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# Graphical abstract

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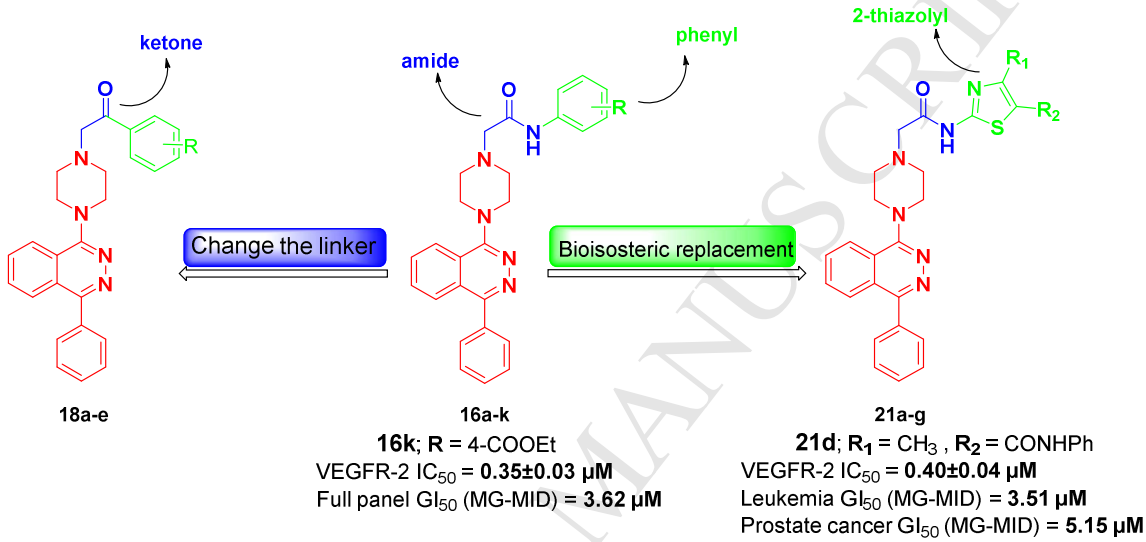

# 1-Piperazinylphthalazines as potential VEGFR-2 inhibitors and anticancer agents: Synthesis and *in vitro* biological evaluation

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**Abstract**

In our endeavor towards the development of effective VEGFR-2 inhibitors, three novel series of phthalazine derivatives based on 1-piperazinyl-4-arylphthalazine scaffold were synthesized. All the newly prepared phthalazines **16a-k**, **18a-e** and **21a-g** were evaluated *in vitro* for their inhibitory activity against VEGFR-2. In particular, compounds **16k** and **21d** potently inhibited VEGFR-2 at sub-micromolar $IC_{50}$ values 0.35±0.03 and 0.40±0.04µM, respectively. Moreover, seventeen selected compounds **16c-e**, **16g**, **16h**, **16j, 16k**, **18c-e** and **21a-g** were evaluated for their *in vitro* anticancer activity according to US-NCI protocol, where compounds **16k** and **21d** proved to be the most potent anticancer agents. While, compound **16k** exhibited potent broad spectrum anticancer activity with full panel $GI_{50}$ (MG-MID) value of 3.62 µM, compound **21d** showed high selectivity toward leukemia and prostate cancer subpanels [subpanel $GI_{50}$ (MG-MID) 3.51 and 5.15 µM, respectively]. Molecular docking of compounds**16k** and **21d** into VEGFR-2 active site was performed to explore their potential binding mode.

*Keywords:* Synthesis; 1-Piperazinylphthalazines; VEGFR-2 inhibitors; Anticancer activity.

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## 1. Introduction



Vascular endothelial growth factors (VEGFs) is a family of members that bind in an overlapping pattern to three diversified but structurally relative VEGF receptors (VEGFRs), which are transmembrane proteins possessing tyrosine kinase domain (TK). VEGFR-1 (Flt-1) is critical for hematopoietic cell development, VEGFR-2 (human KDR or murine Flk-1) for vascular endothelial cell development and angiogenesis, VEGFR-3 (Flt4) for lymphatic endothelial cell development [1]. VEGFR-2, a 210−230 kDa glycoprotein expressed in vascular endothelial cells, is the major regulator of VEGF-driven responses in endothelial cells, including proliferation, migration, tube formation and vascular permeability. VEGFR-2 is considered to be a pivotal signal transducer in both physiologic and pathologic angiogenesis [2].

Angiogenesis is the process of sprouting or splitting of novel capillary blood vessels from the quiescent pre-existing vasculature [3]. Angiogenesis is normally orchestrated *via* a finely balanced equilibrium between pro-angiogenic and anti-angiogenic factors. However, aberrant equilibrium between these factors is associated with several human disorders, like rheumatoid arthritis, psoriasis, diabetic neuropathy and cancer [4]. Mammalian cells need oxygen and nutrients for their survival and are therefore located within 100-200 µm of blood vessels, the diffusion limit for oxygen. To grow beyond this size, tumor cells induce angiogenesis through the secretion of pro-angiogenic growth factors to ensure their oxygen and nutrients [5]. Thence, therapeutic strategies based on the inhibition of VEGFR-2, in the current medical era, have stood out as an attractive approach for discovering therapies for many human malignancies. To date, seven small molecule VEGFR-2 kinase inhibitors (Sorafenib **1** [6], Regorafenib **2** [7], Sunitinib **3** [8], Pazopanib **4** [9], Vandetanib **5** [10], Axitinib **6** [11] and cabozantinib **7** [12]) have been approved for clinical use (Figure 1) [13].

In the last two decades, 1,4-disubstituted phthalazine scaffold has emerged as a promising scaffold for the design and development of potent VEGFR-2 inhibitors. Vatalanib (PTK787) **8** (Figure 2), an 1-anilino-4-substitutedphthalazino derivative brought to light by Novartis and Schering AG in 1998, is one of the most potent and selective first-generation VEGFR kinase inhibitors, with $IC_{50}$ value of 43 nM against VEGFR-2 [14, 15]. Currently, Vatalanib **8** is in phase III clinical trials for the treatment of advanced colorectal cancer [16] and entered phase II studies for malignant mesothelioma [17], metastatic gastrointestinal stromal tumor [18] and metastatic melanoma [19]. Subsequently, extensive studies have been devoted to design and synthesis of different agents with a general structure of 1-anilino-4-substitutedphthalazine as potential VEGFR-2 inhibitors such as IM-023911 **9**, 4-(4-((4-(chlorodifluoromethoxy)phenyl)amino) phthalazin-1-yl)benzamide, with $IC_{50}$ = 190 nM (Figure 2) [16, 20-24]. Unfortunately, these

research efforts failed to develop a clinically approved VEGFR-2 inhibitor based on 1-anilino-4-arylphthalazine scaffold.

Moreover, 1,4-disubstituted phthalazines have attracted considerable attention as effective anticancer agents. Two studies reported the cytotoxicity of novel series of 1-anilino-4-(arylsulfinyl /sulfonylmethyl)phthalazines **10** (Figure 2) against Bel-7402 (human liver cancer cell line) and HT-1080 (human fibro sarcoma cell line) [25, 26]. Recently, Ping Gong and his coworkers explored the *in vitro* anticancer activity of different 1-piperazinyl-4-substitutedphthalazine derivatives against A549 (non-small cell lung cancer cell line), HT-29 (human colorectal cancer cell line) and MDA-MB-231 (breast cancer cell line). Most of the synthesized derivatives showed superior potency to the positive control drug, vatalanib. Among them, 1-piperazinyl-4-benzylphthalazine derivative **11** (Figure 2) possessed $IC_{50}$ values of 2.48, 4.59 and 0.76 μM against A549, HT-29 and MDA-MB-231, respectively, while vatalanib showed values of 21.16, 22.11 and 57.72 μM, respectively [27-29]. Additionally, many synthetic piperazinyl derivatives were developed as potent anticancer agents [30-34].

Interestingly, no attention was paid to investigate the potential inhibitory activity of the 1-piperazinyl-4-substitutedphthalazines towards VEGFR-2.

In view of the previous findings, we herein report the synthesis of three different sets of phthalazine derivatives **16a-k**, **18a-e** and **21a-g** based on the 1-piperazinyl-4-phenylphthalazine scaffold (Figure 2), with the prime aim of developing potent VEGFR-2 inhibitors with good anticancer activity. Firstly, phenyl group was selected to substitute the C-4 of the 1-piperazinylphthalazine core. Then, three strategies were applied on this core. The first one depends on the utilization of different linkers, acetamide (-$CH_2$-CONH-) in the first series **16a-k** and acetyl (-$CH_2$-CO-) in second series **18a-e**, between 1-piperazinyl and aryl moieties. In the second strategy, a bioisosteric approach was adopted to replace the phenyl ring attached to the acetamide linker in **16** with 2-thiazolyl moiety in **21a-g**. The third strategy focused on the usage of different substituents on phenyl and 2-thiazolyl moieties. The substitution pattern on the pendant aryl moieties in **16, 18** and **21** was selected so as to ensure different electronic and lipophilic environments which could manipulate the activity of the target compounds (Figure 2).

