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Design, synthesis and biological evaluation of type-II VEGFR-2 inhibitors based on quinoxaline scaffold

Mai I. Shahin, Dalal A. Abou El Ella, Nasser S.M. Ismail, Khaled A.M. Abouzid *

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abassia, Cairo 11566, Egypt

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ABSTRACT

In an effort to develop ATP-competitive VEGFR-2 selective inhibitors, a series of new quinoxaline-based derivatives was designed and synthesized. The target compounds were biologically evaluated for their inhibitory activity against VEGFR-2. The design of the target compounds was accomplished after a profound study of the structure activity relationship (SAR) of type-II VEGFR-2 inhibitors. Among the synthesized compounds, 1-(2-((4-methoxyphenyl)amino)-3-oxo-3,4 dihydroquinoxalin-6-yl)-3-phenylurea (**VIIa**) displayed the highest inhibitory activity against VEGFR-2. Molecular modeling study involving molecular docking and field alignment was implemented to interpret the variable inhibitory activity of the newly synthesized compounds.

1. Introduction

Inhibition of receptor tyrosine kinase (RTK) signaling pathways is an important area for the development of novel anticancer agents. Vascular endothelial growth factor receptor-2 (VEGFR-2) is the principal mediator of tumor angiogenesis. Angiogenesis, which is the sprouting of new blood vessels from pre-existing ones, is one of the most critical hallmarks of a cancerous cell. Development of selective antiangiogenic agents required thorough study of signal transduction pathways that hold the promise of efficacy with minimal toxicity [1]. Tyrosine kinases were identified as one of the efficient targets for evolving new anticancer agents having the desired selectivity on cancerous cells [2]. Numerous small molecule TK inhibitors were approved to treat many types of tumors of different origins [3]. Tyrosine kinase family comprises prominent members that can effectively induce important cell signaling pathways [4]. Angiogenic switch is a critical element in the growth of primary tumors and formation of metastatic sites [5]. Proliferation and migration of endothelial cells in angiogenesis are provoked by the imbalance between pro-angiogenic factors and anti-angiogenic factors. VEGF was settled to be the most important regulator of angiogenesis. This occurs by binding to the kinase insert domain (KDR) of the receptor tyrosine kinase VEGFR-2 [6]. Small molecule inhibitors targeting KDR proved successful suppression of tumor growth via blocking its signaling pathway [7]. Sutent® (sunitinib) [8] and Nexavar® (sorafenib tosylate) [9], were approved by FDA for treating gastrointestinal stromal tumors and advanced renal cell carcinoma, respectively (Fig. 1).

A literature survey revealed that different scaffolds have been reported as excellent inhibitors of VEGFR-2. Some classes are quinoline amides, [10] quinolinones, [11] anilinophthalazines, [12] and quinazolines [13]. As being one of tyrosine kinases, VEGFR-2 has 2 different conformations; the active and the inactive conformation [14]. This is determined by the movement of the DFG motif (activation loop). The DFG-in (active) conformation makes the active site accessible for ATP binding. Type-I inhibitors can target this active conformation and compete with ATP for binding [15]. Shifting to the DFG-out (inactive) conformation occurs by movement of the DFG motif revealing an extra hydrophobic pocket adjacent to the binding site. Inhibiting the tyrosine kinase activity is achieved through stabilizing this inactive conformation using type-II inhibitors. Type-II inhibitors provide a superior kinetic advantage over type-I inhibitors by avoiding competition with ATP. Also they stabilize the kinase in the DFG-out inactive conformation [16]. In our current study, we synthesized novel quinoxaline derivatives as type II VEGFR-2 inhibitors based on the Structure–Activity Relationship (SAR) study of VEGFR-2 type-I and type-II inhibitors. The newly synthesized compounds were tested for their inhibitory activities on some kinases. The results showed good inhibition percentages of some compounds against VEGFR-2 compared with other tested kinases. The results were interpreted using investigational docking and field alignment studies.

Type-II tyrosine kinase inhibitors, though being highly diverse in structure, revealed common feature pharmacophores for binding. Two simple screening hits, phenylaminopyrimidine in case of

* Corresponding author. Fax: +20 225080728.
E-mail address: Khaled.abouzid@pharma.asu.edu.eg (K.A.M. Abouzid).

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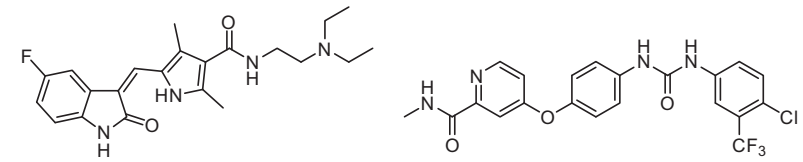


Fig. 1. Sutent® (Sunitinib) and Nexavar® (Sorafenib).

imatinib and biaryl urea in case of both BIRB796 and Sorafenib, were the first type II inhibitors developed by optimization of kinase enzyme activity [17]. As illustrated in Fig. 2, Gleevec®, Nexavar®, and BIRB796 were analyzed for their binding interactions as type-II inhibitors. A flat aromatic ring system of the main scaffold adopts the active site in the same manner as the ATP purine ring does. This occurs along with the essential hydrogen bonding of Cys 919 in the hinge region (presented as the left green side) [18]. Accordingly, we determined the features necessary for a type-I inhibitor which typically forms ~1–3 hydrogen bonds with the kinase hinge residues [15]. In case of type-II inhibitors, it would require an additional moiety to extend into the nearby allosteric site formed by the movement of the DFG motif in the inactive conformation of the enzyme forming two hydrogen bonds with the allosteric site residues: one hydrogen bond with the side chain of a conserved glutamic acid in the C-helix (Glu 883) and the other with the backbone amide of aspartic acid in the DFG motif (Asp 1044) (this is presented by the core group given a yellow color).

All type-II inhibitors also has a hydrophobic moiety that is located just after the hydrogen bond donor–acceptor pair forming van der Waals interactions with the allosteric site (the right pink side) [19].

Our design was based on targeting VEGFR-2 in its DFG-out conformation. Inspection of known type-II inhibitors, especially Sorafenib, revealed that the conserved hydrogen bonds between the ligand and the allosteric site residues were done using urea or amide moieties.

In this respect, we designed novel compounds based on quinoxaline core, with urea, amide and sulfonamide pharmacophoric linking moieties for allosteric site binding with extended alkyl or aryl groups.

2. Results and discussion

2.1. Chemistry

Quinoxaline scaffold in compounds **I** and **XII** was synthesized using the Hinsberg reaction [20] (Schemes 1 and 2). Their nitration was performed using potassium nitrate in sulfuric acid [21] to give 6-nitroquinoxaline derivatives **II** [22] and **XII** [23] respectively. Chlorination of **II** and **XII** was carried out by refluxing with $POCl_3$ either alone in case of **III** [24] or combined with PCl_5 in case of **XIII** [23]. New intermediates **IV** and **XIVa-b** was obtained by reacting the chloroquinoxaline derivative with the appropriate aniline derivative in isopropanol [25] at room temperature giving **IV**. While compounds **XIVa-b** were synthesized using ethanol [24] under reflux with using anhydrous potassium carbonate. In case of compound **IV**, substitution of the chlorine atom at the 2-position of quinoxaline due to regioselectivity created by the electron withdrawing effect of 6-nitro group [26]. Caution was carefully taken by monitoring mild conditions to prevent dinucleophilic substitution of both chlorine atoms. Hydrolysis of the second chlorine atom was accomplished by heating **IV** with conc. HCl to provide **V**. Reduction of the nitro compounds to the corresponding amino derivatives was achieved using 10% Pd-C in THF/MeOH due to their poor solubility in single solvent to give compounds **VI** and **XVa-b**. Moreover, urea and thiourea derivatives were synthesized by stirring the appropriate amine derivative with the cyclohexyl and

