Arch. Pharm. Chem. Life Sci. 2017, *350*, e1600377

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Full Paper

Design, Synthesis, and Cytotoxic Evaluation of Certain 7-Chloro-4-(piperazin-1-yl)quinoline Derivatives as VEGFR-II Inhibitors

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Signaling pathway inhibition of VEGFR-II is visualized as valuable tool in cancer management. In the current study, the synthesis of novel 1-4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-(*N*-substituted-amino)-ethanone derivatives (**4a–t**) was achieved through the amination of 2-chloro-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (**3**) with different secondary amines. The structures of the target compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, HRMS, and microanalysis. Compounds **4a–t** were subjected to *in vitro* anticancer screening against human breast cancer (MCF-7) and prostate cancer (PC3) cell lines. The highest cytotoxicty against both cell lines was displayed by 2-(4-(4-bromobenzyl)piperazin-1-yl)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (**4q**), with IC_{50} values of 6.502 and 11.751 μM against MCF-7 and PC3 cells, respectively, compared with the standard drug doxorubicin (MCF-7: 6.774 μM, PC3: 7.7316 μM). Due to its notable activity toward MCF-7 cells, **4q** was further evaluated as VEGFR-II inhibitor, showing an IC_{50} of 1.38 μM compared to sorafenib (0.33 μM). The docking study proved that **4q** has a binding mode akin to that of VEGFR-II inhibitors.

Keywords: Chloro-4-(piperazin-1-yl)quinolines / Cytotoxic evaluation / Human breast cancer cell line (MCF-7) / Human prostate cancer cell line (PC3) / VEGFR-II

Received: December 13, 2016; Revised: February 19, 2017; Accepted: February 22, 2017

DOI 10.1002/ardp.201600377

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

Cancer is one of the major causes of fatality worldwide. It is a cluster of diseases characterized by uninhibited enlargement. The rising frequency of drug tolerance to anticancer chemotherapy constitutes a serious medical problem [1]. Thus, there is an importunate demand to discover and develop novel chemotherapeutic anticancer agents [2]. The angiogenesis has a critical function in cancer cell survival through tumor cell growth [3, 4]. Consequently, the growth factors including: vascular endothelial growth factor (VEGF) [5], epidermal growth factor (EGF) [6], platelet-derived growth factor (PDGF) [7], and basic fibroblast growth factor (bFGF) [8] play an important role in the regulation of angiogenesis. However, the VEGF is considered as the most crucial factor compared to the other growth factors in the angiogenesis process [9]. The family of VEGF consists of six members namely, VEGF-A, B, C, D, E, and placenta growth factor. They bind to VEGF receptors: VEGFR-I, VEGFR-II, and VEGFR-III, which lead to the proliferation and survival of the endothelial cells and consequently tumor formation [10]. Among VEGFRs, the VEGFR-II is the most significant mediating receptor of all the cellular responses to

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VEGF [11]. Accordingly, the inhibition of VEGFR-II is envisioned to be a target in cancer management.

Noteworthy, VEGFR-II inhibitors having 4-substituted quinoline structural framework have been disclosed as antitumor agents and entered the drug market, such as lenvatinib **(I)** (Lenvima®) [12], cabozantinib **(II)** (Cometriq®) [13] (Fig. 1).

The 4-piperazinoquinoline core in general and 7-chloro-4-(piperazin-1-yl) quinoline in particular have enriched the medicinal chemistry library with many anticancer bioactive candidates. For example, **III** [14], **IV** [15], **V** [16], **VI** [17] (Fig. 2A).

Moreover, the aminoacyl pharmacophoric chain has been perceived to be incorporated in the structural skeleton of numerous potent antitumor compounds such as **VII** [18], **VIII** [19], **IX** [20], as well as the VEGFR-II inhibitor nintedanib **(X)** (Ofev®, Vargatef®) [21] (Fig. 2B).

Thus, the impetus of the present work is the design and synthesis of the hybrids 1-4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-(*N*-substituted-amino)ethanone derivatives **4a–h** and **4i–t** that involve both the 4-piperazinoquinoline and aminoacyl pharmacophoric moieties to be screened for their antitumor effect on breast (MCF-7) and prostate (PC3) cancer cell lines. The VEGFR-II inhibitory effect for the most active compound was assayed, and its molecular docking study was performed as well (Fig. 2C).

Results and discussion

Chemistry

Through the literature survey, it was reported that Singh et al. [22] synthesized 7-chloro-4-piperazin-1-yl-quinoline (**2**) by heating 4,7-dichloroquinoline with anhydrous piperazine in 2-ethoxyethanol. This method was modified and adopted in this work using ethanol instead of 2-ethoxyethanol to obtain **2** in 80% yield. The chloroacetylation of **2** has been achieved through the reaction of **2** with one and half equivalent of chloroacetyl chloride in chloroform to afford **3** in 82% yield (Scheme 1).

A one-pot two-component reaction was carried out for the coupling between the chloroacetyl penultimate synthon **3** with different secondary amines (Scheme 2) in refluxing ethanol to obtain **4a–h** in 68–76% yields (Scheme 2).

Various methods have been cited for the synthesis of monobenzylpiperazines (**5o–t**) using: (i) the corresponding benzyl halides with piperazine [23–27]; (ii) the corresponding aldehydes through reductive amino alkylation [28, 29]; or (iii) through catalytic reduction of benzyldi-(cyanomethyl)-amine [30]. In this work, **5o–t** were achieved in 74–78% yield by refluxing the corresponding benzyl chlorides with 10 equivalents anhydrous piperazine in ethanol. The target dipiperazines **4i–t** have been furnished in 55–78% yields by adopting the previous procedure of refluxing **3** with the appropriate 1-piperazine derivative (**5i–t**) in the presence of sodium carbonate in ethanol (Scheme 3).

Biological activities

In vitro breast (MCF-7) and prostate cancer cell (PC3) cytotoxic activities

The determination of the *in vitro* cytotoxic effect against the human breast (MCF-7) and prostate (PC3) cancer cell lines for the newly synthesized compounds **4a–h** and **4i–t** as well as the reference standard, doxorubicin, was performed (Table 1). Concerning compounds **4a–h**, their cytotoxic effect against breast cancer (MCF-7) cell line showed that they were five- to ninefold less cytotoxic compared to doxorubicin.

Regarding the antitumor activity of **4a–h** against human prostate cancer (PC3) cell line, the data in Table 1 revealed that the cytotoxicity of compounds **4c** and **4b** reached 2.5–3.5-fold less than doxorubicin. In addition, the other derivatives possessed moderate cytotoxic activity from five- to sevenfold less than the reference standard.

Concerning the cytotoxic effect of the target dipiperazine ethanone derivatives **4i–t** against the human breast cancer cell line MCF-7, **4q** exhibited the highest cytotoxicity. It displayed slight higher cytotoxic activity when compared with the reference drug doxorubicin. When the 4-bromobenzylpiperazine moiety of **4q** was replaced by the corresponding 4-methoxy one as in **4s** the cytotoxic effect was reduced to about its half value. Also, the introduction of the 3,4,5-trimethoxybenzyl piperazine in **4t** showed less activity by 3.3-fold than both **4q** and doxorubicin. On the other hand, the introduction of certain other substituted piperazine moieties as in **4i**, **4j**, **4l–p**, and **4r** did not show any interesting activity.

Regarding the cytotoxicity against prostate (PC3) human cancer cell line, the results in Table 1 revealed that **4q** exhibited the highest cytotoxic effect all over series **4i–t**, it was about 0.66-fold that of doxorubicin as reference drug. The

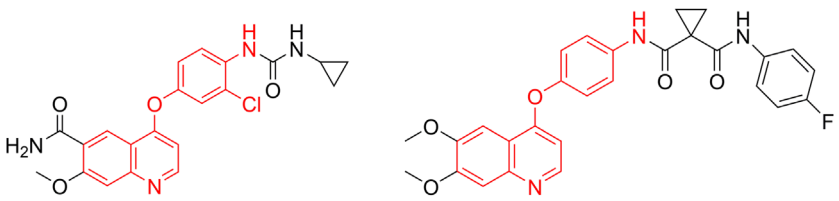

Figure 1. Certain marketed VEGFR-II TK inhibitors having 4-substituted quinoline moiety.

