1018, 916, 841; ^1H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.16–1.83 (m, 14H, cyclohexane–H and pyrrolidine–H), 1.93–1.96 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.47–2.51 (m, 4H, N–CH_2–), 2.57 (t, J = 7.1 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 3.45–3.49 (m, 1H, cyclohexane–H), 4.18 (t, J = 6.4 Hz, 2H, N-CH_2–CH_2–CH_2–O–), 6.01 (d, J = 7.8 Hz, 1H, –NH–), 7.12 (d, J = 2.6 Hz, 1H, Ar-H), 7.20 (dd, J_1 = 9.1 Hz, J_2 = 2.6 Hz, 1H, Ar-H), 7.37 (dd, J_1 = 6.9 Hz, J_2 = 2.0 Hz, 2H, Ar-H), 7.61 (dd, J_1 = 6.9 Hz, J_2 = 2.0 Hz, 2H, Ar-H), 8.24 (s, 1H, –NH–), 8.41 (d, J = 9.3 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 9.51 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 19.05, 22.34, 23.58, 24.69, 27.03, 30.18, 51.35, 54.08, 54.11, 57.28, 69.18, 105.23, 110.29, 117.32, 118.56, 120.15, 123.18, 124.56, 124.69, 130.14, 136.56, 151.42, 153.68, 155.72, 157.25, 163.91; ESI-MS m/z: 487.2 $[M-H]^+$; Anal. Calcd for $C_{28}H_{36}N_6O_2$ (%): C, 68.83; H, 7.43; N, 17.20. Found: C, 68.56; H, 7.32; N, 16.98.

4.1.23. 1-Cyclohexyl-3-(4-((7-(3-(diethylamino)propoxy)-quinazolin-4-yl)amino)phenyl)urea (8g)

Preparation of **8g** is followed the procedure for **7a**. Beige solid, yield: 68%, mp: 115–116 °C. IR (KBr, cm^{-1}): 3423, 3296, 2968, 2931, 2852, 1618, 1578, 1514, 1456, 1417, 1335, 1225, 1130, 1042, 847; ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 0.95 (t, J = 7.1 Hz, 6H, N–CH_2CH_3), 1.12–1.83 (m, 10H, cyclohexane–H), 1.85–1.89 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.43–2.62 (m, 6H, N–CH_2–), 3.45–3.51 (m, 1H, cyclohexane–H), 4.17 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.47 (d, J = 7.0 Hz, 1H, –NH–), 7.11 (d, J = 2.6 Hz, 1H, Ar-H), 7.18 (dd, J_1 = 9.1 Hz, J_2 = 2.6 Hz, 1H, Ar-H), 7.38 (d, J = 9.0 Hz, 2H, Ar-H), 7.61 (d, J = 9.0 Hz, 2H, Ar-H), 8.41 (d, J = 6.3 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 8.76 (s, 1H, –NH–), 9.51 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 11.76, 17.98, 22.36, 24.63, 27.05, 30.19, 46.33, 48.23, 51.36, 57.22, 69.21, 105.25, 110.31, 117.31, 118.54, 120.16, 123.11, 124.53, 124.70, 130.12, 136.52, 151.44, 153.65, 155.78, 157.23, 163.87; ESI-MS m/z: 491.3 $[M+H]^+$; Anal. Calcd for $C_{28}H_{38}N_6O_2$ (%): C, 68.54; H, 7.81; N, 17.13. Found: C, 68.31; H, 7.55; N, 16.93.

4.1.24. 1-Cyclohexyl-3-(4-((7-(3-(ethyl(2-hydroxyethyl)amino)propoxy)quinazolin-4-yl)amino)phenyl)urea (8h)

Preparation of **7r** is followed the procedure for **7a**. Beige solid, yield: 63%, mp: 102–103 °C. IR (KBr, cm^{-1}): 3315, 2961, 1656, 1618, 1574, 1567, 1518, 1452, 1413, 1337, 1226, 1135, 1079, 983; ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 0.96 (t, J = 7.1 Hz, 3H, N–CH_2CH_3), 1.11–1.82 (m, 10H, cyclohexane–H), 1.87–1.91 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.45–2.63 (m, 6H, N–CH_2–), 3.45–3.52 (m, 3H, cyclohexane–H and –CH_2OH), 4.18 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 6.46 (d, J = 7.0 Hz, 1H, –NH–), 7.12 (d,