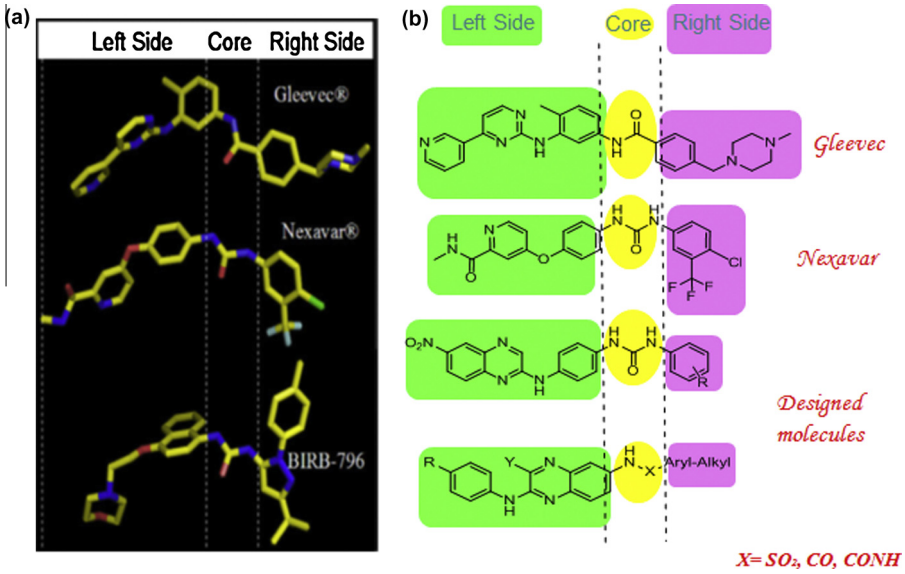
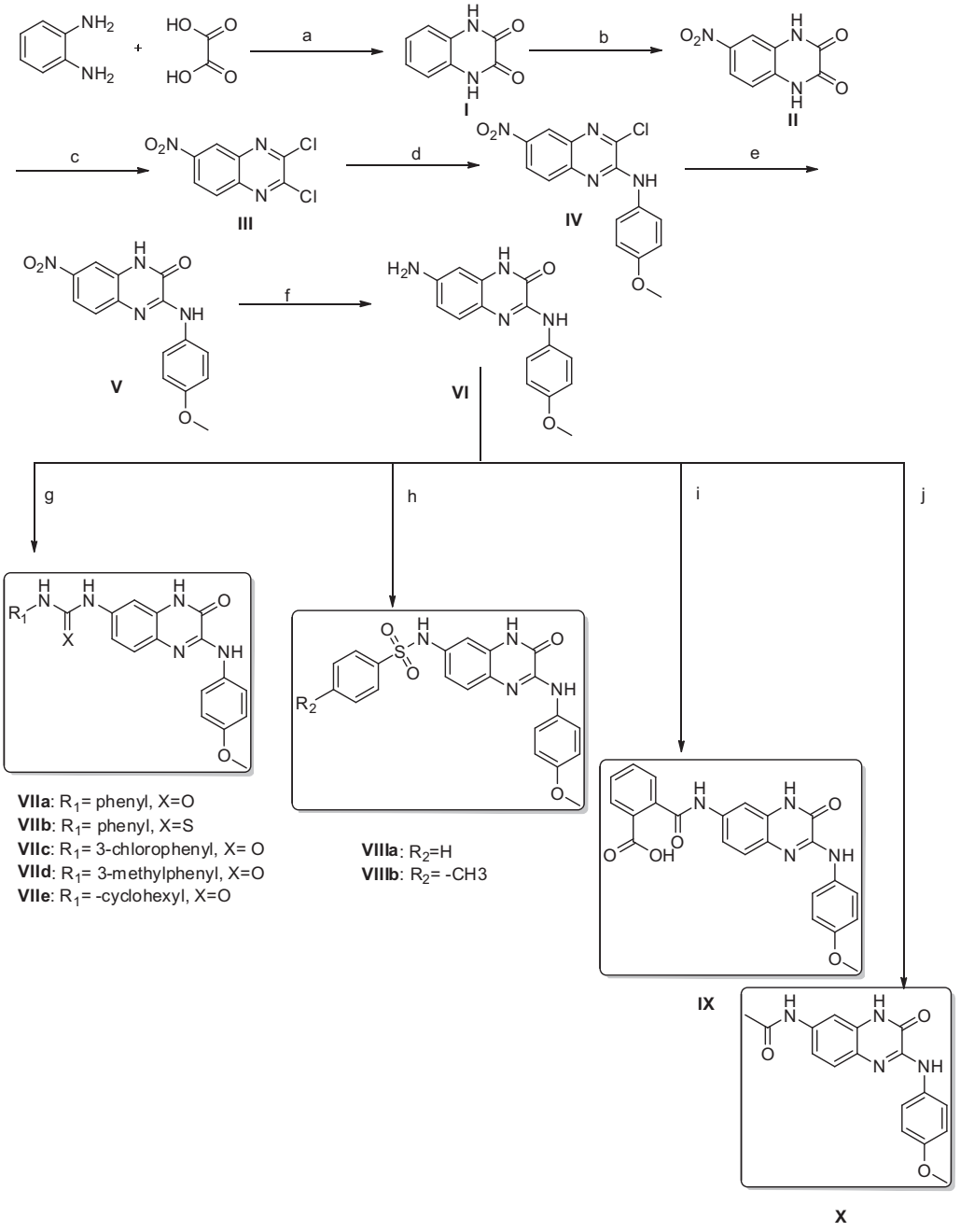


Fig. 2. (a) A diagram illustrating the common features between Gleevec®, Nexavar® and BIRB-769 as type-II inhibitors [18]. (b) Fitness of these essential features on our designed compounds revealing the green left side that presents the main scaffold adopting the active site. Also, the urea, amide or sulfonamide core was presented in yellow color and finally the extra moiety occupying the allosteric site was given in pink color.

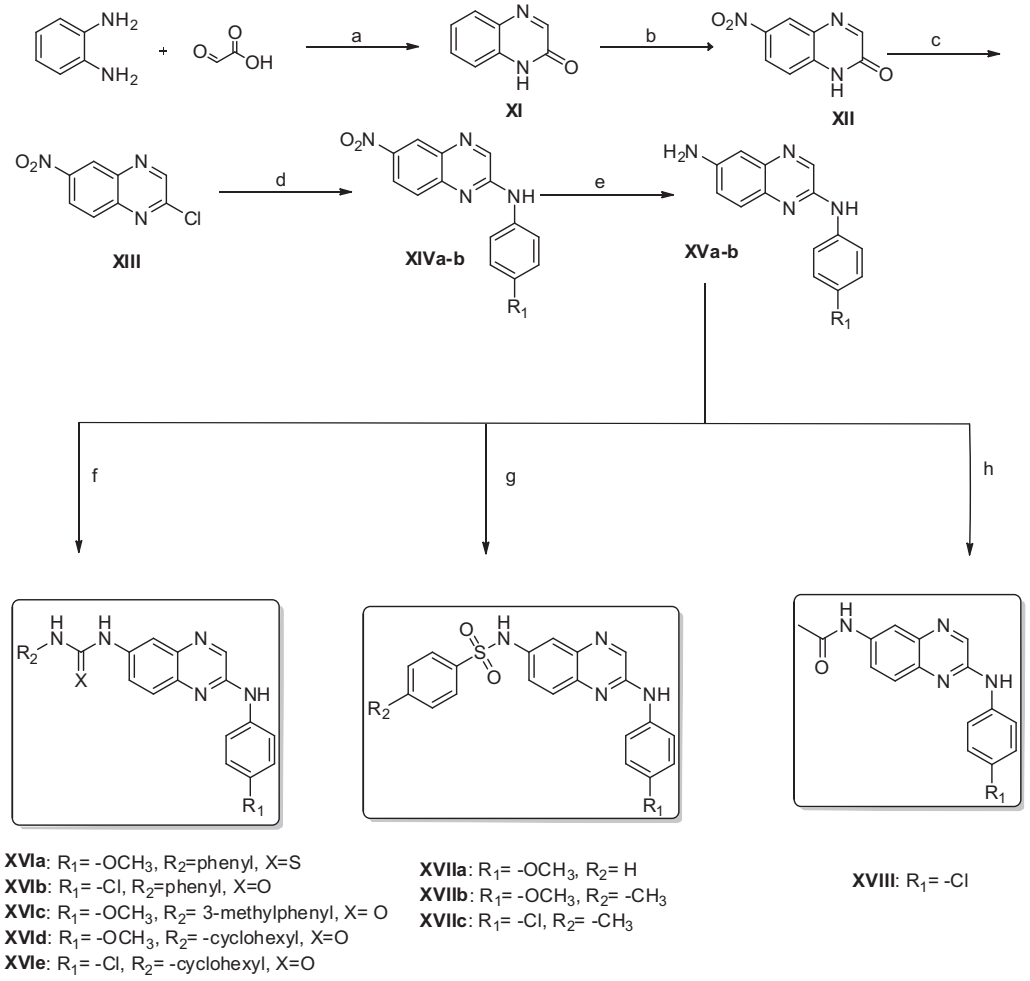


Scheme 1. 3-((4-Methoxyphenyl)amino)-7-(substituted amino)quinoxalin-2(1H)-ones. Reagents and conditions: (a) Aq. HCl, reflux, 2 h, 98%. (b) KNO_3, H_2SO_4, rt, 96%. (c) $POCl_3$, reflux, 6 h, 95%. (d) P-anisidine, isopropanol, rt, 3 h, 90%. (e) Conc. HCl, 50 °C, 8 h, 86%. (f) Pd-C, THF/MeOH, 40 °C, 24 h, 95%. (g) Aryl and cyclohexyl isocyanates and aryl isothiocyanate, THF, 2–24 h, rt, 80–85%. (h) P-substituted benzenesulfonyl chloride, pyridine, reflux, 3 h, 40–58%. (i) Phthalic anhydride, AcOH, rt, 82%. (j) Acetyl chloride, THF, reflux, 24 h, 73%.