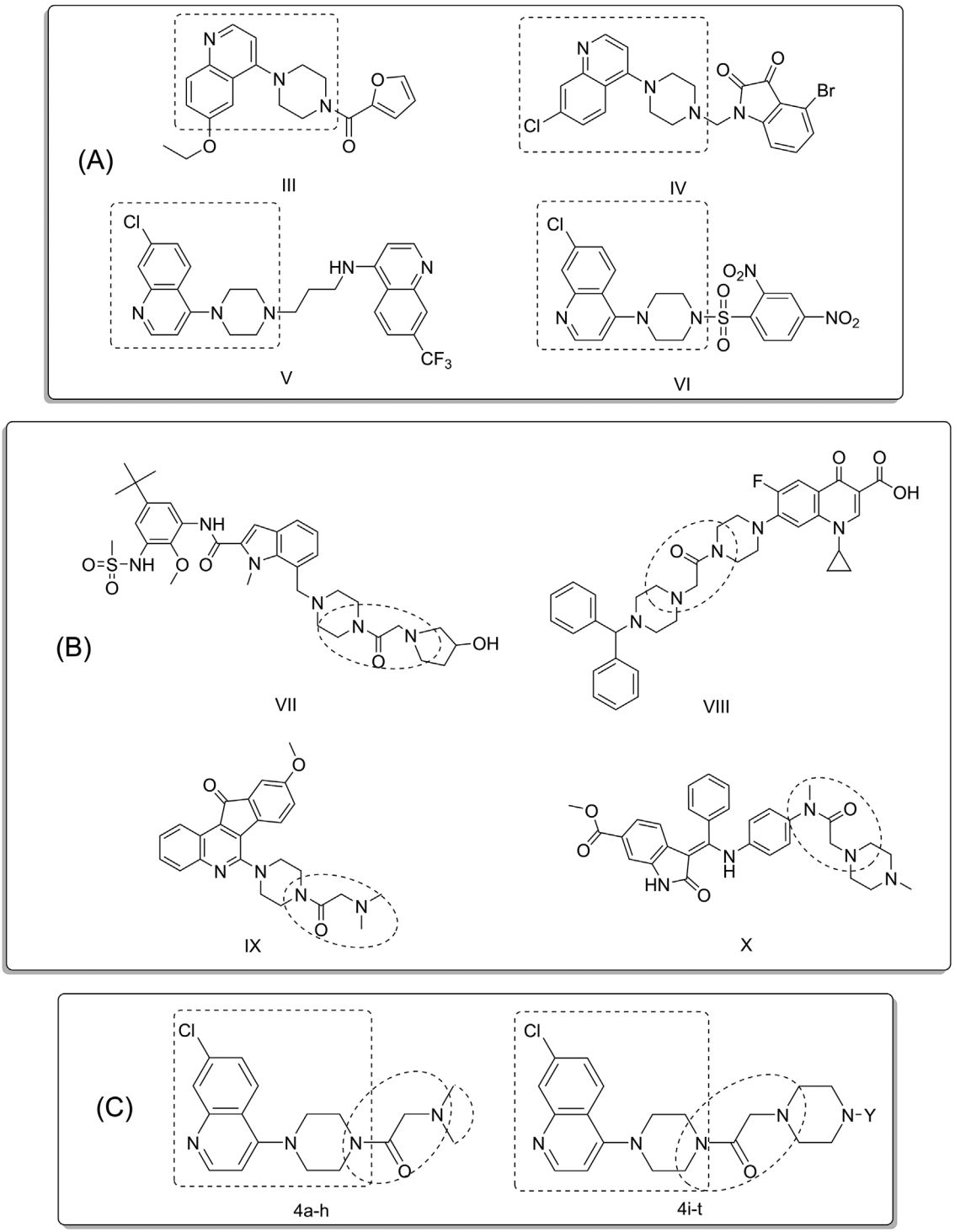

Figure 2. Structures of selected antitumor biocandidates bearing 4-piperazinoquinoline (A), aminoacyl moities (B), and the target compounds **4a–h** and **4i–t** (C).

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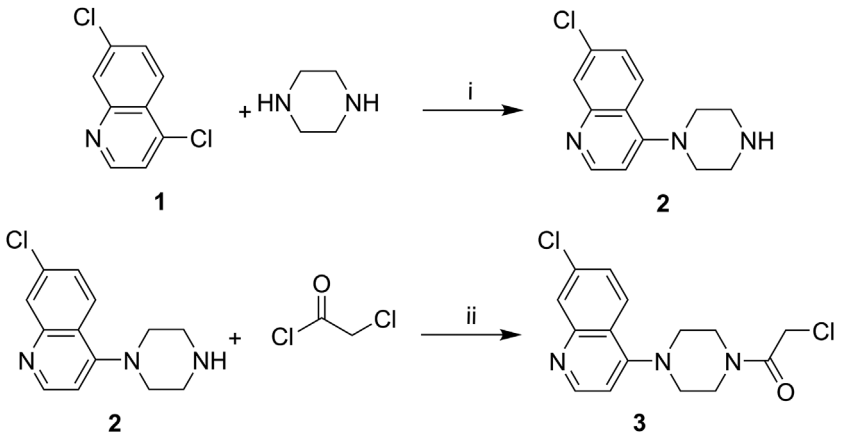


Scheme 1. Synthesis of synthon **3**. Reagants and conditions. (i) EtOH, reflux/8 h, (ii) chloroform/4 h at room temperature.

4-chlorobenzylpiperazine derivative **4p** and 3,4,5-timethoxybenzylpiperazine derivative **4t** as well as 4-methoxybenzylpiperazine derivative **4s** showed nearly similar antitumor activity against PC3 cell line that was less active than the most active candidate **4q** by about 3.4-fold. Whilst, compounds **4i**, **4k**, **4m–o**, and **4r** showed no pharmacological significance in terms of *in-vitro* antitumor activity.

Conclusively, compound **4q** was the most active compound of the series **4a–t** on both breast (MCF-7) and prostate (PC3) human cancer cell lines.

Cytotoxic effect against normal cell lines

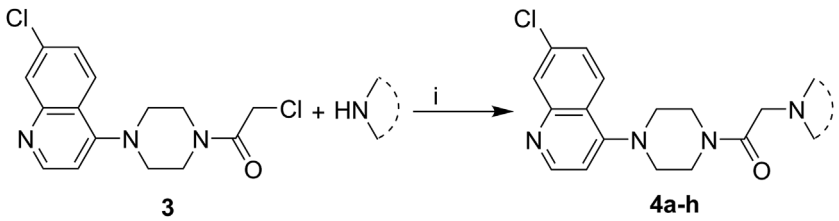
In order to determine the cytotoxic effect of the most active compound **4q** against normal cell, it was evaluated against both normal breast (MCF12A) and primary normal peripheral blood mononuclear (PCS-800-011) cell lines. The obtained IC_{50} values were 37.95 and 33.16 μM against (MCF12A) and (PCS-800-011), respectively. These values represent 5- and 2.8-fold more than the IC_{50} values of **4q** against MCF-7 and PC3, respectively.

In vitro VEGFR-II inhibition assay

The most active compound 2-(4-(4-bromobenzyl)piperazin-1-yl)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (**4q**) was selected to be evaluated for its inhibitory activity against VEGFR-II using Human VEGFR-R2/KDR ELISA (BioVender®, Czech Republic). The result was reported as a 50% inhibition concentration value (IC_{50}) calculated from the concentration–inhibition response curve. Compound **4q** displayed IC_{50} of 1.38 μM, compared with the reference drug sorafenib (IC_{50} of 0.33 μM).

Molecular modeling

It is worth to mention that compound **4q** has relatively similar binding mode to that of sorafenib and lenvatinib at



4	
a	Dimethylamine
b	Diethylamine
c	Dipropylamine
d	Pyrrolidin-1-yl
e	1H-imidazol-1-yl
f	Piperdin-1-yl
g	4-Hydroxypiperdin-1-yl
h	Morpholin-1-yl

Scheme 2. Synthesis of the target compounds **4a–h**. Reagents and conditions. (i) EtOH, reflux/8 h.