The final synthesized 1-piperazinyl-4-phenylphthalazines **16a-k**, **18a-e** and **21a-g** were evaluated for their potential inhibitory activity toward VEGFR-2. Also, they were screened for their *in vitro* anticancer activity against a panel of 56 human cancer cell lines at NCI-USA.

## 2. Results and discussion

### *2.1. Chemistry*

The synthetic strategies adopted for the preparation of the new phthalazines are depicted in Schemes 1−4. In Scheme 1,4-phenyl-1-(2*H*)-phthalazinone **12** was obtained, in 80% yield, by cyclocondensation of 2-benzoylbenzoic acid with hydrazine sulfate in the presence of sodium hydroxide [35]. Next, chlorination of the phthalazinone **12** was carried out via heating with excess of phosphorus oxychloride to furnish 1-chloro-4-phenylphthalazine **13** in 73% yield [36]. The later reacted with excess of anhydrous piperazine in refluxing propan-2-ol to afford the key intermediate 4-phenyl-1-(piperazin-1-yl)phthalazine **14** in 85% yield [37]**.**

The first group of the target phthalazines **16a-k** was obtained in good yields (59-75%) through the reaction of the key intermediate **14** with the appropriate 2-chloro-*N*-(un/substituted phenyl)acetamide **15a-k** in refluxing dry acetone using potassium carbonate as a base (Scheme 2). Alternatively, potassium carbonate catalyzed coupling of the key intermediate **14** with the appropriate phenacyl bromide **17a-e** at room temperature in dry acetone produced the corresponding target derivatives **18a-e** with 58-81% yield (Scheme 3). Finally, the 2-amino function of (un)substituted 2-aminothiazoles **19a-g** was acylated with 2-chloroacetyl chloride to furnish intermediates **20a-g** (yield, 65-79%), which reacted with the piperazinyl phthalazine **14** in dry DMF in presence of potassium carbonate to give the respective phthalazine derivatives **21a-g** in 55-75% yield (Scheme 4).

The structures of all newly prepared compounds were confirmed under the basis of spectral and elemental analyses which were in full agreement with the proposed structures.

### *2.2. Biological evaluations*

#### *2.2.1. VEGFR-2 kinase inhibition assay*

All the newly prepared compounds (**16a-k**, **18a-e** and **21a-g**) were tested *in vitro* for their kinase inhibitory activity against VEGFR-2. Sorafenib was included in the experiments as a reference drug. The results were reported as a 50% inhibition concentration value ($IC_{50}$, determined in triplicate) Table 1. The tested compounds **16, 18** and **21** displayed moderate to good inhibitory activity with $IC_{50}$ values ranging from 4.68±0.47 to 0.35±0.03 µM. In particular, compounds **16k** and **21d** potently inhibited VEGFR-2 at sub-micromolar $IC_{50}$ values (0.35±0.03 and 0.40±0.04 µM, respectively). Also, compounds **16i**, **16j** and **21g** moderately inhibited VEGFR-2 with $IC_{50}$ values of 1.56±0.16, 1.35±0.14

and 1.44±0.14 µM, respectively.

##### *2.2.1.1. Structure activity relationship SAR*

The SAR study depends on the comparison between the inhibitory activity against VEGFR-2 and structural variations resulted from applying the previous three strategies. Initially, we focused

on the impact of substitution on the terminal phenyl on the activity of the first series **16a-k**. Compound **16a** with unsubstituted phenyl group exhibited the least inhibitory action on VEGFR-2 ($IC_{50}$ = 4.68±0.47 µM) relative to the substituted analogs. Introduction of 4-methyl, 3-trifluoromethyl, 4-cyano or 2/3/4-chloro or 2,6-dichloro substituent, compounds **16b-h**, led to non-significant or slight improvement of activity ($IC_{50}$ ranging from 4.35±0.45 to 3.39±0.35 µM). Conversely, appending a 3-$OCH_3$ or 4-$OCH_3$ substituent, compounds **16i** and **16j** ($IC_{50}$ = 1.56±0.16 and 1.35±0.14 µM, respectively), resulted in about 3 fold increase in enzyme inhibition relative to the unsubstituted analog **16a**. Likewise, incorporation of 4-ethyl carboxylate functionality afforded the most active VEGFR-2 inhibitor **16k** ($IC_{50}$ = 0.35±0.03 µM), hinting that substitution with large lipophilic group is more favorable to the enzyme inhibitory activity.

The same trend was observed in the second series **18a-e**, as the unsubstituted phenyl derivative **18a** ($IC_{50}$ = 4.54±0.36 µM) exhibited the lowest activity. Grafting a 4-methyl or 4-chloro substituent in compounds **18b** and **18c**, did not result in significant change of potency ($IC_{50}$ = 4.26±0.44 and 4.29±0.43 µM, respectively), while introduction of a methoxy group at 3- or 4-position, compounds **18d** and **18e**, slightly enhanced the VGFR-2 suppressive activity ($IC_{50}$ = 3.42±0.36 and 2.25±0.22 µM, respectively).

Comparing the activity of compounds **16a-k** and **18a-e** revealed that, the acetamide linker seemed to show better activity than acetyl linker for compounds bearing 3-$OCH_3$ or 4-$OCH_3$ substituent (**16i** and **16j** versus **18d** and **18e**). On the other hand, the type of linker did not show significant effect in case of unsubstituted, 4-$CH_3$ and 4-Cl derivatives (**16a, 16b, 16g** versus **18a-c**).

Finally, we explored the effect of bioisosteric replacement of the pendant phenyl in compound **16a** with 2-thiazolyl group in **21a**. Both compounds displayed similar levels of activity ($IC_{50}$ = 4.68±0.47, 4.16±0.35 µM, respectively), indicating that a 2-thiazolyl group could effectively replace the phenyl ring. Concerning the influence of different substituents on the activity of the third series **21a-g**, substitution at 5- and /or 4-postion of the thiazole ring with different groups proved to be beneficial for activity. The order of activity of the 4-substituted thiazoles **21e-g** was 4-methoxyphenyl **21g** > phenyl **21e** > 4-chlorophenyl **21f** ($IC_{50}$ = 1.44±0.14, 2.38±0.24, 3.11±0.31 µM, respectively). The potency of 4,5-disubstituted thiazole derivatives **21b-d** increased parallel to the increase in lipophilicity of substituent at 5-position, and the order of activity was phenyl carboxamide **21d** > ethyl carboxylate **21c** > acetyl **21b** ($IC_{50}$ = 0.40±0.04, 3.20±0.29, 3.51±0.30 µM, respectively). Highlighting the significance of grafting large lipophilic group on the pendant aryl rings in the first, **16**, and third, **21**, series.

*2.2.2. In vitro anticancer activity.*

The structures of the all synthesized compounds (**16a-k**, **18a-e** and **21a-g**) were submitted to the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov). Seventeen compounds **16c-e**, **16g**, **16h**, **16j, 16k**, **18c-e** and **21a-g** were selected to be screened for their anticancer activity *in vitro*. The anticancer assays were performed in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda [38-40].

*2.2.2.1. Primary single high dose ($10^{-5}$ M) screening.*

The selected compounds were first evaluated at primary anticancer assay against a panel of fifty six cancer lines at concentration $10^{-5}$ M. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. A 48 h drug exposure protocol was used and sulforhodamine B (SRB) protein assay was applied to estimate the cell viability and growth [41]. The mean percentages growth inhibition (GI %) of the tested compounds over the full panel of cell lines are illustrated in Figure 3.

The obtained data revealed that, compounds containing acetamide linker **16c-k** displayed fair anticancer activity (mean % GI = 11-24) except for compound **16k** featuring a lipophilic 4-ethyl carboxylate functionality on the pendant phenyl which elicited the highest activity (mean % GI = 73). Replacing the acetamide linker of the first series with acetyl linker in the second series **18c-e** did not result in significant change of growth inhibitory activity (mean % GI = 14-26 %). On the other hand, replacing the terminal phenyl group in the first series with 2-thiazolyl group in the third series **21a-g** improved the growth inhibitory activity (mean % GI = 23-55 %). The 4-methyl-5-phenyl carboxamide disubstituted thiazole analog **21d** (mean % GI = 55) emerged as the second most potent member in this work.