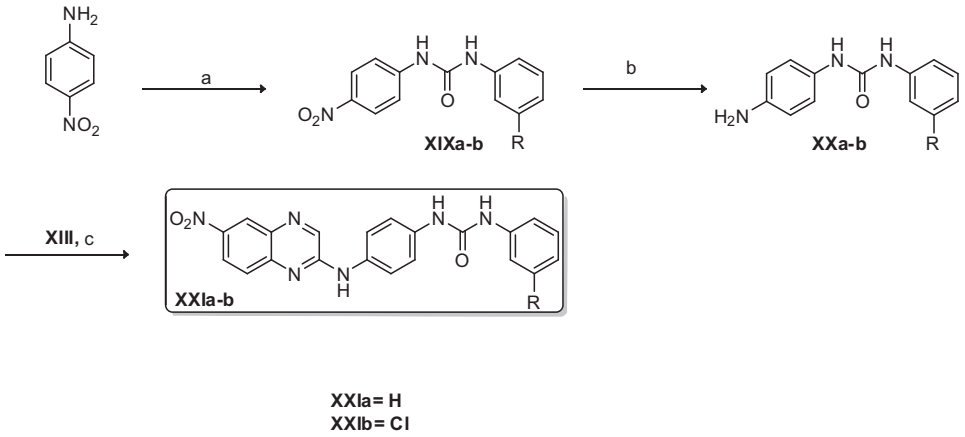
aryl isocyanates and aryl isothiocyanate in THF [27] to yield **VIIa-e**. DCM [28] was used instead to provide **XVIa-e**. Refluxing the amine derivatives with the appropriate sulfonyl chloride in pyridine [29] gave the sulfonamide series **VIIIa-b** and **XVIIa-c**. Synthesis of different amide derivatives was accomplished by stirring phthalic anhydride or acetyl chloride with **VI** and **XVa-b** in glacial acetic acid to give **IX** and **XVIII** [30]. Refluxing acetyl chloride with **VI** in THF gave the corresponding amide derivative **X**. Compounds **XXIa-b** (Scheme 3) were synthesized by refluxing **XIII** with 4-aminophenyl urea derivatives in ethanol [24] and anhydrous potassium carbonate.

2.2. Biological evaluation

Kinase inhibition assay was carried out to evaluate the effect of the synthesized compounds on VEGFR-2 and other selected kinases such as c-Met, EGFR, PDGFRβ and B-raf kinases. The evaluation was performed in KINEXUS Corporation where radiolabeled ATP determination method (^{33}P-ATP) was used. The percent inhibition of the Kinase enzymatic activity of the tested compounds was evaluated against a reference kinase inhibitor at 10 μM. Compounds **VIIa**, **IX**, **XVIc**, **XXIa-b** showed relatively high inhibitory enzyme activity. Among these compounds, **IX** shows the highest percentage of



Scheme 2. N6-Substituted-N2-(4-substituted phenyl)quinoxaline-2,6-diamines. Reagents and conditions: (a) EtOH, rt, 2 h, 98%. (b) KNO_3, H_2SO_4, rt, 96%. (c) $POCl_3$, PCl_5, reflux, 10 h, 86%. (d) P-anisidine, p-chloroaniline, EtOH, reflux, 5 h, 92%. (e) Pd-C, THF/MeOH, 40 °C, 24 h. (f) Aryl and cyclohexyl isocyanates and aryl isothiocyanate, DCM, rt, 2–24 h. (g) P-substituted benzenesulfonyl chloride, pyridine, reflux, 3 h. (h) Acetyl chloride, gl. Acetic acid, rt, 24 h.



Scheme 3. 1-(4-((6-Nitroquinoxalin-2-yl)amino)phenyl)-3-(3-substituted phenyl)ureas. Reagents and conditions: (a) Aryl isocyanate derivatives, THF, 24 h, r.t.75.8–85.3%. (b) $SnCl_2$, EtOH, reflux, 16 h, 50–51.8%. (c) **XIII**, EtOH, anhydrous K_2CO_3, reflux, 6 h. 72–83.7%.

inhibition (69%) on VEGFR-2 while these compounds displayed less significant inhibitory activities on the other tested kinases. The rest of compounds showed weak to moderate activity with exception of compounds **VIId**, **XVIa**, **XVIb** and **XVId** which showed no significant effect (Table 1). The five compounds showing the highest percentage of enzyme inhibition were selected for determination of their IC_{50} on VEGFR-2 (Table 2). Compound **VIIa** showed highest IC_{50} value (10.3 μM) (Fig. 3).

Table 1
VEGFR-2 and other kinases % inhibition achieved by the twenty targeted compounds at 10 μM.

Compound ID	VEGFR-2	c-Met	PDGFRβ	B-raf	EGFR
VIIa	−62	−43	NT	0	NT
VIIb	−23	−47	−8	NT	NT
VIIc	−17	2	−10	NT	−17
VIId	3	−15	−2	NT	NT
VIIe	−52	1	−1	NT	NT
VIIIa	−10	−58	NT	NT	NT
VIIIb	−29	−16	NT	NT	NT
IX	−69	−14	NT	NT	NT
X	−23	−18	NT	NT	NT
XVIa	4	−15	NT	NT	NT
XVIb	3	−1	NT	NT	NT
XVIc	−66	−6	NT	NT	NT
XVId	6	−33	NT	NT	NT
XVIe	−11	−5	NT	NT	NT
XVIIa	−18	−13	NT	NT	NT
XVIIb	−29	−26	NT	NT	NT
XVIIc	−27	−27	NT	NT	NT
XVIII	−5	0	NT	NT	NT
XXIa	−65	−34	NT	NT	NT
XXIb	−61	3	NT	NT	NT

NT: means not tested.

Table 2
IC_{50} values for compounds **VIIa**, **IX**, **XVIc**, **XXIa** and **XXIb**.

Compound ID	IC_{50} values	R^2 values
VIIa	10.3	0.9977
IX	61.8	0.9821
XVIc	210.4	0.9887
XXIa	268.2	0.9664
XXIb	85.4	0.9921

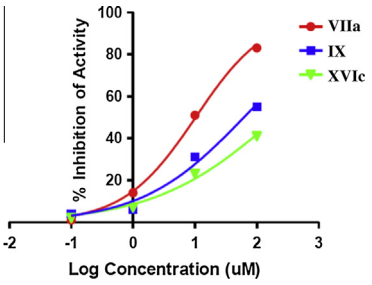


Fig. 3. Graph of log compounds **VIIa**, **IX** and **XVIc** concentrations against % VEGFR-2 activity inhibition.

2.3. Molecular modeling study

Molecular docking study was conducted using Gold 4.1 software in the interface of Accelry's Discovery Studio 2.5 (Accelrys Inc., San Diego, CA, USA). Field alignment study was done using Field Align 2.1 software.