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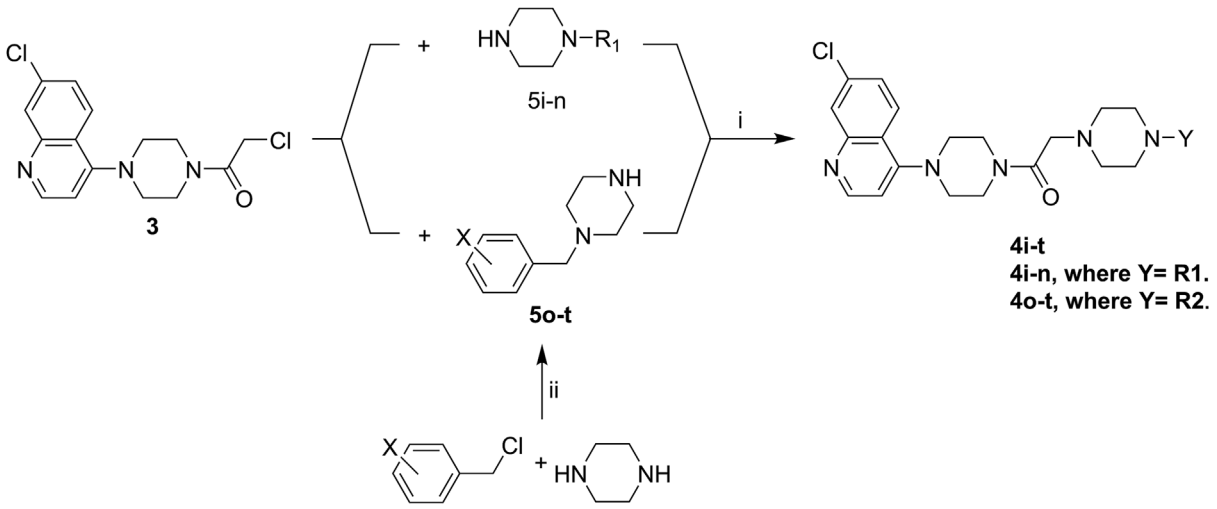

4, 5	R_1
i	Methyl
j	Ethyl
k	Phenyl
l	4-Chlorophenyl
m	4-Methoxyphenyl
n	4-Ethoxyphenyl

4	R_2
o	Benzyl
p	4-Chlorobenzyl
q	4-Bromobenzyl
r	4-Methylbenzyl
s	4-Methoxybenzyl
t	3,4,5-Trimethoxybenzyl

5	X
o	H
p	4-Cl
q	4-Br
r	4-CH_3
s	4-OCH_3
t	3,4,5-$(OCH_3)_3$

Scheme 3. Synthesis of compounds **4i–t**. Reagents and conditions: (i) EtOH and Na_2CO_3 reflux/12 h, (ii) EtOH reflux/12 h.

the ATP binding site of VEGFR-II (Fig. 3), where, both the carbonyl group of the aminoacyl moiety, and the nitrogen atom of the quinoline ring are bound with hydrogen bonds to Asp1046 and Cys919, respectively, while the quinoline ring shows pi-interaction with Ile888, and Phe918. In addition, the docking score of **4q** (−57.5135) was slightly better than that of lenavatinib (−54.0756) but less than sorafenib (−68.8191) (Table 2).

Conclusion

It is worthwhile to mention that compound **4q** displayed the highest cytotoxic activity among the series **4a–t** against both breast (MCF-7) and prostate (PC3) cancer cell lines, with IC_{50} values of 6.502 μM, and 11.751 μM, respectively, compared to doxorubicin (IC_{50}: 6.774 μM for MCF-7, and 7.731 μM for PC3). Since compound **4q** exhibited IC_{50} less than 10 μM on the MCF-7 cell line, it was selected to be further assayed as VEFGR-II inhibitor. It showed IC_{50} of 1.38 μM, compared with sorafenib (0.33 μM). The docking study proved that it has a binding mode akin to that of VEGFR-II inhibitors.

Experimental

Chemistry

General

All melting points were uncorrected and determined with an electrothermal capillary melting point apparatus. Infrared (IR) spactra were recorded on a JASCO FT/IR-6100 spectrometer and values are represented in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were carried out on Bruker Avance 300 and Jeol ECA 500 MHz spectrometers (National Research Centre, Dokki, Egypt) using TMS as an internal standard. Chemical shift values are recorded in ppm δ scale. The ^{1}H-NMR data were represented as follows: chemical shifts, multiplicity (s: singlet, d: doublet, dd: doublet of doublet, t: triplet, q: quartet, m: multiplet, and br: broad), number of protons, and type of protons. ^{13}C-NMR data are represented as chemical shifts. Mass spectral data were obtained with electron impact (EI) ionization technique at 70 eV from a Finnigan Mat SSQ-7000 spectrometer. High resolution mass analysis was performed at the National Research Centre, Dokki, Egypt and the Institute of Organic Chemistry and Biochemistry, the Czech Academy of Sciences, Prague 6, Czech Republic. Elemental analyses were carried out in the Microanalytical

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Table 1. ***In-vitro*** **cytotoxicity assessment of the screened candidates 3 and 4a–t against human breast (MCF7) and prostate (PC3) cancer cell line.**

Compound	Molecular weight (g)	MCF-7		PC3	
		IC_{50} (μg/mL)	IC_{50} (μM)	IC_{50} (μg/mL)	IC_{50} (μM)
4a	332.83	14.5	43.565	18.1	54.382
4b	360.88	18.8	52.094	10	27.710
4c	388.93	16.9	43.452	7.58	19.489
4d	358.87	12.2	33.995	15.1	42.076
4e	355.82	11.5	32.319	N/A	
4f	372.89	15.8	42.371	20.9	56.048
4g	388.89	14.8	38.057	19.1	49.114
4h	374.86	21.7	57.888	17	45.350
4i	387.91	19	48.980	21.6	55.683
4j	401.93	12.1	30.104	14.7	36.573
4k	449.98	17.9	39.779	N/A	
4l	484.42	15.2	31.377	22.3	46.034
4m	480.00	41.2	85.833	N/A	
4n	494.03	22	44.531	38	76.91
4o	464.00	22.9	49.782	40.1	86.422
4p	498.45	16.3	32.701	19.6	39.321
4q	542.9	3.53	6.502	6.38	11.751
4r	478.03	21.4	47.767	40.9	85.559
4s	494.03	6.75	13.633	20.2	40.888
4t	554.08	12.5	22.559	22.3	40.246
Doxorubicin	543.2	3.68	6.774	4.2	7.731

N/A, means the IC_{50} not achieved at 50 μg/mL.
IC_{50} value is the compound concentration needed to inhibit tumor cell line proliferation by 50%.
These values are the means of 0, 5, 12.5, 25, 50 μg/mL experiments.

Unit, National Research Centre, Egypt. Purification has been achieved through column chromatography using silica gel as a stationary phase, and a mixture of ethyl acetate/methanol (8:2) as a mobile phase. All commercially available chemicals and solvents were used without further purification.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*Synthesis of 7-chloro-4-(piperazin-1-yl)quinoline (**2**)*

To a solution of 4,7-dichloroquinoline (**1**) (2.05 g, 10.1 mmol) in ethanol (30 mL), anhydrous piperazine (8.73 g, 101 mmol) was added. The mixture was refluxed under stirring for 8 h, then the solvent was evaporated under vacuum. The residual was treated with an aqueous solution of Na_2CO_3 (10%, 50 mL), then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine (30 mL), dried (anhydrous Na_2SO_4), and evaporated under reduced pressure to afford 2.01 g of **2** as buff powder in 78% yield, m.p. 114°C [24]. IR (KBr cm^{-1}): 3254.29 (NH, secondary amine). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 1.755 (s, 1H, NH, piperazine), 3.019 (br s, 8H, $N(CH_2CH_2)_2NH$), 6.664–6.675 (d, J = 5.35 Hz, $1H_{Ar}$), 7.259–7.277 (d, J = 9.2 Hz, $1H_{Ar}$), 7.791–7.809 (d, J = 9.2 Hz, $1H_{Ar}$), 7.923 (s, $1H_{Ar}$), 8.564–8.575 (d, J = 5.35 Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 46.07, 53.53, 108.95, 121.90, 125.32, 126.01, 128.80, 134.73, 150.13, 151.98, 157.33.