*2.2.2.2. In vitro 5 dose full NCI 56 cell panel assay.*

The preliminary screening results revealed that compounds **16k** (NSC: D-771612/1) and **21d** (NSC: D-771617/1) are the most active members of this study, showing effectiveness toward numerous cell lines that belong to different tumor subpanels (Figure 4). Accordingly, compounds **16k** and **21d** were selected by the NCI for further evaluation at five-dose assay [0.01–100 µM]. The calculated response parameters for both compounds – $GI_{50,}$ TGI and $LD_{50}$– against each cell line are presented in Tables 2 and 3, respectively. Where, $GI_{50}$ represents the growth inhibitory level of effect; TGI reflects cytostatic activity while $LC_{50}$ is viewed as the cytotoxicity parameter for compounds. Furthermore, subpanel and full panel mean graph midpoints (MG-MID) were calculated for $GI_{50}$ parameter, giving an average activity parameter over individual subpanels and

full panel cell lines toward each compound (Table 4). The results of sorafenib as a reference compound were obtained from NCI Data Warehouse index [42], and inserted in Table 2 and 3.

In general, compound **16k** exhibited superior anti-proliferative activity against almost the entire panel of tumor cell lines with $GI_{50}$ range 1.29-9.28 µM (Table 2), but showed non-significant or weak cytostatic activity against the majority of cell lines except for non-small cell lung cancer (NCI-H522), melanoma (SK-MEL-2 and SK-MEL-5) and breast cancer (T-47D) cell lines (TGI =5.03, 6.13, 2.76 and 7.82 µM, respectively) (Table 3). Meanwhile, compound **21d** displayed potent growth inhibitory activity at single digit micro-molar concentration against 35 cell lines belonging to various cancer subpanels (Table 2), but had no cytostatic effect against most of the tested cell lines (TGI > 100 µM) (Table 3). Moreover, both compounds proved to be non-lethal agents where the $LC_{50}$ values for most cell lines exceeded 100 µM.

Regarding sensitivity of different cell lines, compound **16k** had comparatively homogenous anti-proliferative effect throughout the whole NCI panel, with effective growth inhibition full panel $GI_{50}$ (MG-MID) value of 3.62 µM and subpanel $GI_{50}$ (MG-MID) range of 2.37 – 5.47 µM (Table 4). Among the tested cancer subpanels, the leukemia, melanoma and breast cancer subpanels were the most susceptible to the influence of **16k** [$GI_{50}$ (MG-MID) = 2.37, 2.97 and 2.54 µM, respectively] (Table 4). The highest activity was observed against leukemia (SR), Non-small cell lung cancer (NCI-H522), melanoma (SK-MEL-5), breast cancer (T-47D) ($GI_{50}$ = 1.75, 1.41, 1.36 and 1.29 µM, respectively) (Table 2). On the other hand, compound **21d** showed different levels of anti-proliferative activity with $GI_{50}$ (MG-MID) ranging from 3.51 to >100 µM (Table 4). Leukemia was the most sensitive subpanel while renal cancer subpanel was refractory to **21d** (Table 4). Although, compound **21d** showed decreased growth inhibitory potential compared to **16k,** it possessed significant specific influence on some cancer cell lines. It was found especially effective against breast cancer (T-47D) at sub-micromolar level ($GI_{50}$ = 0.60 µM) and revealed a distinctive effectiveness on leukemia (HL-60, MOLT-4 and RPMI-8226), melanoma (SK-MEL-5), ovarian cancer (OVCAR-4) and breast cancer (MDA-MB-231/ATCC and MDA-MB-468) ($GI_{50}$ range 1.65−2.96 µM) (Table 2).

Moreover, the target compounds **16k** and **21d** exhibited superior potency over sorafenib against 10 and 3 cell lines, respectively (Table 2). Of special interest, sorafenib was interrogated for treatment of breast and non-small cell lung cancers showing significant clinical improvement [43-47]. In this regard, compound **16k** showed anticancer activity overcoming sorafenib towards breast (BT-549 and T-47D) as well as NSCL (A549/ATCC and NCI-H522) cancer cells, while compound **21d** was 2.6 times more active than sorafenib against T-47D, offering an additional merit rather sorafenib. Besides, sorafenib is currently involved in clinical trials for treatment of

different hematological malignancies such as Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) [48-51]. Therefore, it is noteworthy to mention that compound **16k** significantly surpassed the activity of sorafenib against SR and MOLT-4 cell lines (Table 2). On the other hand, combination of sorafenib with different therapies did not result in significant clinical benefit for treatment of CNS cancer [52,53]. Accordingly, it is important to point out that **16k** exhibited higher anti-proliferative activity against CNS cancer cells SNB-19 and SNB-75 than sorafenib ($GI_{50}$ = 2.33 and 2.84 µM, respectively) (Table 2).

Concerning selectivity, the index obtained by dividing the full panel MG-MID (µM) of the compounds by their individual subpanel MG-MID (µM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than six indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria are rated as nonselective [54]. In this context, compound **16k** proved to be nonselective with broad spectrum anticancer activity against all tumor subpanels tested at $GI_{50}$ level, with selectivity ratios ranging from 0.66 to 1.52. On the other hand, compound **21d** showed high selectivity toward leukemia and prostate cancer subpanels with selectivity indexes of 9.31 and 6.34 respectively (Table 4). These results suggest that, replacement of the terminal substituted phenyl in **16** with substituted thiazolyl group in **21** may represent a tool to manipulate selectivity of such class of compounds towards different cancer cells.

### 3. Molecular docking

Docking simulation of compounds **16k** and **21d** showed that they fit into the enzyme active site almost at the same position of sorafenib with comparable docking scores (-15.19 kcal/mol for sorafenib, -16.95 and -17.49 kcal/mol for compounds **16k** and **21d**, respectively) (Figure 5A and 5C).

Inspection of the top docking poses of the target compound **16k** revealed that the N–H of the acetamide linker formed one hydrogen bond to the carboxylate of Glu885 (2.81 Å), while the amide carbonyl oxygen identified to be involved in hydrogen bond with the backbone NH of Asp1046 (2.87 Å). Moreover, the complex is stabilized by arene interactions between the terminal phenyl and Asp1046, the piperazine ring and Phe1047 as well as two interactions between 4-phenylphthalazine and Gly922 and Leu840 (Figure 5B). On the other hand, docking of compound **21d** revealed a binding motif in which the N–H of the acetamide linker acted as essential hydrogen bond donor for Glu885 in the active site (2.78 Å). Additionally, **21d** formed arene interactions, two of which occurred between the phthalazine moiety and Leu840, while the piperazine ring interacted with Phe1047. The other two arene interactions occurred between the

thiazolyl rest and Ile1044 and Asp1046, which justifies the fact that the terminal phenyl ring in **16** can be effectively replaced with thiazole ring in **21** (Figure 5D).

Interestingly, the terminal 4-ethyl carboxylate and 4-phenylcarboxamide functionalities of compounds **16k** and **21d**, respectively were accommodated within a lipophilic pocket comprised of the side chains of Ile888, Leu889, Ile892, Val898, Val899, Leu1019, Cys1024 and Ile1044, that may explain their superiority over the other synthesized derivatives in biological activity. Moreover, the involvement of the piperazinyl moiety of both compounds in interaction with Phe1047 within the active site suggested that this ring may positively contribute to VEGFR2 inhibitory activity.

## 4. Conclusion

In summary, we have synthesized three novel series of 1-piperazinyl-4-phenylphthalazine derivatives **16a-k**, **18a-e** and **21a-g** and evaluated their potential inhibitory action against VEGFR-2. Compounds **16k** and **21d** emerged as the most potent VEGFR-2 inhibitors in this study with $IC_{50}$ values of 0.35±0.03 and 0.40±0.04µM, respectively. Whereas, compounds **16i**, **16j** and **21g** moderately inhibited VEGFR-2 with $IC_{50}$ values of 1.56±0.16, 1.35±0.14 and 1.44±0.14 µM, respectively. The SAR and molecular docking studies pointed that, substitution on the pendant aryl moiety with large lipophilic group seems to be the most important factor affecting the activity of a series of *N-(*aryl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide derivatives **16a-k** and their *N-(*substitutedthiazol-2-yl) bioisosteres **21a-g**. Furthermore, seventeen selected compounds **16c-e**, **16g**, **16h**, **16j, 16k**, **18c-e** and **21a-g** were evaluated for their *in vitro* anticancer activity according to US-NCI protocol. The results revealed that compound **16k** exhibited potent broad spectrum anticancer activity with full panel $GI_{50}$ (MG-MID) value of 3.62 µM. Meanwhile, compound **21d** showed high degree of differential selectivity toward leukemia and prostate cancer subpanels [subpanel $GI_{50}$ (MG-MID) 3.51 and 5.15 µM, respectively] (Table 4). Therefore, compounds **16k** and **21d** can be considered as interesting candidates for further development of more potent anticancer agents.