2.3.1. Docking study

A deep docking study was done to investigate the possible binding modes of the newly synthesized compounds inside the ATP binding site of VEGFR-2 using Gold 4.1 software [31]. The X-ray crystal structure of VEGFR-2 bound to naphthamide-based compound was obtained, which represents the protein in its inactive conformation, and prepared for docking by energy minimization. 500,000 iterations were applied to get reliable results by increasing the number of predictions. Docking of Sorafenib as a reference compound was carried out to compare its binding mode with those of the target compounds. Investigation of Sorafenib docking results revealed a hydrogen bond formed with Cys 919 in the hinge region. The urea moiety interacts with the protein through two hydrogen bonds; the NH forms a hydrogen bond with Glu 885 while the urea carbonyl functionality interacts with Asp 1046. The substituted phenyl ring pushes deep into the extended hydrophobic pocket that is formed by the movement of Phe 1047 of the 'DFG' motif to induce the 'DFG-out' conformation. On the other hand, the compounds showing highest inhibition of VEGFR-2 activity (**VIIa**, **IX**, **XVIc**, **XXIa-b**) fulfilled the key features as done by the lead compound in the ATP binding site (Cys 919, Glu 885 and Asp 1046). For **IX**, extra hydrogen bonding with Glu 917 in the hinge region was detected which may explain its high activity relative to other compounds. Also, **XVIc** fulfilled all the features important for being type-II inhibitors which explains its high activity. Compounds showing weak to moderate activity missed one interaction of the features essential to be acting as type-II inhibitors which is either interaction with Glu 885 or Asp 1046 and even some lose the interaction with Cys 919 and rather interact with other amino acids in the hinge region instead as Glu 917 and Thr 916 (Fig. 4).

2.3.2. Field analysis

For understanding the unexpected results of compounds **VIId**, **XVIa**, **XVIb** and **XVId** showing no significant activity, a further study was conducted using field analysis. The field alignment study of compounds **VIId**, **XVIa**, **XVIb** and **XVId** in comparison to Sorafenib as a reference showed that negative field (Hydrogen bond acceptor) around the NH of Sorafenib is present in these compounds but in the totally opposite direction. This negative field represents the interaction to Cys 919 in the hinge region which is the most important key feature of interaction. Upon comparing active compounds **VIIa** and **XVIc** with the reference, the negative field was present in the same direction as Sorafenib. Compound **XVIb** showed no negative field around that of Sorafenib. This proved the importance of this interaction in the inhibitory activity. (Fig. 5)

2.3.3. ADME study

Theoretical kinetic study was performed using Discovery Studio software to predict the ADME of the newly synthesized compounds. All the compounds passed the Lipinski's rule of five except compound **XVIa** which had partition coefficient > 5. Other descriptors as A logP 98 and PSA 2D were calculated to evaluate solubility level and absorption level. Also, CYP2D inhibition was predicted giving score 0 for non-inhibitors and 1 for inhibitors. Most of the compounds showed good absorption levels (score = 0) with relatively low solubility (score of solubility level = 1–2). Our two most active candidates **IX** and **VIIa** showed moderate and good absorption respectively. Compound **IX** is expected to have good solubility, while **VIIa** will be with low solubility. Finally, All the compounds were predicted to be CYP2D non-inhibitors except **XVIII**. (Table 3)

3. Conclusion

Quinoxaline scaffold was explored for its activity against VEGFR-2. The compounds were designed as type-II inhibitors based on comprehensive SAR study. Biological evaluation revealed high inhibitory activity of compounds **IX**, **VIIa**, **XVIc**, **XXIa**, **XXIb**, with highest IC_{50} of compound **VIIa**. The unexplained activity results of inactive compounds **VIId**, **XVIa**, **XIVb** and **XIVd** was adequately explored using field analysis. The results of this study revealed that quinoxaline could be a promising scaffold to be greatly considered for evolving new effective VEGFR-2 inhibitors.

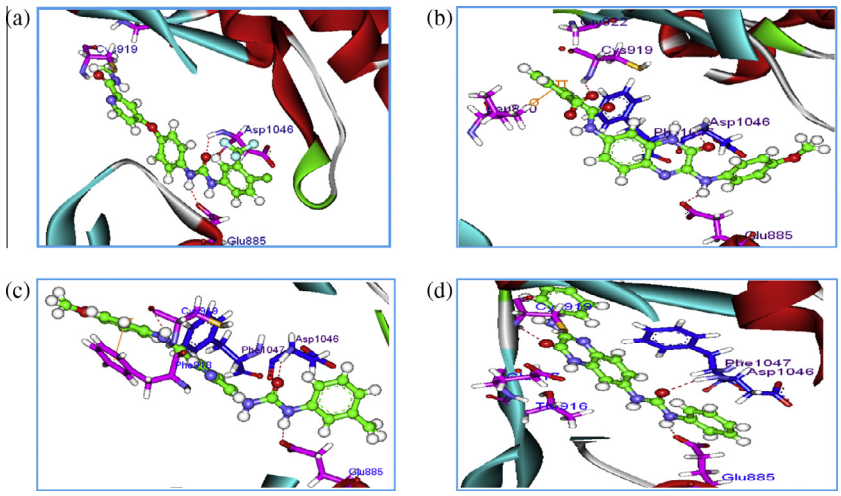


Fig. 4. (a) Sorafenib docking pose showing the same key interactions as reported in the literature (b) The pose adopted by compound **IX** showing excellent binding with VEGFR-2 binding site in its inactive conformation. (c and d) Compounds **XVIc** and **VIIa** in their docked poses respectively revealing that all the essential features of type-II inhibitors are fulfilled.

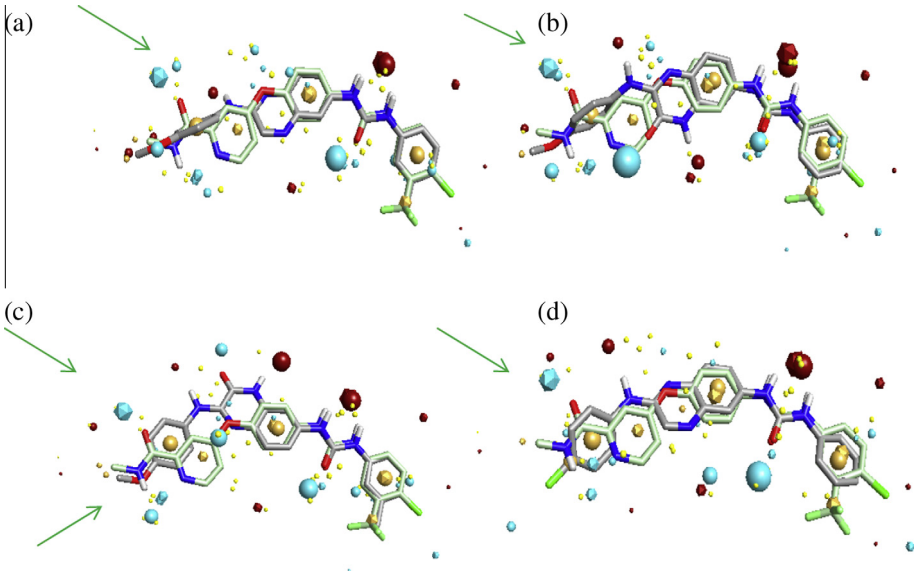


Fig. 5. (a and b) Showing the matching of the negative field (hydrogen bond acceptor) with Sorafenib in both compounds **XVIc**, **VIIa**. (c) Compound **VIId** shows the negative field in the totally opposite directions (d). Compound **XVIb** shows no negative field around that of Sorafenib.

4. Experimental

4.1. Chemistry and analysis

All chemicals either starting materials or reagents used were purchased from Aldrich (USA) or Alfa-Aesar Organics and used without further purification. Melting points were determined using Stuart Scientific apparatus and were uncorrected. The reactions were monitored using analytical thin layer chromatography (TLC) purchased from Merck (Merck, Darmstadt, Germany) and performed on 0.255 mm silica gel plates, with visualization under U.V. light (254 nm). FT-IR spectra were recorded on a PerkineElmer IR spectrophotometer.

1H spectra were run at 300 MHz spectrometer in δ scale (ppm), J (Hz) using TMS as reference at Microanalytical Center at Cairo University. EI-MS spectra were recorded on Finnigan Mat SSQ 7000 (70 eV) mass spectrometer at Microanalytical Center at Cairo University. Elemental analysis was performed at Al-Azhar University. Compounds **I**, **II**, **III**, **XI**, **XII**, **XIII**, **XIXa-b**, **XXa-b** were synthesized according to the reported procedures [22,23,32–34].

*4.1.1. 3-Chloro-N-(4-methoxyphenyl)-6-nitroquinoxalin-2-amine (**IV**)*

A solution of **III** (2 g, 8.2 mmol) in isopropanol was stirred while adding the p-anisidine (1.01 g, 8.2 mmol) portionwise then left with stirring at room temperature for 6 h. Filtration of the resulted solid and stirring it again with absolute ethanol then filtration afforded orange powder of the required compound with a yield of 1.6 g (59%); m.p. 174–176 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.56 (s, 1H), 8.59 (s, 1H), 8.37 (d, J = 8.5 Hz, 1H), 8.33 (d, J = 8.6 Hz, 1H), 7.21 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H),

Table 3
Computer aided ADME study of the targeted compounds.