*Synthesis of 2-chloro-1-(4-(7-chloroquinolin-4-yl)-piperazin-1-yl)ethanone (**3**)*

To a solution of **2** (2.4 gm, 9.7 mmol) in chloroform (30 mL), chloroacetylchloride (1.317 gm ≈ 0.95 mL, 11.66 mmol) was added. The reaction mixture was stirred for 4 h under room temperature. An aqueous solution of 10% NaOH (2 × 30 mL) was added to the mixture. The organic layer was separated, dried (anhydrous Na_2SO_4) and evaporated under vacuum to afford **3** as yellow crystals with 82% yield, m.p. 150°C [24]. IR (KBr cm^{-1}): 1644.98 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$): δ 3.152–3.213 (m, 4H, piperazine), 3.762–3.851 (m, 4H, piperazine), 4.111 (s, 2H, $COCH_2Cl$), 6.785–6.791 (d, J = 2.9 Hz, $1H_{Ar}$), 7.381–7.398 (d, J = 8.6 Hz, $1H_{Ar}$), 7.863–7.879 (d, J = 8 Hz, $1H_{Ar}$), 7.991 (s, $1H_{Ar}$), 8.662–8.668 (d, J = 2.85 Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$): δ 40.95, 42.13, 46.33, 51.86, 52.15, 109.44, 121.74, 124.83, 126.75, 128.88, 135.28, 149.91, 151.83, 156.33, 165.40. MS: 327.63. Anal. calcd. for $C_{15}H_{15}Cl_2N_3O$: C, 55.57; H, 4.66; N, 12.96. Found C, 55.77; H, 4.76; N, 13.16.

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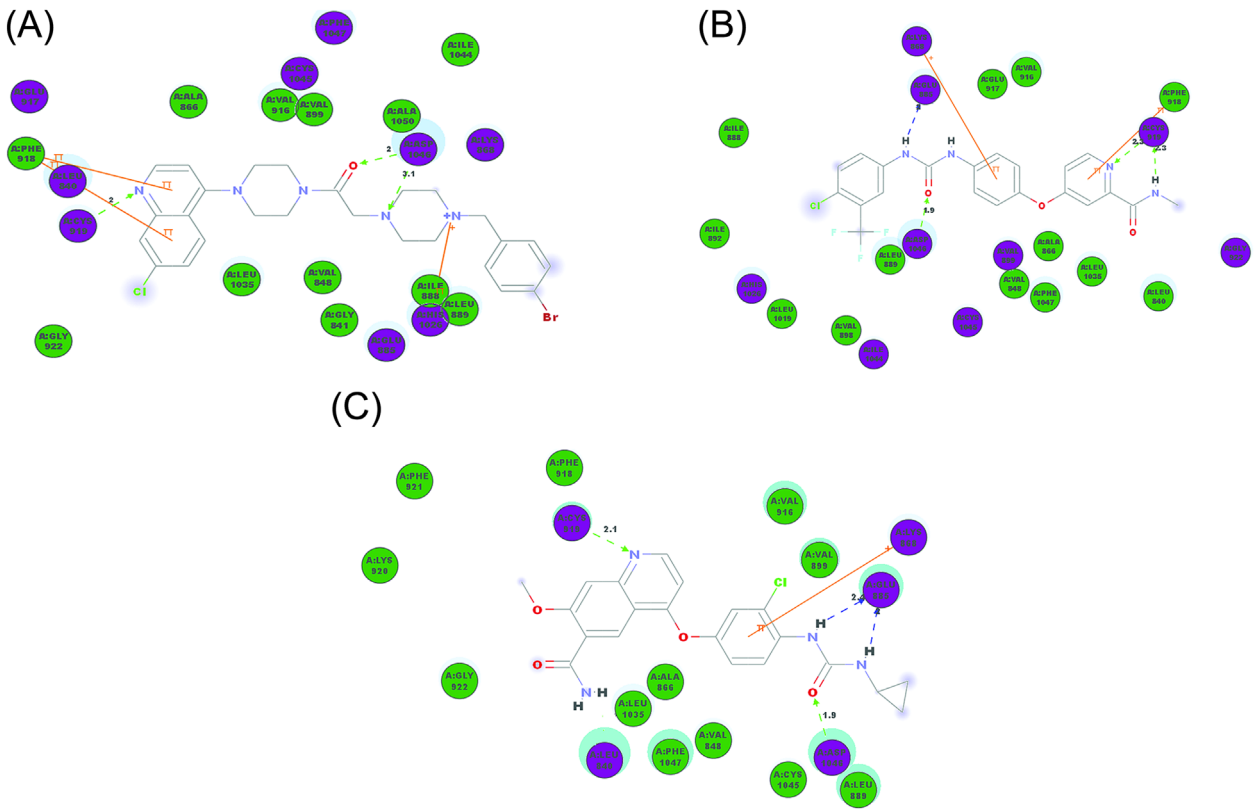

Figure 3. 2D representation of the binding mode of **4q** (A), sorafenib (B), and lenvatinib (C) at their ATP-binding site of VEGFR-II.

*General procedure for the synthesis of 1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-(substituted amino)-ethanones **4a–h***

To a solution of 3 (1.5 g, 4.5 mmol) in ethanol (30 mL), the appropriate amine (13.5 mmol) was added (when hydrochloride salt was used, an equimolar amount of triethylamine was added). The reaction mixture was refluxed under stirring for 12 h. Thereafter, the ethanol was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and washed with water (3×30 mL). The organic layer was separated, dried (anhydrous Na_2SO_4) and evaporated to afford **4a–h**, which were purified through column chromatography.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(dimethylamino)ethanone (**4a**)*

Yield 70%, pale yellow oil. IR (KBr cm^{-1}): 1641.13 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.302 (s, 6H, $N(CH_3)_2$), 3.147 (m, 4H, piperazine), 3.586 (s, 2H, $COCH_2N$), 3.758–3.850 (m, 4H, piperazine), 6.778–6.788 (d, $J = 5.35$ Hz, $1H_{Ar}$), 7.381–7.398 (d, $J = 8.4$ Hz, $1H_{Ar}$), 7.878–7.894 (d, $J = 8.0$ Hz, $1H_{Ar}$), 7.997 (s, $1H_{Ar}$), 8.660–8.671 (d, $J = 5.5$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.92, 44.60, 49.10, 52.13, 53.35, 63.78, 109.13, 121.55, 124.89, 126.12, 128.56, 134.62, 149.77, 151.70, 156.23, 168.52. ESI HRMS (*m/z*) for $C_{17}H_{21}ClN_4O$, calcd. 332.14039, found 333.12119 ($M^+ + 1$).

Table 2. Summary of docking analysis for the docking set at the ATP binding site of VEGFR-II TK.

Compound	Docking scores	H-bond	π-interaction
IVq	−57.5135	Cys919 (2 Å) Asp1046 (2 Å, 3.1 Å)	Ile888 Phe918
Sorafenib	−68.8191	Cys919 (2.3 Å, 2.33 Å) Asp1046 (1.9 Å) Glu885 (1.9 Å)	Lys868 Phe918
Lenvatinib	−54.0756	Cys919 (2.1 Å) Asp1046 (1.9 Å) Glu885 (2 Å, 2.4 Å)	Lys868 Phe918

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Anal. calcd.: C, 61.35; H, 6.36; N, 16.83. Found C, 61.55; H, 6.46; N, 16.93.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(diethylamino)ethanone (**4b**)*