## 5. Experimental

### *5.1. Chemistry*

Melting points were measured with a Stuart melting point apparatus and were uncorrected. Infrared spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer and expressed in wave number ($cm^{-1}$). The NMR spectra were recorded by Varian Gemini-300BB at 300 MHz (Varian Inc., Palo Alto, CA) or Bruker spectrophotometer at 400 MHz. $^{1}H$ NMR spectra were run at 300 or 400 MHz, while $^{13}C$ NMR spectra were run at 75 or 100 MHz in deuterated dimethyl sulfoxide (DMSO-$d_6$) or deuterated chloroform ($CDCl_3$).

Chemical shifts ($\delta_H$) are reported relative to TMS as internal standard. All coupling constant ($J$) values are given in hertz. Chemical shifts ($\delta_C$) are reported relative to DMSO-$d_6$ as internal standard. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Mass spectra were measured on a GCMS-QP1000 EX and Helwett Packard 5988 spectrometers at 70 e.V. Elemental analyses was carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques.

*5.1.1. 4-Phenyl-1(2H)-phthalazinone* **12**.

Compound **12** was prepared according to reported procedure [35] (m.p. 239-241 $^{o}$C, as reported).

*5.1.2. 1-Chloro-4-phenylphthalazine* **13**.

Compound **13** was prepared according to reported procedure [36] (m.p. 158 $^{o}$C, as reported).

*5.1.3. 4-Phenyl-1-(piperazin-1-yl)phthalazine* **14**.

Compound **14** was prepared according to reported procedure [37] (m.p. 176 - 178 $^{o}$C, as reported).

*5.1.4. 2-Chloro-N-(phenyl/substituted phenyl) acetamide derivatives* **15a-k**.

Compounds **15a-k** were prepared according to reported procedures [55-59].

*5.1.5. General procedure for preparation of target compounds* **16a-k**.

To a stirred mixture of 1-phenyl-4-(1-piperazinyl)phthalazine **14** (0.58 g, 2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in dry acetone, a solution of the appropriate 2-chloro-*N*-(substituted phenyl) acetamide derivative **15a-k** (2.0 mmol) in dry acetone was added. The mixture was heated under reflux for 5 h, filtered while hot and the residue was washed with hot acetone. The combined filtrate and wash were evaporated under vacuum. The residue was collected, washed with water, dried and crystallized from an appropriate solvent.

*5.1.5.1. N-Phenyl-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16a)***:

Crystallized from ethanol/water mixture (yield 71 %); m.p.187-189 °C; IR (KBr, ν cm $^{-1}$): 3251 (NH), 3028 (aromatic CH), 1689 (C=O); $^{1}$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.88 ( s, br, 4H, 2$CH_2$ piperazine)[*], 3.30 (s, 2H, $COCH_2$), 3.55 ( s, br, 4H, 2$CH_2$ piperazine),7.04 ( t, 1H, H-4 of NH-$C_6H_5$, $J$= 8.1 Hz), 7.29 ( t, 2H, H-3 and H-5 of NH-$C_6H_5$, $J$= 8.4 Hz), 7.56 – 7.61 (m, 3H, H-3,

[*] In the present study, the piperazine protons were detected as two broad singlet signals instead of two triplets. This is in agreement with the published $^{1}$ H NMR spectral data of other piperazine derivatives [28, 29].

H-4 and H-5 of $C_6H_5$), 7.66 – 7.70 ( m, 4H, H-2, H-6 of $C_6H_5$ and H-2, H-6 of NH-$C_6H_5$), 7.89 (d, 1H, H-5 phthalazine, *J*= 7.5 Hz), 7.95 – 8.01 (m, 2H, H-6 and H-7 phthalazine), 8.17 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 9.83 (s, 1H, NH, $D_2O$ exchangable); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 51.18 ($2CH_2$ piperazine), 53.12 ($2CH_2$ piperazine), 62.25 (CO$\underline{CH_2}$), 119.97, 121.23, 123.87, 125.04, 126.65, 126.96, 128.93, 129.13, 129.30, 130.15, 132.24, 132.60, 136.85, 139.12, 156.15, 159.38, 168.69 (C=O); MS, *m/z* (%): $M^+$ 423 (6.3); Anal. Calcd. for $C_{26}H_{25}N_5O$: C, 73.74; H, 5.95; N, 16.54; Found C, 73.78; H, 5.96 ; N, 16.59.

*5.1.5.2. 2-[4-(4-Phenylphthalazin-1-yl)piperazin-1-yl]-N-4-tolylacetamide* ***(16b)*****:**

Crystallized from ethanol/water mixture (yield 68 %); m.p.211-213 °C; IR (KBr, ν cm $^{-1}$): 3248 (NH), 3039 (aromatic CH), 1689 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm* :2.26 (s, 3H, $CH_3$), 2.87 (s, br, 4H, $2CH_2$ piperazine), 3.25 (s, 2H, $COCH_2$), 3.54 (s, br, 4H, $2CH_2$ piperazine), 7.11 (d, 2H, H-3 and H-5 of 4-$CH_3$-$C_6H_4$, *J*= 8.4 Hz), 7.54 – 7.63 (m, 5H, H-3, H-4, H-5 of $C_6H_5$ and H-2, H-6 of 4-$CH_3$-$C_6H_4$ ), 7.66 – 7.70 (m, 2H, H-2 and H-6 of $C_6H_5$), 7.89 (d, 1H, H-5 phthalazine, *J*= 7.2 Hz), 7.92 – 8.01 (m, 2H, H-6 and H-7 phthalazine), 8.17( d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 9.73 (s, 1H, NH, $D_2O$ exchangable); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 20.92 ($CH_3$), 51.18 ($2CH_2$ piperazine), 53.13 ($2CH_2$ piperazine), 62.22 (CO$\underline{CH_2}$), 119.98, 121.23, 125.06, 126.67, 126.97, 128.94, 129.31, 129.50, 130.16, 132.27, 132.64, 132.78, 136.61, 136.85, 156.16, 159.39, 168.44 (C=O); MS, *m/z* (%): $M^+$ 437 (7.9); Anal. Calcd. for $C_{27}H_{27}N_5O$: C, 74.17; H, 6.22; N, 16.01; Found C, 74.17; H, 6.21 ; N, 16.05.

*5.1.5.3. 2-[4-(4-Phenylphthalazin-1-yl)piperazin-1-yl]-N-(3-(trifluoromethyl)phenyl)acetamide* ***(16c):***

Crystallized from ethanol (yield 61 %); m.p. 108-111 °C; IR (KBr, ν cm $^{-1}$): 3369 (NH), 3074 (aromatic CH), 1685 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.88 (s, br, 4H, $2CH_2$ piperazine), 3.33 (s, 2H, $COCH_2$), 3.56 (s, br, 4H, $2CH_2$ piperazine), 7.41 (d, 1H, H-4 of 3-$CF_3$-$C_6H_4$, *J*= 7.5 Hz), 7.54 – 7.63 (m, 4H, H-3, H-4, H-5 of $C_6H_5$ and H-5 of 3-$CF_3$-$C_6H_4$), 7.67 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.88 – 7.92 (m, 2H, H-6 of 3- $CF_3$-$C_6H_4$ and H-5 phthalazine), 7.95 – 8.02 (m, 2H, H-6 and H-7 phthalazine), 8.17 – 8.20 (m, 2H, H-2 of 3-$CF_3$-$C_6H_4$ and H-8 phthalazine), 10.18 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50.59 ($2CH_2$ piperazine), 52.56 ($2CH_2$ piperazine), 61.67 (CO$\underline{CH_2}$), 115.61, 120.73, 123.09, 124.53, 126.14, 126.46, 128.39, 128.77, 129.61, 129.80, 131.73, 132.10, 136.34, 139.39, 155.63, 158.87, 168.93 (C=O); MS, *m/z* (%): $M^+$ 491 (3.2); Anal. Calcd. for $C_{27}H_{24}F_3N_5O$: C, 65.98; H, 4.92; N, 14.25; Found C, 66.03; H, 4.95; N, 14.33.