	A log *P* 98[a]	PSA 2D[b]	Absorption level[c]	Solubility[d]	Solubility level[e]	CYP 2D6[f]	CYP 2D6 probability[g]
VIIa	2.564	106.095	0	−4.009	2	0	0.277
VIIb	3.463	88.794	0	−4.794	2	0	0.237
VIIc	3.229	106.095	0	−4.921	2	0	0.178
VIId	3.05	106.095	0	−4.428	2	0	0.178
VIIe	2.846	106.095	0	−4.255	2	0	0.485
VIIIa	2.359	110.586	0	−4.217	2	0	0.465
VIIIb	2.845	110.586	0	−4.635	2	0	0.386
IX	2.286	131.401	1	−3.977	3	0	0.336
X	0.992	93.285	0	−2.698	3	0	0.475
XVIa	4.959	69.882	0	−6.132	1	0	0.435
XVIb	4.74	78.253	0	−6.066	1	0	0.247
XVIc	4.546	87.183	0	−5.752	2	0	0.415
XVId	4.341	87.183	0	−5.582	2	1	0.643
XVIe	5.022	78.253	0	−6.32	1	0	0.475
XVIIa	3.854	91.674	0	−5.426	2	0	0.485
XVIIb	4.34	91.674	0	−5.85	2	0	0.396
XVIIc	5.021	82.744	0	−6.595	1	0	0.356
XVIII	3.168	65.443	0	−4.68	2	1	0.514
XXIa	3.97	121.076	1	−5.519	2	0	0.267
XXIb	4.635	121.076	2	−6.338	1	0	0.158

[a] Lipophilicity descriptor.
[b] Polar surface area.
[c] Absorption level (0 = good, 1 = moderate, 2 = low, 3 = very low).
[d] Solubility parameter.
[e] Solubility level (0 = extremely low, 1 = very low but soluble, 2 = low, 3 = good, 4 = optimal).
[f] CYP2D inhibition (0 = non inhibitor, 1 = likely to inhibit).
[g] CYP2D inhibition probability.

3.75 (s, 3H); FT-IR (*ν* max, cm^{-1}): 3320 (NH), 3032 (CH aromatic), 2947 (CH aliphatic), 1587, 1390 (NO_2); MS: (Mwt.: 330): *m/z*, 332 [M + 2, (2.46%)], 330 [M^+, (9.66%)], 79 (100%); Anal. Calcd for $C_{15}H_{11}ClN_4O_3$: C, 54.47; H, 3.35; N, 16.94; Found: C, 54.08; H, 3.04; N, 16.84.

*4.1.2. 3-((4-Methoxyphenyl)amino)-7-nitroquinoxalin-2(1H)-one (**V**)*

This compound was obtained with a yield of 2.1 g (74%) by heating **IX** (3 g, 9.07 mmol) in conc. HCl (30 ml) at 50 °C for 12hrs then filtration, washing the solid thoroughly with water and finally triturating it with diethyl ether to give orange powder of **V**; m.p. 282 °C; ^{1}H NMR (300 MHz, DMSO-d6) *δ* 12.70 (s, 1H), 9.87 (s, 1H), 8.92 (s, 1H), 8.06(s,1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 3.75 (s, 3H); FT-IR (*ν* max, cm^{-1}): 3363 (NH), 3032 (CH aromatic), 2910 (CH aliphatic), 1673 (C═O amide), 1580, 1383 (NO_2); MS: (Mwt.: 312): *m/z*, 313 [M+1, (16.5%)], 312 [M^+, (100%)]; Anal. Calcd for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.94; Found: C, 57.69; H, 3.58; N, 17.63.

*4.1.3. 7-Amino-3-((4-methoxyphenyl)amino)quinoxalin-2(1H)-one (**VI**)*

To a solution of **X** (1 g, 3.2 mmol) in THF/MeOH (50 ml) was added 10% Pd-C wet (0.1 g) and then the mixture was stirred at 40 °C under H_2. After removing the catalyst by filtration with celite, the filtrate was concentrated *in vacuo*, dried giving brownish powder of **VI** at 0.8 g (88%) yield; m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d6) *δ* 12.11 (s, 1H), 9.86 (s, 1H), 7.98 (s, 1H), 7.95 (s,2H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.54 (d, *J* = 8.8 Hz, 2H), 3.75 (s, 3H); FT-IR (*ν* max, cm^{-1}): 3440 (forked NH_2), 3120 (CH aromatic), 2954 (CH aliphatic), 1620 (C═O amide); MS: (Mwt.: 282): *m/z*, 283 [M+1, (16.5%)], 282 [M^+, (2.2%)], 154 (100%); Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85; Found: C, 63.35; H, 4.98; N, 19.42.

*4.1.4. General procedure for the preparation of compounds (**VIIa-e**)*

To a solution of **VI** (0.1 g, 0.35 mmol) in THF was added the appropriate isocyanate or isothiocyanate (0.7 mmol; 2 eq.) (*viz*; 3-chlorophenyl isocyanate, phenyl isothiocyanate, 3-methyl phenyl isocyanate and cyclohexyl isocyanate) and stirred for 2–24 h. The mixture was added to hexane and stirred for several minutes. The resulting solid was filtered, washed with hexane, recrystallized from ethanol to afford the titled compounds **(VIIa-e)**.

*4.1.4.1. 1-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4 dihydroquinoxalin-6-yl)-3-phenylurea (**VIIa**).* The titled compound was separated as buff solid 0.1 g (70%); m.p 238 °C; ^{1}H NMR (300 MHz, DMSO-d6) *δ* 12.43 (s, 1H), 11.57 (s, 1H), 9.28 (s, 1H), 9.13 (s, 1H), 8.94 (s, 1H), 8.05 – 7.94 (m, 5H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 2H), 6.62 (d, *J* = 8.1 Hz, 2H), 3.74 (s, 3H); FT-IR (*ν* max, cm^{-1}): 3363 (NH), 3032 (CH aromatic), 2910 (CH aliphatic), 1673 (C═O amide); MS: (Mwt.: 401): *m/z*, 402 [M+1, (19.80%)], 401 [M^+, (1.17%)], 93 (100%); Anal. Calcd for $C_{22}H_{19}N_5O_3$: C, 65.83; H, 4.77; N, 17.45; Found: C, 65.97; H, 4.82; N, 17.58.

*4.1.4.2. 1-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-phenylthiourea (**VIIb**).* The titled compound was afforded as yellow solid 0.12 g (81%); m.p. 168 °C; ^{1}H NMR (300 MHz, DMSO-d6) *δ* 12.59 (s, 1H), 10.70 (s, 1H), 9.71 (s, 1H), 9.21 (s, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.45–7.30 (m, 5H), 7.28 (s, 1H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 3.76 (s, 3H); FT-IR (*ν* max, cm^{-1}): 3395 (NH), 3084 (CH aromatic), 2984 (CH aliphatic), 1665 (C═O amide); MS: (Mwt.: 417): *m/z*, 418 [M+1, (27.23%)], 417 [M^+, (1.98%)], 296 (100%); Anal. Calcd for $C_{22}H_{19}N_5O_2S$: C, 63.29; H, 4.59; N, 16.78; Found: C, 63.17; H, 4.65; N, 16.59.

*4.1.4.3. 1-(3-Chlorophenyl)-3-(2-((4-methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)urea (**VIIc**).* The titled compound was afforded as buff solid 0.08 g (52%); m.p. 192 °C; ^{1}H NMR (300 MHz, DMSO-d6) *δ* 12.30 (s, 1H), 9.13 (s, 1H), 8.92 (s, 1H), 8.87 (s, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 8.6 Hz, 1H), 7.56 (s, 1H), 7.40–7.35 (m, 3H), 7.28 (s, 1H), 7.18 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 3.75 (s, 3H); FT-IR (*ν* max, cm^{-1}): 3280 (NH), 3084 (CH aromatic), 2946 (CH aliphatic), 1662 (C═O amide);

MS: (Mwt.: 435): *m/z*, 437 [M+2, (72.34%)], 417 [M^+, (57.45%)], 361 (100%); Anal. Calcd for $C_{22}H_{18}ClN_5O_3$: C, 60.62; H, 4.16; N, 16.07; Found: C, 60.71; H, 4.23; N, 16.19.