Yield 70%, dark yellow oil. IR (KBr cm^{-1}): 1644.02 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 0.923–0.951 (t, $J = 6.9$ Hz, 6H, $(CH_3CH_2)_2N$), 2.447–2.490 (q, $J = 7.65$ Hz, 4H, $(CH_3CH_2)_2N$), 3.045–3.087 (m, 4H, piperazine), 3.204 (s, 2H, $COCH_2N$), 3.768–3.861 (m, 4H, piperazine), 6.701–6.711 (d, $J = 5.35$ Hz, $1H_{Ar}$), 7.298–7.312 (d, $J = 6.75$ Hz, $1H_{Ar}$), 7.810–7.827 (d, $J = 8.4$ Hz, $1H_{Ar}$), 7.908–7.911 (d, $J = 1.55$ Hz, $1H_{Ar}$), 8.579–8.590 (d, $J = 5.35$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 11.57, 41.56, 45.36, 47.20, 51.94, 52.45, 57.25, 109.15, 121.62, 124.95, 126.21, 128.59, 134.75, 149.79, 151.74, 156.38, 169.64. ESI HRMS (*m/z*) for $C_{19}H_{25}ClN_4O$, calcd. 360.17169, found 361.15931 (M^++1). Anal. calcd. C, 61.24; H, 6.98; N, 15.53. Found C, 61.56; H, 7.06; N, 15.75.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(dipropylamino)ethanone (**4c**)*

Yield 70%, yellow oil. IR (KBr cm^{-1}): 1637.27 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 0.804–0.833 (t, $J = 6.9$ Hz, 6H, $(CH_3CH_2CH_2)_2N$), 1.402–1.446 (m, 4H, $(CH_3CH_2CH_2)_2N$), 2.381–2.397 (t, $J = 7.65$ Hz, 4H, $(CH_3CH_2CH_2)_2N$), 3.10–3.135 (m, 4H, piperazine), 3.275 (s, 2H, $COCH_2N$), 3.810–3.898 (m, 4H, piperazine), 6.757–6.768 (d, $J = 5.35$ Hz, $1H_{Ar}$), 7.358–7.381 (d, $J = 9.15$ Hz, $1H_{Ar}$), 7.865–7.884 (d, $J = 9.2$ Hz, $1H_{Ar}$), 7.963–7.966 (d, $J = 1.5$ Hz, $1H_{Ar}$), 8.643–8.654 (d, $J = 5.35$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 12.05, 19.91, 41.69, 45.47, 52.14, 52.59, 56.31, 58.82, 109.31, 121.84, 124.98, 126.56, 128.94, 135.08, 150.07, 151.98, 156.58, 169.86. ESI HRMS (*m/z*) for $C_{21}H_{29}ClN_4O$, calcd. 388.20299, found 389.19308 (M^++1). Anal. calcd. C, 64.85; H, 7.52; N, 14.41. Found C, 65.02; H, 7.61; N, 14.68.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(pyrrolidin-1-yl)ethanone (**4d**)*

Yield 79%, dark yellow oil. IR (KBr cm^{-1}): 1644.98 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 1.796 (br s, 4H, N $(CH_2CH_2)_2$), 2.683 (br s, 4H, $N(CH_2CH_2)_2$), 3.132–3.157 (m, 4H, piperazine), 3.409–3.507 (m, 2H, $COCH_2N$), 3.843 (br s, 4H, piperazine), 6.785–6.795 (d, $J = 4.75$ Hz, $1H_{Ar}$), 7.394–7.406 (d, $J = 6.7$ Hz, $1H_{Ar}$), 7.883–7.899 (d, $J = 8.6$ Hz, $1H_{Ar}$), 7.997–8.001 (d, $J = 1.9$ Hz, $1H_{Ar}$), 8.652–8.685 (d, $J = 4.8$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 23.85, 41.68, 45.48, 52.136, 52.61, 53.98 (2C), 58.30, 109.35, 121.83, 124.94, 126.58, 128.90, 135.16, 150.03, 151.93, 156.57, 168.65. ESI HRMS (*m/z*) for $C_{19}H_{23}ClN_4O$, calcd. 358.15604, found 359.16386 (M^++1). Anal. calcd. C, 63.59; H, 6.46; N, 15.61. Found C, 63.77; H, 6.55; N, 15.83.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(1H-imidazol-1-yl)ethanone (**4e**)*

Yield 68%, yellow crystals from ethyl acetate/methanol, m.p. 208°C. IR (KBr cm^{-1}): 1660.41 (CO amide). ^{1}H-NMR 300 MHz ($CDCl_3$) δ ppm 3.17 (br s, 4H, piperazine), 3.77 (br s, 4H, piperazine), 5.11 (s, 2H, $COCH_2N$), 6.89 (s, $1H_{Ar}$), 7.02–7.04 (d, $J = 6.0$ Hz, $1H_{Ar}$), 7.09 (s, $1H_{Ar}$), 7.57 (s, $2H_{Ar}$), 8.01 (s, $1H_{Ar}$), 8.10–8.13 (d, $J = 9.0$ Hz, $1H_{Ar}$), 8.74–8.75 (d, $J = 3.0$ Hz, $1H_{Ar}$). ^{13}C-NMR 75 MHz ($CDCl_3$): δ 42.30, 45.07, 48.15, 51.88, 52.02, 109.54, 120.47, 121.78, 124.75, 126.92, 128.95, 129.13, 135.42, 138.11, 149.99, 151.94, 156.24, 165.03. ESI HRMS (*m/z*) for $C_{18}H_{18}ClN_5O$, calcd. 355.11999, found 356.12488 (M^++1). Anal. calcd. C, 60.76; H, 5.10; N, 19.68. Found C, 60.93; H, 5.18; N, 19.86.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(piperidin-1-yl)ethanone (**4f**)*

Yield 72%, yellow crystals, m.p. 118°C. IR (KBr cm^{-1}): 1650.77 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 1.295 (br s, 2H, N $(CH_2CH_2)_2CH_2$), 1.437 (br s, 4H, $N(CH_2CH_2)_2CH_2$), 2.300 (br s, 4H, $N(CH_2CH_2)_2CH_2$), 3.022–3.062 (m, 6H, piperazine (4H) & $COCH_2N$), 3.748–3.826 (m, 4H, piperazine), 6.809–6.820 (d, $J = 5.4$ Hz, $1H_{Ar}$), 7.412–7.433 (dd, $J = 9.15$, 2.3 Hz, $1H_{Ar}$), 7.920–7.939 (d, $J = 9.15$ Hz, $1H_{Ar}$), 8.025–8.028 (d, $J = 1.55$ Hz, $1H_{Ar}$), 8.702–8.711 (d, $J = 4.60$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 13.91, 26.02, 41.68, 45.69, 52.13, 52.77, 54.38 (2C), 62.63, 109.27, 121.79, 124.99, 126.39, 128.85, 134.95, 150.02, 151.91, 156.52, 168.82. ESI HRMS (*m/z*) for $C_{20}H_{25}ClN_4O$, calcd. 372.17169, found 373.17897 (M^++1). Anal. calcd. C, 64.42; H, 6.76; N, 15.03. Found C, 64.63; H, 6.83; N, 15.25.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-hydroxypiperidin-1-yl)ethanone (**4g**)*

Yield 71%, yellow crystals, m.p. 170°C. IR (KBr cm^{-1}): 1644.02 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 1.576 (m, 2H, $CH_2CH(OH)CH_2$), 1.865 (br s, 2H, $CH_2CH(OH)CH_2$), 2.198–2.400 (t, $J = 10.5$ Hz, 2H, CH_2NCH_2), 2.752 (br s, 3H, CH_2NCH_2 & $CH_2CH(OH)CH_2$), 3.132–3.199 (m, 4H, piperazine), 3.687–3.899 (m, 4H, piperazine (2H) & $COCH_2N$), 6.795 (s, $1H_{Ar}$), 7.399–7.418 (d, $J = 9.5$ Hz, $1H_{Ar}$), 7.898–7.917 (d, $J = 9.5$ Hz, $1H_{Ar}$), 8.012 (s, $1H_{Ar}$), 8.678 (s, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 34.59, 41.80, 45.78, 51.24, 52.24, 52.77, 53.07, 61.75, 67.35, 109.36, 121.87, 124.94, 126.65, 128.96, 135.23, 150.08, 151.94, 156.61, 168.72. ESI HRMS (*m/z*) for $C_{20}H_{25}ClN_4O_2$, calcd. 388.16660, found 389.17388 (M^++1 Anal. calcd. C, 61.77; H, 6.48; N, 14.41. Found C, 61.94; H, 6.66; N, 14.62.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-morpholinoethanone (**4h**)*

Yield 73%, yellow crystals, m.p. 131°C. IR (KBr cm^{-1}): 1645.95 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.470 (br s, 4H, N $(CH_2)_2$, morpholine), 3.099–3.187 (m, 6H, piperazine (4H) & $COCH_2N$), 3.644–3.661 (t, $J = 4.5$ Hz, 4H, piperazine), 3.835 (m, 4H, CH_2OCH_2, morpholine), 6.759–6.768 (d, $J = 4.6$ Hz, $1H_{Ar}$), 7.357–7.379 (dd, $J = 9.15$ Hz, 2.3 Hz, $1H_{Ar}$), 7.859–7.876 (d, $J = 8.4$ Hz, $1H_{Ar}$), 7.971–7.975 (d, $J = 2.3$ Hz, $1H_{Ar}$), 8.644–8.653 (d, $J = 4.6$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.59, 45.52, 51.92, 52.51, 53.40, 61.52, 66.72, 109.25, 121.65, 124.94, 125.83, 128.61, 134.79, 149.81, 151.78, 156.31, 167.87. ESI HRMS (*m/z*) for $C_{19}H_{23}ClN_4O_2$, calcd. 374.15095, found

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375.15823 (M^++1). Anal. calcd. C, 60.88; H, 6.18; N, 14.95. Found C, 61.05; H, 6.25; N, 15.12.