*5.1.5.4. N-(4-Cyanophenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16d)**:*

Crystallized from ethanol (yield 75 %); m.p. 140-141 °C; IR (KBr, ν cm $^{-1}$): 3302 (NH), 2225 (C≡N), 3034 (aromatic CH), 1685 (C=O); $^{1}$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.89 (s, br, 4H, 2$CH_2$ piperazine), 3.27 (s, 2H, CO$CH_2$), 3.57 (s, br, 4H, 2$CH_2$ piperazine), 7.55 – 7.62 (m, 3H, H-3, H-4, H-5 of $C_6H_5$), 7.66 ( d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.76 (d, 2H, H-3 and H-5 of 4-CN-$C_6H_4$, *J*= 9.0 Hz), 7.87 (m, 3H, H-2, H-6 of 4-CN-$C_6H_4$and H-5 phthalazine), 7.92 – 7.99 (m, 2H, H-6 and H-7 phthalazine), 8.16 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 10.21 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50.58 (2$CH_2$ piperazine), 52.53 (2$CH_2$ piperazine), 61.70 (CO$\underline{CH_2}$), 105.11 (C≡N), 118.96, 119.48, 120.71, 124.50, 126.12, 126.45, 128.39, 128.76, 129.61, 131.70, 132.07, 133.11, 136.33, 142.82, 155.62, 158.84, 169.13 (C=O); MS, *m/z* (%): $M^+$ 448 (3.5); Anal. Calcd. for $C_{27}H_{24}N_6O$: C, 72.30; H, 5.39; N, 18.74; Found C, 72.32; H, 5.38; N, 18.78.

*5.1.5.5. N-(2-Chlorophenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16e)**:*

Crystallized from ethanol (yield 71 %); m.p. 184-187 °C; IR (KBr, ν cm $^{-1}$): 3315 (NH), 3032 (aromatic CH), 1695 (C=O); $^{1}$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.93 (s, br, 4H, 2$CH_2$ piperazine), 3.36 (s, 2H, CO$CH_2$), 3.59 (s, br, 4H, 2$CH_2$ piperazine), 7.15 (t, 1H, H-4 of 2-Cl-$C_6H_4$, *J*= 7.8 Hz), 7.37 (t, 1H, H-5 of 2-Cl-$C_6H_4$, *J*= 8.4 Hz), 7.53 (d, 1H, H-3 of 2-Cl-$C_6H_4$, *J*= 8.4 Hz), 7.57 – 7.66 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.67 – 7.70 (m, 2H, H-2 and H-6 of $C_6H_4$), 7.88 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.94 – 8.02 (m, 2H, H-6 and H-7 phthalazine), 8.19 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 8.28 (d, 1H, H-6 of 2-Cl-$C_6H_4$, *J*= 8.4 Hz), 10.01 (s, 1H, NH, $D_2O$ exchangable); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 51.56 (2$CH_2$ piperazine), 53.16 (2$CH_2$ piperazine), 61.78 (CO$\underline{CH_2}$), 121.25, 121.76, 123.27, 125.03, 125.47, 126.71, 127.00, 128.40, 128.95, 129.34, 129.76, 130.15, 132.34, 132.70, 134.91, 136.83, 156.32, 159.34, 168.84 (C=O); MS, *m/z* (%): $M^+$ 457 (4.7), $M^+$+2 459 (1.4); Anal. Calcd. for $C_{26}H_{24}ClN_5O$: C, 68.19; H, 5.28; N, 15.29; Found C, 68.21; H, 5.30; N, 15.38.

*5.1.5.6. N-(3-Chlorophenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16f)**:*

Crystallized from ethanol (yield 74 %); m.p. 129- 131 °C; IR (KBr, ν cm $^{-1}$): 3325 (NH), 3062 (aromatic CH), 1689 (C=O); $^{1}$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.87 (s, br, 4H, 2$CH_2$ piperazine), 3.31 (s, 2H, CO$CH_2$), 3.56 (s, br, 4H, 2$CH_2$ piperazine), 7.11 (d, 1H, H-4 of 3-Cl-$C_6H_4$, *J*= 8.4 Hz), 7.32 (t, 1H, H-5 of 3-Cl-$C_6H_4$, *J*= 8.4 Hz), 7.56 – 7.61 (m, 4H, H-3, H-4, H-5 of $C_6H_5$ and H-6 of 3-Cl-$C_6H_4$), 7.67 – 7.70 (m, 2H, H-2 and H-6 of $C_6H_5$), 7.88 – 7.92 ( m, 2H, H-5 phthalazine and H-2 of 3-Cl-$C_6H_4$), 7.95 – 8.01 (m, 2H, H-6 and H-7 phthalazine), 8.17( d, 1H, H-8 phthalazine, *J*= 8.1 Hz), 10.02 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50. 59 (2$CH_2$ piperazine), 52.56 (2$CH_2$ piperazine), 61.66 (CO$\underline{CH_2}$), 117.89, 119.00, 120.73, 123.04, 124.51,

126.12, 126.46, 128.39, 128.75, 129.61, 130.26, 131.70, 132.06, 132.95, 136.35, 140.05, 155.61, 158.85, 168.66 (C=O); MS, *m/z* (%): $M^+$ 457 (3.3), $M^+$+2 459 (1.3); Anal. Calcd. for $C_{26}H_{24}ClN_5O$: C, 68.19; H, 5.28; N, 15.29; Found C, 68.21; H, 5.31; N, 15.34.

*5.1.5.7. N-(4-Chlorophenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16g)**:*

Crystallized from ethanol (yield 71 %); m.p.213-215 °C; IR (KBr, ν cm $^{-1}$): 3244 (NH), 3032 (aromatic CH), 1693 (C=O); $^1$H NMR ($CDCl_3$, 300 MHz) δ *ppm*: 2.96 (s, br, 4H, $2CH_2$ piperazine), 3.31 (s, 2H, $COCH_2$), 3.69 (s, br, 4H, $2CH_2$ piperazine), 7.31 (d, 2H, H-3 and H-5 of 4-Cl-$C_6H_4$, *J*= 8.7 Hz), 7.55 – 7.59 (m, 5H, H-3, H-4, H-5 of $C_6H_5$ and H-2, H-6 of 4-Cl-$C_6H_4$), 7.73 – 7.70 (m, 2H, H-2 and H-6 of $C_6H_5$), 7.80 – 7.87 (m, 2H, H-6 and H-7 phthalazine), 8.04 (d, 1H, H-5 phthalazine, *J*= 8.1 Hz), 8.12 (d, 1H, H-8 phthalazine, *J*= 8.1 Hz), 9.26 (s, 1H, NH, $D_2O$ exchangable); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 51.14 ($2CH_2$ piperazine), 53.10 ($2CH_2$ piperazine), 62.22 ($CO\underline{CH_2}$), 121.23, 121.57, 125.06, 126.68, 126.97, 127.43, 128.94, 129.02, 129.31, 130.15, 132.27, 132.64, 136.85, 138.11, 156.16, 159.38, 168.93 (C=O); MS, *m/z* (%): $M^+$ 457 (5.6), $M^+$+2 459 (2.6); Anal. Calcd. For $C_{26}H_{24}ClN_5O$: C, 68.19; H, 5.28; N, 15.29; Found C, 68.23; H, 5.27; N, 15.38.

*5.1.5.8. N-(2,6-dichlorophenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16h)**:*

Crystallized from ethanol (yield 75 %); m.p.215-217 °C; IR (KBr, ν cm $^{-1}$): 3269 (NH), 3066 (aromatic CH), 1707 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.94 (s, br, 4H, $2CH_2$ piperazine), 3.26 (s, 2H, $COCH_2$), 3.59 (s, br, 4H, $2CH_2$ piperazine), 7.32 (t, 1H, H-4 of 2,6-$Cl_2$-$C_6H_3$, *J*= 7.8 Hz), 7.53 ( d, 2H, H-3 and H-5 of 2,6-$Cl_2$-$C_6H_3$, *J*= 7.8 Hz), 7.57 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.66 – 7,70 (d, 2H, H-2 and H-6 of $C_6H_5$), 7.87 ( d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.94–8.01 (m, 2H, H-6 and H-7 phthalazine), 8.18 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 9.76 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50. 55 ($2CH_2$ piperazine), 52.76 ($2CH_2$ piperazine), 61.07 ($CO\underline{CH_2}$), 120.73, 124.55, 126.12, 126.46, 128.36, 128.40, 128.77, 129.05, 129.61, 131.72, 132.09, 133.05, 133.75, 136.33, 155.61, 158.87, 168.40 (C=O); MS, *m/z* (%): $M^+$ 491 (4.33), $M^+$+2 493 (2.63), $M^+$+4 495 (0.51); Anal. Calcd. for $C_{26}H_{23}Cl_2N_5O$: C, 63.42; H, 4.71; N, 14.22; Found C, 63.46; H, 4.74; N, 14.29.