*4.1.4.4. 1-(2-((3-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(p-tolyl)urea (**VIId**).* The titled compound was obtained as buff powder 0.12 g (81%); m.p. 292 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.29 (s, 1H), 9.11 (s, 1H), 8.80 (s, 1H), 8.53 (s, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.23 (s, 1H), 7.18 (d, *J* = 8.7 Hz, 2H), 7.12 (s, 1H), 6.96–6.87 (m, 3H), 3.75 (s, 3H), 2.27 (s, 3H); FT-IR (ν max, cm^{-1}): 3275 (NH), 3054 (CH aromatic), 2936 (CH aliphatic), 1685 (C═O amide); MS: (Mwt.: 415): *m/z*, 416 [M^++1, (1.8%)], 415 [M^+, (9.3%)], 77 (100%); Anal. Calcd for $C_{23}H_{21}N_5O_3$: C, 66.49; H, 5.09; N, 16.86; Found: C, 60.63; H, 4.04; N, 16.03.

*4.1.4.5. 1-Cyclohexyl-3-(2-((4-methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)urea (**VIIe**).* The titled compound was separated as yellow solid 0.12 g (81%); m.p. 237 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.25 (s, 1H), 9.02 (s, 1H), 8.76 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.36 (s, 1H), 7.29 (s, 1H), 7.09 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 3.74 (s, 3H), 2.03 – 0.89 (m, 5H); FT-IR (ν max, cm^{-1}): 3328 (NH), 3035 (CH aromatic), 2929 (CH aliphatic), 1627 (C═O amide); MS: (Mwt.: 407): *m/z*, 408 [M+1, (24.2%)], 407 [M^+, (3.8%)], 281 (100%); Anal. Calcd for $C_{22}H_{25}N_5O_3$: C, 64.85; H, 6.18; N, 17.19; Found: C, 64.97; H, 6.15; N, 17.32.

*4.1.5. General procedure for preparation of compounds (**VIIIa-b**)*

To a solution of **VI** (0.1 g, 0.35 mmol) in dry pyridine (10 mL) was added the appropriate sulfonyl chloride (1.05 mmol; 3 eq.) (*viz*; benzenesulfonyl chloride and 4-methylbenzenesulfonyl chloride) and heated at reflux for 2 h. The mixture was added to cold water (30 mL) and stirred for several minutes. The resulted solid was filtered, washed with water, dried and recrystallized from ethanol to afford the titled compounds.

*4.1.5.1. N-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)benzenesulfonamide (**VIIIa**).* The titled compound was separated as brown solid 0.06 g (40%); m.p. 122 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.29 (s, 1H), 10.35 (s, 1H), 9.27 (s, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.61 – 7.50 (m, 5H), 7.29 (d, *J* = 8.6 Hz, 2H), 7.08 (s, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 3.73 (s, 3H); FT-IR (ν max, cm^{-1}): 3442 (NH), 3075 (CH aromatic), 2930 (CH aliphatic), 1625 (C═O amide), 1335 & 1160 (SO2); MS: (Mwt.: 422): *m/z*, 423 [M+1, (3.16%)], 422 [M^+, (9.47%)], 77 (100%); Anal. Calcd for $C_{21}H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26; Found: C, 59.79; H, 4.33; N, 13.41.

*4.1.5.2. N-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-4-methylbenzene sulfonamide (**VIIIb**).* The titled compound was separated as brownish black solid 0.09 g (58%); m.p.116 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.27 (s, 1H), 10.26 (s, 1H), 9.21 (s, 1H), 7.97 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 7.07 (s, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 2H), 3.73 (s, 3H), 2.50 (s, 3H); FT-IR (ν max, cm^{-1}): 3404 (NH), 3062 (CH aromatic), 2926 (CH aliphatic), 1658 (C═O amide), 1335 & 1160 (SO2); MS: (Mwt.: 436): *m/z*, 437 [M^++1, (13.96%)], 436 [M^+, (28.83%)], 281 (100%); Anal. Calcd for $C_{21}H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26; Found: C, 59.79; H, 4.33; N, 13.41.

*4.1.6. 2-((2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)carbamoyl)benzoic acid (**IX**)*

A mixture of compound **VI** (0.1 g, 0.35 mmol) and phthalic anhydride (0.1 g, 0.7 mmol) in gl. acetic acid was stirred for 48 h at room temperature. The formed precipitate was filtered, washed with gl. acetic acid then water thoroughly. The solid separated was black powder with a yield of 0.1 g (65%); m.p. > 300; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.35 (s, 1H), 10.44 (s, 1H), 9.40 (s, 1H), 9.17 (s, 1H), 8.08–7.94 (m, 4H), 7.87 (s, 1H), 7.65 (d, *J* = 7.4 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 2H), 3.75 (s, 3H); FT-IR (ν max, cm^{-1}): 3390 (NH), 3400–2900 (OH carboxylic), 1685 (C═O acid), 1665 (C═O amide), 3032 (CH aromatic), 2929 (CH aliphatic); MS: (Mwt.: 430): *m/z*, 431 [M+1, (75.53%)], 430 [M^+, (55.32%)], 243 (100%); Anal. Calcd for $C_{23}H_{18}N_4O_5$ C, 64.18; H, 4.22; N, 13.02; Found: C, 64.03; H, 4.13; N, 12.95.

*4.1.7. N-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)acetamide (**X**)*

The titled compound was afforded by refluxing **VI** (0.1 g, 0.35 mmol) with acetyl chloride (0.083 g, 1.06 mmol) in THF for 24 h. It was filtered, washed with water, dried. 0.08 g (70%) of black solid was obtained; m.p. 176 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.68 (s, 1H), 10.23 (s, 1H), 9.65 (s, 1H), 7.95 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 8.6 Hz, 1H), 7.41 (d, *J* = 8.7 Hz, 2H), 7.31 (s, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 3.76 (s, 3H), 2.06 (s, 3H); FT-IR (ν max, cm^{-1}): 3447 (NH), 3035 (CH aromatic), 2984 (CH aliphatic), 1642 (C═O amide); MS: (Mwt.: 324): *m/z*, 325 [M+1, (18.7%)], 324 [M^+, (53.45%)], 281 (100%); Anal. Calcd for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.97; N, 17.27; Found: C, 63.04; H, 4.99; N, 17.34.

*4.1.8. General procedure for preparation of compounds (**XIVa-b**)*

(2 g, 9.54 mmol) of **XIII** were refluxed in ethanol (30 ml) with the appropriate amine (19.08 mol; 2 eq.) (*viz:* p-anisidine, p- chloroaniline) for 5 h, allowed to cool then finally filtered. The crude products **XIVa-b** were purified by recrystallization from ethanol.

*4.1.8.1. N-(4-Methoxyphenyl)-6-nitroquinoxalin-2-amine (**XIVa**).* The titled compound was obtained as red powder 1.8 g (63%); m.p. 218 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.31 (s, 1H), 8.61 (s, 1H), 8.56 (d, *J* = 2.6 Hz, 1H), 8.33 (dd, *J* = 9.1, 2.7 Hz, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 9.1 Hz, 2H), 6.99 (d, *J* = 9.0 Hz, 2H), 3.77 (s, 3H); FT-IR (ν max, cm^{-1}): 3447 (NH), 3035 (CH aromatic), 2984 (CH aliphatic), 1587, 1390 (NO_2); MS: (Mwt.: 296): *m/z*, 297 [M+1, (17.7%)], 296 [M^+, (84.64%)], 137 (100%); Anal. Calcd for $C_{15}H_{12}N_4O_3$: C, 60.81; H, 4.08; N, 18.91; Found C, 60.63; H, 3.96; N, 18.71.

*4.1.8.2. N-(4-Chlorophenyl)-6-nitroquinoxalin-2-amine (**XIVb**).* The titled compound was obtained as yellow powder 1.5 g (52%); m.p. 142 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.27 (s, 1H), 8.56 (s, 1H), 8.32 (d, *J* = 2.6 Hz, 1H), 8.12 (dd, *J* = 8.6, 2.7 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.64 (d, *J* = 9.1 Hz, 2H), 6.84 (d, *J* = 8.9 Hz, 2H); FT-IR (ν max, cm^{-1}): 3390 (NH), 3015 (CH aromatic), 1583, 1380 (NO_2); MS: (Mwt.: 300): *m/z*, 302 [M + 2, (32.2%)], 300 [M^+, (4.3%)], 141 (100%); Anal. Calcd for $C_{14}H_9ClN_4O_2$: C, 55.92; H, 3.02; N, 18.63; Found C, 55.75; H, 2.96; N, 18.23.