*General procedure for the synthesis of 1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-(4-substituted piperazin-1-yl)ethanones **4i–t***

To a solution of **3** (1.5 g, 4.5 mmol) in ethanol (30 mL), the appropriate piperazine derivative **5i–t** (6.75 mmol) and anhydrous sodium carbonate (0.95 g, 9 mmol) were added. The mixture was stirred under reflux for 12 h. Thereafter, ethanol was evaporated under reduced pressure, and then the residue was dissolved in ethyl acetate (30 mL) and washed with water (3 × 30 mL). The organic layer was separated, dried (anhydrous Na_2SO_4) and evaporated to afford the desired compounds, which were purified through column chromatography.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-methylpiperazin-1-yl)ethanone (**4i**)*

Yield 74%, yellow oil. IR (KBr cm^{-1}): 1644.02 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.26 (s, 3H, CH_3N), 2.541 (br s, 8H, piperazine), 3.092–3.183 (m, 8H, piperazine), 3.872 (s, 2H, $COCH_2N$), 6.84–6.85 (s, 1H_{Ar}), 7.412–7.429 (d, J = 8.5 Hz, 1H_{Ar}), 7.909–7.928 (d, J = 9.5 Hz, 1H_{Ar}), 8.022 (s, 1H_{Ar}), 8.702 (s, 1H_{Ar}). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.78, 45.71, 45.91, 52.15, 52.83, 52.99, 54.98, 61.49, 109.36, 121.88, 124.91, 126.65, 129.03, 135.23, 150.14, 152.00, 156.57, 168.34. ESI HRMS (*m/z*) for $C_{20}H_{26}ClN_5O$, calcd. 387.18259, found 388.16058 (M^++1). Anal. calcd. C, 61.93; H, 6.76; N, 18.05. Found C, 62.04; H, 6.88; N, 18.22.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-ethylpiperazin-1-yl)ethanone (**4j**)*

Yield 77%, dark yellow oil. IR (KBr cm^{-1}): 1643.05 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 1.002–1.026 (t, J = 7.0 Hz, 3H, CH_3CH_2N), 2.345–2.358 (d, J = 6.5 Hz, 2H, CH_3CH_2N), 2.519 (br s, 8H, piperazine), 3.136–3.183 (m, 6H, piperazine, $COCH_2N$), 3.819–3.838 (m, 4H, piperazine), 6.757–6.765 (d, J = 4.0 Hz, 1H_{Ar}), 7.359–7.376 (d, J = 8.5 Hz, 1H_{Ar}), 7.861–7.878 (d, J = 8.5 Hz, 1H_{Ar}), 7.969 (s, 1H_{Ar}), 8.645–8.654 (d, J = 4.5 Hz, 1H_{Ar}). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 11.83, 41.69, 45.60, 52.06, 52.22, 52.65, 52.73, 52.92, 60.37, 109.27, 121.79, 124.83, 126.52, 128.97, 135.07, 150.09, 151.92, 156.44, 168.25. ESI HRMS (*m/z*) for $C_{21}H_{28}ClN_5O$, calcd. 401.19824, found 402.20606 (M^++1). Anal. calcd. C, 62.75; H, 7.02; N, 17.42. Found C, 62.93; H, 7.21; N, 17.59.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-phenylpiperazin-1-yl)ethanone (**4k**)*

Yield 73%, dark yellow oil. IR (KBr cm^{-1}): 1655.59 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$): δ ppm 2.713–2.732 (t, J = 5.0 Hz, 4H, piperazine), 3.189–3.235 (m, 8H, piperazine), 3.340 (s, 2H, $COCH_2N$), 3.924–3.950 (m, 4H, piperazine), 6.831–6.881 (m, 2H_{Ar}), 6.92 (s, 1H_{Ar}), 6.936 (s, 1H_{Ar}), 7.248–7.280 (m, 2H_{Ar}), 7.445–7.467 (dd, J = 9.0 Hz, 2.0 Hz, 1H_{Ar}), 7.944–7.962 (d, J = 9.0 Hz, 1H_{Ar}), 8.069–8.073 (d, J = 2.0 Hz, 1H_{Ar}), 8.725–8.735 (d, J = 5.0 Hz, 1H_{Ar}). ^{13}C-NMR: 125 MHz ($CDCl_3$) δ ppm 41.75, 45.7, 49.16, 52.18, 52.68, 53.16, 61.48, 109.25, 116.13, 119.96, 121.74, 124.87, 126.6, 128.8, 128.85, 129.14, 135.22, 149.92, 151.09, 151.76, 156.52, 168.09. ESI HRMS (*m/z*) for $C_{25}H_{28}ClN_5O$, calcd. 449.19832, found 450.20633 (M^++1). Anal. calcd. C, 66.73; H, 6.27; N, 15.56. Found C, 66.92; H, 6.35; N, 15.74.

*2-(4-(4-Chlorophenyl)piperazin-1-yl)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (**4l**)*

Yield 76%, white crystals from ethyl acetate, m.p. 169°C. IR (KBr cm^{-1}): 1665.23 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.679–2.719 (t, J = 6.5 Hz, 4H, piperazine), 3.157–3.199 (m, 8H, piperazine), 3.304 (s, 2H, $COCH_2N$), 3.908 (br s, 4H, piperazine), 6.799–6.852 (m, 3H_{Ar}), 7.179–7.161 (d, J = 8.6 Hz, 2H_{Ar}), 7.422–7.445 (d, J = 11.5 Hz, 1H_{Ar}), 7.919–7.938 (d, J = 9.55 Hz, 1H_{Ar}), 8.037 (s, 1H_{Ar}), 8.708–8.717 (d, J = 4.5 Hz, 1H_{Ar}). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.83, 45.78, 49.26, 52.24, 52.32, 53.11, 61.51, 109.38, 117.39, 121.87, 124.79, 124.88, 126.69, 129.05, 135.24, 149.79, 149.89, 150.18, 152.03, 156.49, 168.14. ESI HRMS (*m/z*) for $C_{25}H_{27}Cl_2N_5O$, calcd. 483.15927, found 484.13785 (M^++1). Anal. calcd. C, 61.99; H, 5.62; N, 14.46. Found C, 62.18; H, 5.70; N, 14.65.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-(4-methoxyphenyl)piperazin-1-yl)ethanone (**4m**)*