*5.1.5.9. N-(3-methoxyphenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(16i)**:*

Crystallized from ethanol/water mixture (yield 59 %); m.p. 187-188 °C; IR (KBr, ν cm $^{-1}$): 3313 (NH), 3062 (aromatic CH), 1681 (C=O); $^1$H NMR ($CDCl_3$, 300 MHz) δ *ppm*: 2.92 (s, br, 4H, $2CH_2$ piperazine), 3.26 (s, 2H, $COCH_2$), 3.64 ( s, br, 4H, $2CH_2$ piperazine), 3.79 (s, 3H, $OCH_3$), 6.63 (d, 1H, H-4 of 3-$OCH_3$-$C_6H_4$, *J*= 7.8 Hz), 7.02 (d, 1H, H-6 of 3-$OCH_3$-$C_6H_4$, *J*= 7.8 Hz), 7.21 (t, 1H,

H-5 of 3-$OCH_3$-$C_6H_4$, *J*= 7.8 Hz), 7.33 (s, 1H, H-2 of 3-$OCH_3$-$C_6H_4$), 7.50 – 7.56 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.67 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.2 Hz), 7.72 – 7.86 (m, 2H, H-6 and H-7 phthalazine), 7.99 (d, 1H, H-5 phthalazine, *J*= 8.1 Hz), 8.10 (d, 1H, H-8 phthalazine, *J*= 7.2 Hz), 9.17 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 51.17 ($2CH_2$ piperazine), 53.10 ($2CH_2$ piperazine), 55.49 ($OCH_3$), 62.26 ($CO\underline{CH_2}$), 105.68, 109.38, 112.18, 121.23, 125.06, 126.67, 126.97, 128.94, 129.31, 129.93, 130.15, 132.28, 132.64, 136.85, 140.30, 156.16, 159.39, 159.98, 168.77 (C=O); MS, *m/z* (%): $M^+$ 453 (3.6); Anal. Calcd. for $C_{27}H_{27}N_5O_2$: C, 71.50; H, 6.00; N, 15.44; Found C, 71.52; H, 6.03; N, 15.51.

*5.1.5.10. N-(4-Methoxyphenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide* ***(16j)****:*
Crystallized from ethanol/water mixture (yield 60 %); m.p.188-190 °C; IR (KBr, ν $cm^{-1}$): 3313 (NH), 3035 (aromatic CH), 1681 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.87 (s, br, 4H, $2CH_2$ piperazine), 3.26 (s, 2H, $COCH_2$), 3.56 (s, br, 4H, $2CH_2$ piperazine), 3.73 (s, 3H, $OCH_3$), 6.88 (d, 2H, H-3 and H-5 of 4-$OCH_3$-$C_6H_4$, *J*= 6.9 Hz), 7.57 – 7.62 (m, 5H, H-3, H-4, H-5 of $C_6H_5$ and H-2, H-6 of 4-$OCH_3$-$C_6H_4$), 7.67 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.2 Hz), 7.89 (d, 1H, H-5 phthalazine, *J*= 7.2 Hz), 7.94 – 8.00 (m, 2H, H-6 and H-7 phthalazine), 8.16 (d, H, H-8 phthalazine, *J*= 7.8 Hz), 9.66 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50.63 ($2CH_2$ piperazine), 52.62 ($2CH_2$ piperazine), 55.12 ($OCH_3$), 61.67 ($CO\underline{CH_2}$), 113.73, 120.71, 121.08, 124.52, 126.13, 126.50, 128.39, 128.76, 129.61, 131.74, 132.08, 136.34, 155.32, 155.61, 158.85, 167.65 (C=O); MS, *m/z* (%): $M^+$ 453 (9.5); Anal. Calcd. for $C_{27}H_{27}N_5O_2$: C, 71.50; H, 6.00; N, 15.44; Found C, 71.53; H, 6.04; N, 15.51.

*5.1.5.11. Ethyl 4-{2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamido}benzoate* ***(16k)***:
Crystallized from ethanol (yield 69 %); m.p.175-177 °C; IR (KBr, ν $cm^{-1}$): 3275 (NH), 3030 (aromatic CH), 1750 (C=O ester), 1689 (C=O amide); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 1.29 (t, 3H, $CH_2\underline{CH_3}$, *J*= 7.2 Hz), 2.89 (s, br, 4H, $2CH_2$ piperazine), 3.34 (s, 2H, $COCH_2$), 3.56 (s, br, 4H, $2CH_2$ piperazine), 4.26 (q, 2H, $\underline{CH_2}CH_3$, *J*= 7.2 Hz), 7.54 – 7.65 (m, 3H, H-3, H-4, H-5 of $C_6H_5$), 7.67 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.82 (d, 2H, H-2 and H-6 of 4-COOEt-$C_6H_4$, *J*= 9.0 Hz), 7.88 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.92 (d, 2H, H-3 and H-5 of 4-COOEt-$C_6H_4$, *J*= 9.3 Hz), 7.95 –8.01 (m, 2H, H-6 and H-7 phthalazine), 8.17 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 10.16 (s, 1H, NH, $D_2O$ exchangable); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 14.69 ($CH_2\underline{CH_3}$), 51.16 ($2CH_2$ piperazine), 53.06 ($2CH_2$ piperazine), 60.91 ($\underline{CH_2}CH_3$), 62.22 ($CO\underline{CH_2}$), 119.29, 121.23, 124.85, 125.06, 126.68, 126.97, 128.94, 129.31, 130.15, 130.62, 132.28, 132.64, 136.84, 143.49, 156.17, 159.38, 165.79 (C=O), 169.41 (C=O); MS, *m/z* (%): $M^+$

495 (7.4); Anal. Calcd. for $C_{29}H_{29}N_5O_3$: C, 70.28; H, 5.90; N, 14.13; Found C, 70.31; H, 5.89; N, 14.20.

*5.1.6. substituted 2-bromo-1-phenylethanone* **17a-e**.

Compounds **17a-e** were prepared according to reported procedures [60].

*5.1.7. General procedure for preparation of target compounds* **18a-e**.

To a stirred mixture of 1-phenyl-4-(1-piperazinyl)phthalazine **14** (0.58 g, 2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in dry acetone, a solution of the appropriate 2-bromo-1-phenylethanone derivative **17a-e** (2.0 mmol) in dry acetone was added. The reaction mixture was stirred for 3 h at room temperature. The solid product was collected by filtration, washed with water, dried and crystallized from an appropriate solvent.

*5.1.7.1. 1-Phenyl-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]ethanone **(18a)**:*

Crystallized from ethanol (yield 77 %); m.p.186-188 °C; IR (KBr, ν cm $^{-1}$): 3057 (aromatic CH), 1697 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.90 (s, br, 4H, $2CH_2$ piperazine), 3.51 (s, br, 4H, $2CH_2$ piperazine), 4.00 (s, 2H, $COCH_2$), 7.52 – 7.63 (m, 6H, H-3, H-4, H-5 of $C_6H_5$ and H-3, H-4, H-5 of $COC_6H_5$), 7.66 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.87 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.95 – 8.00 (m, 2H, H-6 and H-7 phthalazine), 8.04 (d, 2H, H-2 and H-6 of $COC_6H_5$, *J*= 9.0 Hz), 8.17 (d, 1H, H-8 phthalazine, *J*= 8.4 Hz); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50.74 ($2CH_2$ piperazine), 52.49 ($2CH_2$ piperazine), 63.64 ($CO\underline{CH_2}$), 120.70, 124.50, 126.13, 126.46, 128.05, 128.38, 128.57, 128.75, 129.61, 131.71, 132.07, 133.19, 135.92, 136.35, 155.60, 158.82, 196.98 (C=O); MS, *m/z* (%): $M^+$ 408 (14.0); Anal. Calcd. for $C_{26}H_{24}N_4O$: C, 76.45; H, 5.92; N, 13.72; Found C, 76.49; H, 5.94; N, 13.79.