*4.1.9. General procedure for preparation of compounds (**XVa-b**)*

Reduction of **XIVa-b** (1 g, 3.33 mmol) was carried out by adding (0.1 g) 10% Pd-C wet to 50 ml of their solutions in THF/MeOH then stirring under H_2 at room temperature for 8 h. After removing the catalyst by filtration with celite and evaporation of the filtrate under reduced pressure, we afforded the titled compounds **XVa-b**.

*4.1.9.1. N2-(4-Methoxyphenyl)quinoxaline-2,6-diamine (**XVa**).* The titled compound was obtained as brown solid 0.6 g (67%); m.p. 247 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.27 (s, 1H), 8.56 (s, 1H), 8.32 (d, *J* = 2.6 Hz, 1H), 8.12 (dd, *J* = 7.5, 2.7 Hz, 1H), 7.76

(d, J = 8.5 Hz, 1H), 7.64 (d, J = 9.1 Hz, 2H), 6.84 (d, J = 7.6 Hz, 2H), 5.37 (s, 2H), 3.80 (s, 3H); FT-IR (ν max, cm^{-1}): 3420 (forked NH_2), 3115 (CH aromatic), 2932 (CH aliphatic); MS: (Mwt.: 266): *m/z*, 267 [M+1, (16.4%)], 266 [M^+, (1.7%)], 145 (100%); Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04; Found: C, 67.36; H, 5.12; N, 20.93.

*4.1.9.2. N2-(4-Chlorophenyl)quinoxaline-2,6-diamine (**XVb**).* The titled compound was separated as brown solid 0.75 g (83%); m.p.159 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.13 (s, 1H), 8.23 (s, 1H), 8.06 (d, J = 2.6 Hz, 1H), 7.89 (dd, J = 7.5, 2.7 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 9.1 Hz, 2H), 6.54 (d, J = 7.6 Hz, 2H), 4.96 (s, 2H); FT-IR (ν max, cm^{-1}): 3420 (forked NH_2), 3115 (CH aromatic); MS: (Mwt.: 270): *m/z*, 272 [M + 2, (15.3%)], 270 [M^+, (32%)], 141 (100%); Anal. Calcd for $C_{14}H_{11}ClN_4$: C, 62.11; H, 4.10; N, 20.70; Found: C, 62.01; H, 4.00; N, 20.52.

*4.1.10. General procedure for preparation of compounds (**XVIa-e**)*

To a solution of **XVa-b** (0.1 g, 0.37 mmol) in methylene chloride was added the appropriate isocyanate or isothiocyanate (0.7 mmol; 2 eq.) (*viz*; phenyl isothiocyanate, phenyl isocyanate, 3-methyl phenyl isocyanate, and cyclohexyl isocyanate) and stirred overnight. The resulted solid was filtered, triturated with diethyl ether and recrystallized from ethanol to afford the titled compounds. (**XVIa-e**)

*4.1.10.1. 1-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)-3-phenylthiourea (**XVIa**).* The titled compound was separated as yellow solid; m.p. 249 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.01 (s, 1H), 9.92 (s, 1H), 9.72 (s, 1H), 8.48 (s, 1H), 7.98 (d, J = 2.4 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.73 (dd, J = 8.9, 2.4 Hz, 1H), 7.61 (d, J = 8.9 Hz, 2H), 7.54–7.45 (m, 2H), 7.38–7.29 (m, 1H), 7.15 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 9.1 Hz, 2H), 3.76 (s, 3H); FT-IR (ν max, cm^{-1}): 3383 (NH), 3054 (CH aromatic), 2952 (CH aliphatic), 1660 (C═O amide),; MS: (Mwt.: 401): *m/z*, 402 [M^++1, (26.4%)], 401 [M^+, (5.2%)], 166 (100%); Anal. Calcd for $C_{22}H_{19}N_5OS$: C, 65.81; H, 4.77; N, 17.44; Found: C, 65.63; H, 4.54; N, 17.21.

*4.1.10.2. 1-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)-3-phenylurea (**XVIb**).* The titled compound was obtained as yellow powder; m.p. 134 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.63 (s, 1H), 8.59 (s, 1H), 8.52 (s, 1H), 8.37 (s, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 2.4 Hz, 1H), 7.27 (dd, J = 8.6, 2.3 Hz, 1H), 7.06–6.88 (m, 5H), 6.56 (d, J = 8.6 Hz, 2H), 6.48 (d, J = 8.3 Hz, 2H); FT-IR (ν max, cm^{-1}): 3383 (NH), 3054 (CH aromatic), 1660 (C = O amide),; MS: (Mwt.: 389): *m/z*, 391 [M + 2, (32.4%)], 389 [M^+, (22.9%)], 269 (100%); Anal. Calcd for $C_{21}H_{16}ClN_5O$: C, 64.70; H, 4.14; N, 17.96; Found: C, 64.52; H, 4.03; N, 17.64.

*4.1.10.3. 1-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)-3-(m-tolyl)urea (**XVIc**).* The titled compound was separated as buff solid; m.p.216 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.61 (s, 1H), 8.89 (s, 1H), 8.66 (s, 1H), 8.46 (s, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.62 (s, 1H), 7.33 (s, 1H), 7.27 (d, J = 8.1 Hz, 1H), 7.22–7.12 (m, 3H), 6.95 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 7.4 Hz, 2H), 3.75 (s, 3H), 2.29 (s, 3H); FT-IR (ν max, cm^{-1}): 3383 (NH), 3054 (CH aromatic), 2935 (CH aliphatic), 1685 (C═O amide); MS: (Mwt.: 399): *m/z*, 400 [M+1, (26.8%)], 399 [M^+, (2.3%)], 164 (100%); Anal. Calcd for $C_{23}H_{21}N_5O_2$: C, 69.16; H, 5.30; N, 17.53; Found: C, 69.28; H, 5.28; N, 17.69.

*4.1.10.4. 1-Cyclohexyl-3-(2-((4-methoxyphenyl)amino)quinoxalin-6-yl)urea (**XVId**).* The titled compound was separated as yellowish solid; m.p. 258 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.55 (s, 1H), 8.51 (d, J = 8.8 Hz 1H), 8.43 (s, 1H), 7.96 (s, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.54 (s, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.12 (d, J = 7.8 Hz, 2H), 3.81 (s, 1H), 3.75 (s, 3H), 1.95–1.05 (m, 5H); FT-IR (ν max, cm^{-1}): 3380 (NH), 3020 (CH aromatic), 2926 (CH aliphatic), 1680 (C═O amide); MS: (Mwt.: 391): *m/z*, 392 [M+1, (25.6%)], 391 [M^+, (3.2%)], 265 (100%); Anal. Calcd for $C_{22}H_{25}N_5O_2$: C, 67.50; H, 6.44; N, 17.89; Found: C, 67.61; H, 6.48; N, 18.02.

*4.1.10.5. 1-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)-3-cyclohexylurea (**XVIe**).* The titled compound was separated as yellow solid; m.p. 194 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.55 (s,1H), 8.51 (s,1H), 8.40 (s, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.38(d, J = 8.6 Hz, 1H), 7.36 (s, 1H), 7.24 (d, J = 8.9 Hz, 2H), 6.07 (d, J = 7.8 Hz, 2H), 5.75 (s, 1H), 1.91–1.00 (m, 5H); FT-IR (ν max, cm^{-1}): 3430 (NH), 3016 (CH aromatic), 2920 (CH aliphatic), 1670 (C═O amide); MS: (Mwt.: 395): *m/z*, 397 [M+2, (32.4%)], 395 [M^+, (1.6%)], 269 (100%); Anal. Calcd for $C_{21}H_{22}ClN_5O$: C, 63.71; H, 5.60; N, 17.69; Found: C, 63.61; H, 5.48; N, 17.52.

*4.1.11. General procedure for preparation of compounds (**XVIIa-c**)*

To a solution of the appropriate amine **XVa-b** (0.1 g, 0.37 mmol) in dry pyridine (10 mL) the appropriate sulfonyl chloride was added (1.05 mmol; 3 eq.) (*viz*; benzenesulfonyl chloride and 4- methylbenzenesulfonyl chloride) and heated at reflux for 2 h. The mixture was added to cold water (30 mL) and stirred for several minutes. The resulted solid was filtered, washed with water, dried and recrystallized from ethanol to afford the titled compounds.