J = 2.6 Hz, 1H, Ar-H), 7.19 (dd, J_1 = 9.0 Hz, J_2 = 2.6 Hz, 1H, Ar-H), 7.39 (d, J = 9.0 Hz, 2H, Ar-H), 7.61 (d, J = 9.0 Hz, 2H, Ar-H), 8.42 (d, J = 6.3 Hz, 1H, Ar-H), 8.43 (s, 1H, Ar-H), 8.78 (s, 1H, –NH–), 9.52 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 11.97, 22.33, 24.65, 27.01, 30.13, 47.65, 49.78, 51.33, 57.22, 59.31, 69.29, 105.23, 110.32, 117.31, 118.55, 120.14, 123.15, 124.54, 124.73, 130.19, 136.50, 151.43, 153.66, 155.79, 157.28, 163.90; ESI-MS m/z: 507.3 [M+H]$^+$; Anal. Calcd for $C_{28}H_{38}N_6O_3$ (%): C, 66.38; H, 7.56; N, 16.59. Found: C, 66.52; H, 7.68; N, 16.53.

4.1.25. 1-Cyclohexyl-3-(4-((7-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8i)

Preparation of **8i** is followed the procedure for **7a**. Beige solid, yield: 68%, mp: 100–101 °C. IR (KBr, cm^{-1}): 3332, 2947, 2803, 1683, 1615, 1585, 1554, 1517, 1458, 1421, 1336, 1314, 1227, 1135, 1001, 849; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.18–1.83 (m, 10H, cyclohexane–H), 1.92–1.95 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.32–2.57 (m, 12H, N–CH_2–), 3.50–3.55 (m, 3H, cyclohexane–H and –CH_2OH), 4.17 (t, J = 6.3 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.39 (br, 1H, –OH), 6.01 (d, J = 7.6 Hz, 1H, –NH–), 7.13 (s, 1H, Ar-H), 7.20 (d, J = 9.2 Hz, 1H, Ar-H), 7.38 (d, J = 9.0 Hz, 2H, Ar-H), 7.63 (d, J = 8.9 Hz, 2H, Ar-H), 8.27 (s, 1H, –NH–), 8.42 (d, J = 9.2 Hz, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 9.53 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.38, 24.69, 26.85, 30.16, 43.78, 51.36, 52.75, 58.53, 60.08, 69.93, 105.25, 110.28, 117.36, 118.59, 120.15, 123.10, 124.58, 124.68, 130.23, 136.55, 151.46, 153.68, 155.65, 157.28, 163.89; ESI-MS m/z: 548.3 [M+H]$^+$; Anal. Calcd for $C_{30}H_{41}N_7O_3$ (%): C, 65.79; H, 7.55; N, 17.90. Found: C, 65.83; H, 7.51; N, 17.59.

4.1.26. 1-Cyclohexyl-3-(4-((7-(3-(4-(hydroxymethyl)piperidin-1-yl)propoxy)quinazolin-4-yl)amino)phenyl)urea (8j)