*5.1.7.2. 2-[4-(4-Phenylphthalazin-1-yl)piperazin-1-yl]-1-4-tolylethanone **(18b):***

Crystallized from ethanol (yield 79 %); m.p.181-183 °C; IR (KBr, ν cm $^{-1}$): 3051 (aromatic CH), 1693 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.39 (s, 3H, $CH_3$), 2.85 (s, br, 4H, $2CH_2$ piperazine), 3.49 (s, br, 4H, $2CH_2$ piperazine), 3.97 (s, 2H, $COCH_2$), 7.33 (d, 2H, H-3 and H-5 of 4-$CH_3$-$C_6H_4$, *J*= 7.8 Hz), 7.55 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.66 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.5 Hz), 7.87 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.94 – 8.01 (m, 4H, H-6, H-7 phthalazine and H-2, H-6 of 4-$CH_3$-$C_6H_4$), 8.16 (d, 1H, H-8 phthalazine, *J*= 8.1 Hz); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 21.65 ($CH_3$), 51.27 ($2CH_2$ piperazine), 53.04 ($2CH_2$ piperazine), 64.12 ($CO\underline{CH_2}$), 121.21, 125.01, 126.64, 126.95, 128.70, 128.91, 129.28, 129.62, 130.14, 132.21,

132.57, 133.91, 136.85, 144.08, 156.11, 159.33, 196.94 (C=O); MS, *m/z* (%): $M^+$ 422 (12.3); Anal. Calcd. for $C_{27}H_{26}N_4O$: C, 76.75; H, 6.20; N, 13.26; Found C, 76.74; H, 6.21; N, 13.37.

*5.1.7.3. 1-(4-Chlorophenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]ethanone **(18c)**:*
Crystallized from ethanol (yield 81 %); m.p.205-207 °C; IR (KBr, ν cm $^{-1}$): 3064 (aromatic CH), 1695 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.88 (s, br, 4H, $2CH_2$ piperazine), 3.51 (s, br, 4H, $2CH_2$ piperazine), 3.97 (s, 2H, $COCH_2$), 7.57 – 7.61 (m, 5H, H-3, H-4, H-5 of $C_6H_5$ and H-3, H-5 of 4-Cl-$C_6H_4$), 7.66 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.89 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.94 – 7.99 (m, 2H, H-6 and H-7 phthalazine), 8.07 (d, 2H, H-2 and H-6 of 4-Cl -$C_6H_4$, *J*= 8.4 Hz), 8.16 (d, 1H, H-8 phthalazine, *J*= 8.1 Hz); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50.70 ($2CH_2$ piperazine), 52.46 ($2CH_2$ piperazine), 63.76 ($CO\underline{CH_2}$), 120.71, 124.48, 126.12, 126.47, 128.38, 128.67, 128.74, 129.60, 130.07, 131.69, 132.06, 134.54, 136.36, 138.09, 155.60, 158.80, 196.13 (C=O); MS, *m/z* (%): $M^+$ 442 (13.2), $M^+$+2 444 (4.8); Anal. Calcd. for $C_{26}H_{23}ClN_4O$: C, 70.50; H, 5.23; N, 12.65; Found C, 70.56; H, 5.28; N, 12.74.

*5.1.7.4. 1-(3-Methoxyphenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]ethanone **(18d)**:*
Crystallized from ethanol/water mixture (yield 68 %); m.p.103-106 °C; IR (KBr, ν cm $^{-1}$): 1681 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.89 (s, br, 4H, $2CH_2$ piperazine), 3.51 (s, br, 4H, $2CH_2$ piperazine), 3.84 (s, 3H, $OCH_3$), 4.02 (s, 2H, $COCH_2$), 7.02 (d, 1H, H-4 of 3-$OCH_3$-$C_6H_4$, *J*= 8.4 Hz), 7.44 (t, 1H, H-5 of 3-$OCH_3$-$C_6H_4$, *J*= 8.4 Hz), 7.56 – 7.61 (m, 4H, H-3, H-4, H-5 of $C_6H_5$ and H-6 of 3-$OCH_3$-$C_6H_4$), 7.63 (s, 1H, H-2, of 3-$OCH_3$-$C_6H_4$), 7.66 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.2 Hz), 7.89 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.9 – 8.00 (m, 2H, H-6 and H-7 phthalazine), 8.17 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 51.29 ($2CH_2$ piperazine), 52.98 ($2CH_2$ piperazine), 55.79 ($OCH_3$), 64.23 ($CO\underline{CH_2}$), 113.23, 119.66, 121.04, 121.21, 125.03, 126.67, 126.97, 128.93, 129.30, 130.15, 130.29, 132.26, 132.62, 136.85, 137.79, 156.14, 159.35, 1593.80, 197.29 (C=O); MS, *m/z* (%): $M^+$ 438 (12.5); Anal. Calcd. for $C_{27}H_{26}N_4O_2$: C, 73.95; H, 5.98; N, 12.78; Found C, 74.01; H, 6.00; N, 12.75.

*5.1.7.5. 1-(4-Methoxyphenyl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]ethanone **(18e)**:*
Crystallized from ethanol/water mixture (yield 58 %), m.p.155-157 °C; IR (KBr, ν cm $^{-1}$): 3070 (aromatic CH), 1687 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.87 (s, br, 4H, $2CH_2$ piperazine), 3.50 (s, br, 4H, $2CH_2$ piperazine), 3.86 (s, 3H, $OCH_3$), 3.91 (s, 2H, $COCH_2$), 7.05 (d, 2H, H-3 and H-5 of 4-$OCH_3$-$C_6H_4$, *J*= 8.7 Hz), 7.56 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.66 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.87 (d, 1H, H-5 phthalazine, *J*= 7.8 Hz), 7.95 – 8.08 (m, 2H, H-6 and H-7 phthalazine), 8.04 (d, 2H, H-2 and H-6 of 4-$OCH_3$-$C_6H_4$, *J*= 9.0 Hz),

8.16 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 51.28 ($2CH_2$ piperazine), 53.08 ($2CH_2$ piperazine), 55.99 ($OCH_3$), 64.12 ($CO\underline{CH_2}$), 114.28, 121.21, 125.03, 126.66, 126.96, 128.93, 129.30, 129.32, 130.15, 131.01, 132.25, 132.61, 136.85, 156.13, 159.35, 163.64, 195.85 (C=O); MS, *m/z* (%): $M^+$ 438 (14.0); Anal. Calcd. for $C_{27}H_{26}N_4O_2$: C, 73.95; H, 5.98; N, 12.78; Found C, 73.78; H, 5.97; N, 12.83.

*5.1.8. Substituted 2-aminothiazoles* **19a-g**.

Compounds **19a-g** were prepared according to reported procedures [61-63].

*5.1.9. 2-Chloro-N-(substituted thiazol-2-yl)acetamides* **20a-g.**

Compounds **20a-c** and **20e-g** were prepared according to reported procedures [63-66].

*5.1.9.1. 2-(2-Chloroacetamido)-4-methyl-N-phenylthiazole-5-carboxamide **(20d):***

A mixture of 2-amino-4-methyl-*N*-phenylthiazole-5-carboxamide **19d** (0.47 g, 2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in dry toluene (15 ml) was stirred at room temperature, while 2-chloroacetyl chloride (0.23 g, 0.16 mL, 2.0 mmol) was added dropwise. The reaction mixture was heated under reflux for 3 h, solvent was then removed *in vacuo* and the solid obtained was triturated with water, filtered, dried and recrystallized from methanol to produce **20d** (yield 75 %); m.p.180-182 °C; IR (KBr, ν cm $^{-1}$): 3170 (NH), 3039 (aromatic CH), 1705 (C=O amide); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ *ppm*: 2.54 (s, 3H, $CH_3$), 4.42 (s, 2H, $COCH_2$), 7.06 (t, 1H, H-4 of $NHC_6H_5$, *J*= 7.36 Hz), 7.30 (t, 2H, H-3 and H-5 of NH-$C_6H_5$, *J*= 8.0 Hz), 7.67 (d, 2H, H-2 and H-6 of NH-$C_6H_5$, *J*= 7.76 Hz), 9.99 (s, 1H, NH), 12.75 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 17.52 ($CH_3$), 42.75 ($CO\underline{CH_2}$), 119.91, 120.87, 124.21, 128.92, 129.02, 139.28, 152.04, 157.32, 160.96 (C=O), 165.97 (C=O).

*5.1.10. General procedure for preparation of target compounds* **21a-g**.

To a stirred mixture of 1-phenyl-4-(1-piperazinyl)phthalazine **14** (0.58 g, 2.0 mmol) and anhydrous potassium carbonate (0.55 g, 4.0 mmol) in dry DMF, a solution of the appropriate 2-chloro-*N*-(substituted thiazol-2-yl)acetamide derivatives **20a-g** (2.0 mmol) in dry DMF was added. The mixture was heated at 100 $^0$C for 3 h. The reaction mixture was cooled then poured over crushed ice. The precipitated product was filtered, washed with water, dried and crystallized from an appropriate solvent.