*4.1.11.1. N-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)benzenesulfonamide (**XVIIa**).* The titled compound was separated as black solid; m.p. 218 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.58 (s, 1H), 8.94 (d, J = 3.4 Hz, 1H), 8.63 (s, 1H), 8.53 (s, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.60–7.46 (m, 5H), 7.41 (dd, J = 8.7, 2.5 Hz, 1H), 7.31 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 3.74 (s, 3H); FT-IR (ν max, cm^{-1}): 3390 (NH), 3045 (CH aromatic), 2926 (CH aliphatic),1330 & 1150 (SO2); MS: (Mwt.: 406): *m/z*, 407 [M+1, (25.1%)], 406 [M^+, (5.7%)], 251 (100%); Anal. Calcd for $C_{21}H_{18}N_4O_3S$: C, 62.05; H, 4.46; N, 13.78; Found: C, 62.13; H, 4.49; N, 13.86.

*4.1.11.2. N-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)-4-methylbenzenesulfonamide (**XVIIb**).* The titled compound was obtained as brownish solid; m.p. 166 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.40 (s, 1H), 9.74 (s, 1H), 8.75 (d, J = 3.2 Hz, 1H), 8.44 (s, 1H), 7.81 (d, J = 8.9 Hz, 2H), 7.66 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.38 (dd, J = 8.9, 2.5 Hz, 1H), 7.33 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 9.0 Hz, 2H), 3.74 (s, 3H), 2.30 (s, 3H); FT-IR (ν max, cm^{-1}): 3350 (NH), 3092 (CH aromatic), 2910 (CH aliphatic),1350 & 1165 (SO2); MS: (Mwt.: 420): *m/z*, 421 [M+1, (24.1%)], 420 [M^+, (4.5%)], 185 (100%); Anal. Calcd for $C_{22}H_{20}N_4O_3S$: C, 62.84; H, 4.79; N, 13.32; Found: C, 62.97; H, 4.84; N, 13.39.

*4.1.11.3. N-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)-4-methylbenzenesulfonamide (**XVIIc**).* The product was separated as brownish black solid; m.p. 268 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.52 (s, 1H), 10.23 (s, 1H), 8.55 (s, 1H), 7.98 (d, J = 2.3 Hz, 1H), 7.95 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.43 (dd, J = 8.9, 2.6 Hz, 1H), 7.36 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 2.29 (s, 3H); FT-IR (ν max, cm^{-1}): 3420 (NH), 3055 (CH aromatic), 2905 (CH aliphatic),1400 & 1160 (SO2); MS: (Mwt.: 424): *m/z*, 426 [M+2, (36.5%)], 424 [M^+, (23.8%)], 127 (100%); Anal. Calcd for $C_{21}H_{17}ClN_4O_2S$: C, 59.36; H, 4.03; N, 13.19; Found: C, 59.25; H, 4.01; N, 13.08.

*4.1.12. N-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)acetamide (**XVIII**)*

The titled compound was afforded by stirring **XVb** (0.1 g, 0.37 mmol) with acetyl chloride (0.083 g, 1.06 mmol) in glacial acetic acid (10 ml) overnight. It was filtered, washed with water thoroughly and dried, giving brownish black solid with a yield of 0.06 g (52%); m.p. 176 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.17

(s, 1H), 9.95 (s, 1H), 8.51 (s, 1H), 8.24 (d, J = 2.3 Hz, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.77 (dd, J = 8.9, 2.4 Hz, 1H), 7.68 (d, J = 8.9 Hz, 2H), 7.40 (d, J = 8.9 Hz, 2H), 2.10 (s, 3H); FT-IR (ν max, cm^{-1}): 3380 (NH), 3032 (CH aromatic), 2932 (CH aliphatic), 1665 (C=O amide); MS: (Mwt.: 312): m/z, 314 [M^++2, (17.5%)], 312 [M^+, (5.6%)], 255 (100%); Anal. Calcd for $C_{16}H_{13}ClN_4O$: C, 61.44; H, 4.19; N, 17.91; Found: C, 61.36; H, 4.02; N, 17.85.

*4.1.13. General procedure for preparation of compounds (**XXIa-b**)*

The final compounds **XXIa-b** were synthesized by refluxing **XIII** (0.5 g, 2.39 mmol) with the 4-aminophenyl urea derivatives **XXa-b** (1 g, 4.4 mmol) in ethanol (20 ml) and in presence of anhydrous K_2CO_3 (0.6 g, 4.4 mmol) for 6 h. The reaction mixture is allowed to cool, filtered and the resulted solid product is washed thoroughly with water, left to dry.

*4.1.13.1. 1-(4-((6-Nitroquinoxalin-2-yl)amino)phenyl)-3-phenylurea (**XXIa**).* The titled compound was obtained as red powder, 0.8 g (83.7%); m.p. 258 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.97 (s, 1H), 8.61 (s, 1H), 8.57 (s, 1H), 8.33 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 8.3 Hz, 2H),7.52 (s,1H), 7.49 (d, J = 8.1 Hz, 2H), 7.40–7.18 (m, 5H), 6.95 (s, 1H); FT-IR (ν max, cm^{-1}): 3410 (NH), 3015 (CH aromatic), 1660 (C=O amide), 1540, 1350 (NO_2); MS: (Mwt.: 400): m/z, 401 [M+1, (25.2%)], 400 [M^+, (2.5%)], 226 (100%); Anal. Calcd for $C_{21}H_{16}N_6O_3$: C, 62.99; H, 4.03; N, 20.99; Found: C, 63.10; H, 4.07; N, 21.13.

*4.1.13.2. 1-(3-Chlorophenyl)-3-(4-((6-nitroquinoxalin-2-yl)amino)-phenyl)urea (**XXIb**).* Compound **XXIb** was obtained as red–orange powder in 0.75 g (72%); m.p. 280 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.85 (s,1H), 8.68 (s, 1H), 8.3 (s,1H,8.19 (s, 1H), 7.69 (s, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.34–7.18 (m, 3H), 7.06 (d, J = 8.3 Hz, 1H), 6.97 (d, J = 8.3 Hz, 2H), 6.51 (d, J = 8.2 Hz, 2H), 4.77 (s, 1H); FT-IR (ν max, cm^{-1}): 3400 (NH), 3010 (CH aromatic), 1650 (C=O amide), 1530, 1320 (NO_2); MS: (Mwt.: 434): m/z, 436 [M + 2, (33.1%)], 434 [M^+, (25%)], 175 (100%); Anal. Calcd for $C_{21}H_{15}ClN_6O_3$: C, 58.00; H, 3.48; N, 19.33; Found C, 58.09; H, 3.46; N, 19.51.

4.2. In vitro enzyme inhibition assay

In vitro kinase inhibition assay of the twenty final compounds was performed by KINEXUS Corporation, Vancouver, British Columbia, Canada. In a designated radioactive working area, a radioactive assay format for profiling evaluation of protein kinase targets is performed. Assays are performed for 15 min duration, at 30 °C, with 50 μM of ^{33}P-ATP in a final volume of 25 μl. The assays are terminated by spotting 20 μl of the reaction mixture onto a phosphocellulose P81 plate. The P81 plate was washed 3 times for approximately 15 min each in a 1% phosphoric acid solution to remove excess unreacted ^{33}P-ATP. The radioactivity on the ^{33}P-labeled peptide/protein in the P81 plate was counted in the presence of scintillation fluid in a Trilux scintillation counter. Blank control was set up for each protein kinase target which included all the assay components except the addition of appropriate substrate in which we replace with equal volume of kinase assay buffer. The corrected activity for the target was determined by removing the blank control value.

4.3. Molecular docking

The X-ray crystal structure of VEGFR-2 kinase was obtained from Protein Data Bank (accession code is 3B8Q). The protein structure was prepared according to the standard protein preparation procedure integrated in Accelry's discovery studio 2.5. This was accomplished by adding hydrogen atoms, completing the missing loops and applying force field parameters by using CHARMm force field. Water molecules were preserved because of their importance in ligand interaction to VEGFR-2 enzyme. The protein structure was minimized using steepest descent minimization algorithm. The integrated naphthyl-based compound was removed from the binding site. Our ligands were prepared using Accelry's discovery studio prepare ligands protocol. This adds hydrogen atoms and minimizes them. Docking was accomplished using GOLD 4.1 software in the interface of Accelry's discovery studio 2.5. The default values of GOLD were used but with enabling early termination and allowing generating diverse solutions to get more possible docking solutions. Also the number of docking iterations was raised to 500,000 ones so high number of predictions was obtained. 10 Different docking solutions were generated and were inspected thoroughly for getting the best binding mode.

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