Preparation of **7t** is followed the procedure for **8j**. Beige solid, yield: 65%, mp: 120–121 °C. IR (KBr, cm^{-1}): 3311, 2927, 2852, 1676, 1620, 1579, 1555, 1514, 1458, 1417, 1335, 1225, 1130, 1039, 839; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 1.11–1.89 (m, 15H, cyclohexane–H and piperidine–H), 1.91–1.96 (m, 2H, N–CH_2–CH_2–CH_2–O–), 2.49–2.56 (m, 6H, N–CH_2–), 3.29 (t, J = 5.8 Hz, 2H, –CH_2–OH), 3.44–3.50 (m, 1H, cyclohexane–H), 4.17 (t, J = 6.4 Hz, 2H, N–CH_2–CH_2–CH_2–O–), 4.35–4.37 (m, 1H, –OH), 6.02 (d, J = 7.8 Hz, 1H, –NH–), 7.12 (d, J = 2.6 Hz, 1H, Ar-H), 7.19 (dd, J_1 = 9.1 Hz, J_2 = 2.6 Hz, 1H, Ar-H), 7.36 (dd, J_1 = 7.0 Hz, J_2 = 2.0 Hz, 2H, Ar-H), 7.61 (dd, J_1 = 7.0 Hz, J_2 = 2.0 Hz, 2H, Ar-H), 8.26 (s, 1H, –NH–), 8.41 (d, J = 9.3 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 9.51 (s, 1H, –NH–); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.33, 24.71, 25.06, 26.89, 30.15, 30.35, 33.98, 51.38, 53.44, 54.52, 56.15, 68.95, 105.26, 110.23, 117.31, 118.52, 120.11, 123.16, 124.61, 124.65, 130.29, 136.58, 151.47, 153.68, 155.63, 157.29, 163.91; ESI-MS m/z: 533.37 [M+H]$^+$; Anal. Calcd for $C_{30}H_{40}N_6O_3$ (%): C, 67.64; H, 7.57; N, 15.78. Found: C, 67.66; H, 7.69; N, 15.55.

4.2. Biological evaluation

4.2.1. In vitro antiproliferative activity against 6 human cancer cell lines

All of the target compounds were evaluated against human colon cancer cell lines (HT29 and LoVo), human chronic myeloid leukemia cell line (K562), human acute monocytic myeloid leukemia cell line (U937) and human lung cancer cell lines (A549 and NCI-H661). The cell viability was determined employing the MTT-based assay using WST-8 reagent, a reagent solution prepared as an aqueous solution containing 5 mM WST-8 (Sigma), 0.2 mM 1-methoxyphenazinium salt (Sigma) and 150 mM NaCl.[41] Briefly, the tumor cell lines in RPMI1640 medium with 10% fetal bovine serum were plated in 96-well microtiter plates (5.0×10^3 cells/well), and allowed to adhere at 37 °C with 5% CO_2 for 4 h. The test compound was then added, and the cells were incubated at 37 °C with 5% CO_2 for 72 h later. Subsequently, cell growth medium was removed, and WST-8 was added to each well for another incubation of 1.5 h at 37 °C. Absorbance was finally measured with a plate reader at 650 nm. The results were expressed as the percentage of absorbance of treated wells versus that of the vehicle control. IC_{50}, the drug concentration causing 50% growth inhibition, was calculated via sigmoid curve fitting using GraphPad Prism 5.0.

4.2.2. In vitro kinase assays

The kinase activities of Aurora A and B were measured using HTRF-KinEASE-STK discovery kit (Cisbio, Bedford, MA, USA). Briefly, Aurora A (65 nM) or Aurora B (316 nM) (Millipore, Billerica, MA, USA) was incubated with 1 μM STK substrate 2-biotin (Cisbio) and 15 μM ATP (Sigma–Aldrich) in the presence of tested compounds for 1 h at room temperature. The reaction was terminated by adding 200 mM EDTA. Phosphorylated substrates were incubated with STK antibody labeled with Eu^{3+}-cryptate and streptavidin-XL665. The Eu^{3+}-cryptate-conjugated antibody was excited at 340 nm, and cryptate and fluorescence resonance energy transfer emissions were detected at 615 and 665 nm, respectively. GRAPHPAD PRISM was used to generate IC_{50} values.

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

4.2.3. Procedure of docking simulation

Crystal structures of Aurora kinase A (PDB ID: 3D15)[18] and Aurora kinase B (PDB ID: 2VRX)[39] were imported to Maestro[42] and prepared for docking simulations using Protein Preparation Wizard in Schrodinger Suite.[43] In the protein preparation step, water molecules were removed, bond orders assigned, hydrogen atoms added, H-bonds assigned, and the structure relaxed with the OPLS2005 force field. The receptor grid was generated in Glide:[44] grid center was defined by selecting the ligand of the structure in the workspace, the limit of the ligand length was $\leqslant$20 Å, and the dimension of the grid box was $10 \times 10 \times 10$. Ligands in the SDF format were imported to Maestro and prepared using LigPrep:[45] possible ionized states of the compounds were generated at target pH 7 ± 2. Docking simulations of the compounds prepared with LigPrep were performed in Glide.

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