Yield 69%, dark yellow oil. IR (KBr cm^{-1}): 1645.95 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.742 (br s, 4H, piperazine), 3.099–3.225 (m, 8H, piperazine), 3.327 (s, 2H, $COCH_2N$), 3.834 (s, 3H, OCH_3), 3.896–3.946 (m, 4H, piperazine), 6.811–6.845 (m, 2H_{Ar}), 6.883–6.912 (dd, J = 9.0, 7.0 Hz, 2H_{Ar}), 6.962–6.974 (d, J = 6 Hz, 1H_{Ar}), 7.416–7.438 (dd, J = 9.0, 2.0 Hz, 1H_{Ar}), 7.927–7.945 (d, J = 9 Hz, 1H_{Ar}), 8.039–8.043 (d, J = 2 Hz, 1H_{Ar}), 8.702–8.712 (d, J = 5.0 Hz, 1H_{Ar}). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.73, 45.69, 50.57, 52.12, 52.75, 53.39, 55.40, 61.58, 109.25, 111.35, 118.16, 120.97, 121.77, 123.06, 124.89, 126.51, 128.88, 135.11, 141.05, 150.01, 151.81, 152.24, 156.50, 168.20. ESI HRMS (*m/z*) for $C_{26}H_{30}ClN_5O_2$, calcd. 479.20880, found 480.18621 (M^++1). Anal. calcd. C, 65.06; H, 6.30; N, 14.59. Found C, 65.24; H, 6.58; N, 14.77.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-(4-ethoxyphenyl)piperazin-1-yl)ethanone (**4n**)*

Yield 68%, dark yellow oil. IR (KBr cm^{-1}): 1637.27 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 1.365–1.393 (t, J = 7.0 Hz, 3H, CH_3CH_2O), 2.725 (br s, 4H, piperazine), 3.088–3.200 (m, 8H, piperazine), 3.317 (s, 2H, $COCH_2N$), 3.855–3.905 (m, 4H, piperazine), 3.972–4.014 (q, 2H, J = 7.0 Hz, CH_3CH_2O), 6.772–6.789 (m, 2H_{Ar}), 6.830–6.846 (m, 2H_{Ar}), 6.883–6.917 (m, 1H_{Ar}), 7.385–7.407 (dd, J = 9.0, 2.0 Hz, 1H_{Ar}), 7.887–7.905 (d, J = 9 Hz, 1H_{Ar}), 8.015–8.019 (d, J = 2.0 Hz, 1H_{Ar}), 8.667–8.677 (d, J = 5.0 Hz, 1H_{Ar}). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 14.94, 41.75, 45.70, 50.38, 52.14, 52.73, 53.39, 61.48, 63.64, 109.19, 112.64, 118.15, 121.00, 121.71, 122.96, 124.88, 126.63, 128.72, 135.34, 141.02, 149.78, 151.57, 151.59, 156.66, 168.06. ESI HRMS (*m/z*) for $C_{27}H_{32}ClN_5O_2$, calcd. 493.22445, found 494.20270 (M^++1). Anal. calcd. C, 66.73; H, 6.27; N, 15.56. Found C, 66.92; H, 6.35; N, 15.74.

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*2-(4-Benzylpiperazin-1-yl)-1-(4-(7-chloroquinolin-4-yl)-piperazin-1-yl)ethanone (**4o**)*
Yield 71%, buff powder, m.p. 171°C. IR (KBr cm^{-1}): 1639.20 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.55 (m, 8H, piperazine), 3.17 (m, 4H, piperazine), 3.26 (s, 2H, $COCH_2N$), 3.55 (s, 2H, $PhCH_2N$), 3.90 (m, 4H, piperazine), 6.82–6.84 (d, $J = 3.0$ Hz, $1H_{Ar}$), 7.31–7.32 (m, $5H_{Ar}$), 7.44–7.46 (d, $J = 6.0$ Hz, $1H_{Ar}$), 7.93–7.99 (d, $J = 6.0$ Hz, $1H_{Ar}$), 8.07–8.09 (d, $J = 6.0$ Hz, $1H_{Ar}$), 8.73–8.75 (d, $J = 6.0$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.74, 45.62, 52.08, 52.27, 52.89, 61.35, 62.80, 109.29, 121.83, 124.88, 126.54, 127.28, 128.30, 128.92, 129.54, 135.14, 137.36, 150.07, 151.89, 156.91, 168.31. ESI HRMS (*m/z*) for $C_{26}H_{30}ClN_5O$, calcd. 463.21389, found 464.20662 (M^++1). Anal. calcd. C, 67.30; H, 6.52; N, 15.09. Found C, 67.51; H, 6.73; N, 15.30.

*2-(4-(4-Chlorobenzyl)piperazin-1-yl)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (**4p**)*
Yield 58%, dark yellow oil. IR (KBr cm^{-1}): 1644.98 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.560 (br s, 8H, piperazine), 3.140–3.184 (m, 4H, piperazine), 3.245 (s, 2H, $COCH_2N$), 3.444 (s, 2H, $PhCH_2N$), 3.792–3.859 (m, 4H, piperazine), 6.519 (s, $1H_{Ar}$), 6.541 (s, $1H_{Ar}$), 6.806–6.815 (d, $J = 4.6$ Hz, $1H_{Ar}$), 7.253 (s, $1H_{Ar}$), 7.274 (s, $1H_{Ar}$), 7.412–7.429 (d, $J = 8.6$ Hz, $1H_{Ar}$), 7.901–7.920 (d, $J = 9.5$ Hz, $1H_{Ar}$), 8.0312 (s, $1H_{Ar}$), 8.706–8.716 (d, $J = 4.8$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.99, 45.66, 52.10, 52.83, 60.96, 61.88, 109.47, 121.75, 123.71, 124.92, 126.77, 128.85, 129.76, 130.98, 132.52, 135.48, 149.85, 151.86, 156.68, 167.93. ESI HRMS (*m/z*) for $C_{26}H_{29}Cl_2N_5O$, calcd. 463.21389, found 464.20662 (M^++1). Anal. calcd. C, 62.65; H, 5.86; N, 14.05. Found C, 62.83; H, 5.93; N, 14.21.

*2-(4-(4-Bromobenzyl)piperazin-1-yl)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone (**4q**)*
Yield 71%, buff crystals from ethyl acetate, m.p. 184°C. IR (KBr cm^{-1}): 1651.73 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.463–2.549 (m, 8H, piperazine), 3.149–3.172 (t, $J = 4.4$ Hz, 4H, piperazine), 3.250 (s, 2H, $COCH_2N$), 3.436 (s, 2H, $PhCH_2N$), 3.889–3.922 (m, 4H, piperazine), 6.825–6.837 (d, $J = 4.8$ Hz, $1H_{Ar}$), 7.171–7.192 (d, $J = 8.4$ Hz, AB system, $2H_{Ar}$), 7.401–7.423 (d, $J = 8.8$ Hz, AB system, $2H_{Ar}$), 7.455–7.461 (d, $J = 2.4$ Hz, $1H_{Ar}$), 7.940–7.962 (d, $J = 8.8$ Hz, $1H_{Ar}$), 8.053–8.058 (d, $J = 2$ Hz, $1H_{Ar}$), 8.734–8.746 (d, $J = 4.8$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.71, 45.68, 52.88, 53.00, 53.88, 61.61, 62.12, 109.28, 120.84, 121.88, 124.85, 126.51, 129.05, 130.71, 131.33, 135.08, 137.20, 150.18, 151.97, 156.44, 168.30. ESI HRMS (*m/z*) for $C_{26}H_{29}BrClN_5O$, calcd. 541.12440, found 544.12256 (M^++ 3). Anal. calcd. C, 57.72; H, 5.38; N, 12.90. Found C, 57.70; H, 5.46; N, 13.02.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-(4-methylbenzyl)piperazin-1-yl)ethanone (**4r**)*
Yield 73%, buff powder, m.p. 184°C. IR (KBr cm^{-1}): 1654.62 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.35 (s, 3H, $PhCH_3$), 2.50–2.56 (m, 8H, piperazine), 3.18–3.22 (m, 4H, piperazine), 3.26 (s, 2H, $COCH_2N$), 3.48 (s, 2H, $PhCH_2N$), 3.91 (m, 4H, piperazine), 6.84–6.85 (d, $J = 3.0$ Hz, $1H_{Ar}$), 7.12–7.20 (m, $4H_{Ar}$), 7.46–7.48 (d, $J = 6.0$ Hz, $1H_{Ar}$), 7.96–7.98 (d, $J = 6.0$ Hz, $1H_{Ar}$), 8.08 (s, $1H_{Ar}$), 8.75–8.76 (d, $J = 3.0$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 21.63, 42.25, 46.21, 52.62, 53.51, 53.61, 62.16, 63.20, 109.81, 122.37, 125.38, 127.05, 129.45, 129.55, 129.66, 135.33, 135.64, 137.22, 150.70, 152.46, 157.02, 168.91. ESI HRMS (*m/z*) for $C_{27}H_{32}ClN_5O$, calcd. 477.22954, found 478.23087 (M^++1). Anal. calcd. C, 67.84; H, 6.75; N, 14.65. Found C, 68.02; H, 6.82; N, 14.83.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-(4-methoxybenzyl)piperazin-1-yl)ethanone (**4s**)*
Yield 56%, dark yellow oil. IR (KBr cm^{-1}): 1643.05 (CO amide) ^{1}H-NMR 500 MHz ($CDCl_3$) δ ppm 2.633 (br s, 8H, piperazine), 3.155–3.189 (m, 4H, piperazine), 3.266 (s, 2H, $COCH_2N$), 3.591 (s, 2H, $PhCH_2N$), 3.774–3.834 (m, 4H, piperazine), 3.858 (s, 3H, OCH_3), 6.823–6.848 (m, $3H_{Ar}$), 7.211–7.228 (d, $J = 8.6$ Hz, $2H_{Ar}$), 7.437–7.454 (d, $J = 8.6$ Hz, $1H_{Ar}$), 7.911–7.928 (d, $J = 8.6$ Hz, $1H_{Ar}$), 8.056–8.062 (d, $J = 2.9$ Hz, $1H_{Ar}$), 8.733–8.742 (d, $J = 4.8$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$) δ ppm 41.77, 45.54, 52.13, 52.24, 52.66, 55.37, 60.89, 61.58, 109.34, 113.868, 121.824, 124.92, 126.38, 127.38, 128.65, 131.18, 135.47, 149.74, 151.59, 151.72, 156.80, 159.30, 168.18. ESI HRMS (*m/z*) for $C_{27}H_{32}ClN_5O_2$, calcd. 493.22445, found 494.21948 (M^++1). Anal. calcd. C, 65.64; H, 6.53; N, 14.18. Found C, 65.82; H, 6.60; N, 14.36.

*1-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-2-(4-(3,4,5-trimethoxybenzyl)piperazin-1-yl)ethanone (**4t**)*
Yield 55%, dark yellow oil. IR (KBr cm^{-1}): 1641.13 (CO amide). ^{1}H-NMR 500 MHz ($CDCl_3$): δ ppm 2.585 (br s, 8H, piperazine), 3.134–3.176 (m, 8H, piperazine), 3.482 (s, 2H, $COCH_2N$), 3.801 (m, 11H, $PhCH_2N$ & $(OCH_3)_3$), 6.519 (s, $2H_{Ar}$), 6.798–6.810 (d, $J = 5.75$ Hz, $1H_{Ar}$), 7.399–7.416 (d, $J = 8.6$ Hz, $1H_{Ar}$), 7.888–7.905 (d, $J = 8.6$ Hz, $1H_{Ar}$), 8.0221 (s, $1H_{Ar}$), 8.697–8.706 (d, $J = 4.75$ Hz, $1H_{Ar}$). ^{13}C-NMR 125 MHz ($CDCl_3$): δ 41.75, 45.58, 52.10, 52.60, 52.99, 56.16, 60.93, 62.75, 106.18, 109.34, 121.79, 124.95, 126.73, 128.63, 132.34, 135.39, 137.16, 149.74, 151.73, 153.16, 156.76, 168.10. ESI HRMS (*m/z*) for $C_{29}H_{36}ClN_5O_4$, calcd. 553.24558, found 554.34191 (M^++1). Anal. calcd. C, 62.86; H, 6.55; N, 12.64. Found C, 63.04; H, 6.42; N, 12.82.

*Synthesis of 1-(un)substituted-benzylpiperazines **5o–t***
To a solution of the appropriate benzylchloride (40 mmol) in ethanol (30 mL), anhydrous piperazine (8.73 g, 400 mmol) was added. The mixture was refluxed under stirring for 8 h, then ethanol was evaporated under vacuum. To the residue an aqueous solution of Na_2CO_3 (10%, 50 mL) was added, and extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine (30 mL), separated, dried (anhydrous Na_2SO_4) and evaporated under reduced pressure; then the residue was crystallized from petroleum ether (40:60) to afford **5o–t**.

- 1-Benzylpiperazine (**5o**) [23], colorless oil used as such, 77.5% yield.

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- 1-(4-Chlorobenzyl)piperazine (**5p**) [29], white solid, m.p. 96°C, 75% yield.
- 1-(4-Bromobenzyl)piperazine (**5q**) [30], white solid, m.p. 58–60°C, 76.5% yield.
- 1-(4-Methylbenzyl)piperazine (**5r**) [31], yellow solid, m.p. 40°C, 74% yield.
- 1-(4-Methoxybenzyl)piperazine (**5s**) [29], yellow solid, m.p. 100°C, 78% yield.
- 1-(3,4,5-Trimethoxybenzyl)piperazine (**5t**) [32], yellow solid, m.p. 69°C, 78% yield.

Biological evaluation

The human tumor cell lines (MCF-7) and (PC3) were obtained from NCI, MD, USA. All chemicals and solvents were purchased from Sigma–Aldrich.

Procedures

The pharmacology unit of the National Cancer Institute, Cairo University performed the *in-vitro* anticancer screening, where both breast (MCF7) and prostate (PC3) human cancer cell lines were used. The Skehan et al. method adopting the Sulfo-Rhodamine-B stain (SRB) assay was performed to determine the cytotoxic effect of the screened compounds [33, 34].

This assay depends on the capability of SRB to bind to cells protein components which were fixed by trichloroacetic acid (TCA) to tissue-culture plates. The bright-pink aminoxanthene dye (SRB) possesses two sulfonic acid groups which under mild acidic conditions are connected to the basic amino acid residues, and are dissociated under basic conditions. The SRB binding status is stoichiometric 1, so the relation is directly proportional between the amount of dye extracted from stained cells and the cell mass.

Before treatment with the tested compounds, the cells were plated in 96-multiwell plates (10^4 cells/well) for 24 h, which allows the attachment of cells to the plate wall. Different concentrations (0, 5, 12.5, 25, 50 μg/mL) for each compound were added to the monolayer triplicate well. Then, the monolayer cells including the screened compounds were allowed for incubation at 37°C in atmosphere of 5% CO_2 for 48 h. Then, cells were fixed, held and stained with 0.4% (wt/vol) SRB dissolved in 1% acetic acid, for 30 min. Four washes with 1% acetic acid were carried out to remove the excess unbound dye, and then, Tris-EDTA buffer was used to recover the attached stain. ELISA reader was used to measure the color intensity at a wave length of 570 nm.

After the specific time, the survival curves for both human tumor cell lines (MCF-7 and PC3) are plotted to illustrate the relation between drug concentration and the screened surviving fraction, using GraphPad Prism 5. The IC_{50} values were calculated for compounds **4a–t** (Table 1).

In vitro VEGFR-II enzyme assay

As compound **4q** exhibited the highest cytotoxicity on MCF-7 cell line among the synthesized compounds, therefore, it was chosen to be examined for its inhibitory effect on VEGFR-II. The inhibitory activity of both **4q** and sorafenib as reference drug was examined by Human VEGFR-R2/KDR ELISA according to the manufacturer instructions (BioVendor®, Czech Republic).

Molecular modeling

A molecular docking study was carried out using Discovery Studio 2.5 in order to explore the binding mode of **4q** in comparison with the marketed VEGFR-II inhibitors, sorafenib and lenvatinib. The automated docking study was performed using the crystal structure of VEGFR-II (4ASD) complexed with its ligand sorafenib. The CDOCKER protocol was performed for all conformers of **4q** to the selected active site. The redocking of lenvatinib and the co-crystallized ligand (sorafenib) was used to evaluate the docking method. Each docked compound was assigned a score according to its binding mode onto the binding active site after energy minimization according to the prepared ligand protocol Table 2.

The authors would like to thank the National Research Centre, Dokki, Giza, Egypt, through project No.10010302 (2013–2016), and the Science and Technology Development Fund in Egypt (STDF-STF) through project No. 6458, for the support of this research. Also, special thanks to Dr. Jaroslav Sebestik and Martin Safarik at the Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic for performing some HRMS analysis.

The authors have declared no conflict of interest.

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