*5.1.10.1. 2-[4-(4-Phenylphthalazin-1-yl)piperazin-1-yl]-N-(thiazol-2-yl)acetamide **(21a)**:*

Crystallized from ethanol (yield 69 %); m.p.233-234 °C; IR (KBr, ν cm $^{-1}$): 3284 (NH), 3111 (aromatic CH), 1695 (C=O); $^{1}$H NMR ($CDCl_3$, 300 MHz) δ *ppm*: 2.91 (s, br, 4H, $2CH_2$ piperazine), 3.38 (s, 2H, $COCH_2$), 3.67(s, br, 4H, $2CH_2$ piperazine), 6.98 (d, 1H, H-5 thiazole, *J*= 3.3 Hz), 7.43 (d, 1H, H-4 thiazole, *J*= 3.3 Hz), 7.46 – 7.55 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.69 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.75 – 7.85 (m, 2H, H-6 and H-7 phthalazine), 7.98 (d, 1H, H-5 phthalazine *J*= 8.1 Hz), 8.10 (d, 1H, H-8 phthalazine, *J*= 8.4 Hz), 10.45 (s, 1H, NH); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ: 51.12 ($2CH_2$ piperazine), 53.75 ($2CH_2$ piperazine), 61.47 ($CO\underline{CH_2}$), 113.95, 121.96, 124.57, 127.22, 127.66, 128.61, 129.13, 130.14, 131.37, 131.70, 136.70, 137.82, 156.76, 159.30, 169.50 (C=O); MS, *m/z* (%): $M^+$+1 431 (12.7); Anal. Calcd. for $C_{23}H_{22}N_6OS$: C, 64.16; H, 5.15; N, 19.52; Found C, 64.19; H, 5.14; N, 19.55.

*5.1.10.2. N-(5-Acetyl-4-methylthiazol-2-yl)-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide* ***(21b):***

Crystallized from ethanol (yield 72 %); m.p.157-158 °C; IR (KBr, ν cm $^{-1}$): 3298 (NH), 3059 (aromatic CH), 1681 (C=O amide),1658 (C=O ketone); $^{1}$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.48 (s, 3H, $CH_3$), 2.55 (s, 3H, $COCH_3$), 2.89 (s, br, 4H, $2CH_2$ piperazine), 3.49 (s, 2H, $COCH_2$), 3.53 (s, br, 4H, $2CH_2$ piperazine), 7.53 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.66 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.88 (d, 1H, H-5 phthalazine *J*= 7.8 Hz), 7.93 – 7.99 (m, 2H, H-6 and H-7 phthalazine), 8.16 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 12.75 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 17.92 ($CH_3$), 29.99 ($CO\underline{CH_3}$), 50.66 ($2CH_2$ piperazine), 52.27 ($2CH_2$ piperazine), 60.02 ($CO\underline{CH_2}$), 120.71, 124.49, 125.36, 126.10, 126.47, 131.68, 132.05, 133.49, 136.34, 154.20, 155.61, 158.81, 158.93, 169.25 (C=O), 190.54 (C=O); MS, *m/z* (%): $M^+$ 486 (3.4); Anal. Calcd. for $C_{26}H_{26}N_6O_2S$: C, 64.18; H, 5.39; N, 17.27; Found C, 64.23; H, 5.40; N, 17.24.

*5.1.10.3. Ethyl 4-methyl-2-{2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamido} thiazole-5-carboxylate* ***(21c)****:*

Crystallized from ethanol (yield 75 %); m.p.140-143 °C; IR (KBr, ν cm $^{-1}$): 3169 (NH), 3041(aromatic CH), 1712 (C=O ester), 1676 (C=O amide); $^{1}$H NMR ($CDCl_3$, 300 MHz) δ *ppm*: 1.35 (t, 3H, $CH_2\underline{CH_3}$, *J*= 6.9 Hz), 2.67 (s, 3H, $CH_3$), 2.94 (s, br, 4H, $2CH_2$ piperazine), 3.42 (s, 2H, $COCH_2$), 3.71 (s, br, 4H, $2CH_2$ piperazine), 4.32 (q, 2H, $\underline{CH_2}CH_3$, *J*= 6.9 Hz), 7.53 – 7.60 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.74 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.79 – 7.88 (m, 2H, H-6 and H-7 phthalazine), 8.04 (d, 1H, H-5 phthalazine *J*= 6.9 Hz), 8.10 (d, 1H, H-8 phthalazine, *J*= 7.2 Hz), 10.50 (s, 1H, NH); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ: 14.58 ($CH_2\underline{CH_3}$), 17.36 ($CH_3$), 51.10 ($2CH_2$ piperazine), 53.80 ($2CH_2$ piperazine), 61.12 ($\underline{CH_2}CH_3$), 61.48 ($CO\underline{CH_2}$), 116.54, 121.97,124.55, 127.31, 127.68, 128.68, 129.20, 130.19, 131.42, 131.74, 136.68, 156.74, 156.90,

158.73, 159.27, 162.92 (C=O), 168.94 (C=O); MS, *m/z* (%): $M^+$ 516 (3.2); Anal. Calcd. for $C_{27}H_{28}N_6O_3S$: C, 62.77; H, 5.46; N, 16.27; Found C, 62.81; H, 5.44; N, 16.30.

*5.1.10.4. 4-Methyl-N-phenyl-2-{2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamido}thiazole-5-carboxamide **(21d)**:*

Crystallized from ethanol (yield 69 %); m.p.175-177 °C; IR (KBr, ν cm $^{-1}$): 3242 (NH), 3061 (aromatic CH), 1693 (C=O amide), 1674 (C=O amide); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.49 (s, 3H, $CH_3$), 2.90 (s, br, 4H, $2CH_2$ piperazine), 3.50 (s, 2H, $COCH_2$), 3.54 (s, br, 4H, $2CH_2$ piperazine), 7.09 (t, 1H, H-4 of $NHC_6H_5$, *J*= 7.2 Hz), 7.35 (t, 2H, H-3 and H-5 of NH-$C_6H_5$, *J*= 7.8 Hz), 7.53 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.66 – 7.70 (m, 4H, H-2, H-6 of $C_6H_5$ and H-2, H-6 of NH-$C_6H_5$), 7.87 (d, 1H, H-5 phthalazine *J*= 8.1 Hz), 7.94 – 8.00 (m, 2H, H-6 and H-7 phthalazine), 8.16 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 9.92 (s, 1H, NH), 12.23 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 17.54 ($CH_3$), 51.20 ($2CH_2$ piperazine), 52.18 ($2CH_2$ piperazine), 60.56 ($CO\underline{CH_2}$), 119.42, 120.90, 121.22, 124.14, 125.02, 126.65, 126.96, 128.92, 129.04, 129.29, 130.14, 132.23, 132.59, 136.84, 139.38, 151.96, 156.14, 157.31, 159.34, 161.11(C=O), 169.49 (C=O); MS, *m/z* (%): $M^+$ 563 (1.5); Anal. Calcd. for $C_{31}H_{29}N_7O_2S$: C, 66.05; H, 5.19; N, 17.39; Found C, 66.10; H, 5.21; N, 17.36.

*5.1.10.5. 2-[4-(4-Phenylphthalazin-1-yl)piperazin-1-yl]-N-(4-phenylthiazol-2-yl)acetamide **(21e)**:*

Crystallized from ethanol (yield 63 %); m.p.241-243 °C; IR (KBr, ν cm $^{-1}$): 3356 (NH), 3109 (aromatic CH), 1697 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.90 (s, br, 4H, $2CH_2$ piperazine), 3.50 (s, 2H, $COCH_2$), 3.52 (s, br, 4H, $2CH_2$ piperazine), 7.32 (t, 1H, H-4 of phenylthiazole, *J*= 8.4 Hz), 7.44 (t, 2H, H-3 and H-5 of phenylthiazole, *J*= 8.4 Hz), 7.55 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.66 (s, 1H, H-5 thiazole), 7.67 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 8.1 Hz), 7.87 – 7.93 (m, 3H, H-5 phthalazine and H-2 and H-6 of phenylthiazole), 7.95 – 8.00 (m, 2H, H-6 and H-7 phthalazine), 8.17 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 12.20 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ: 50.73 ($2CH_2$ piperazine), 52.33 ($2CH_2$ piperazine), 60.05 ($CO\underline{CH_2}$), 108.07, 120.71, 124.53, 125.65, 126.12, 126.46, 127.72, 128.39, 128.65, 128.75, 129.61, 131.71, 132.07, 134.22, 136.35, 148.82, 155.63, 157.44,158.84, 168.62 (C=O); MS, *m/z* (%): $M^+$ 506 (3.4); Anal. Calcd. for $C_{29}H_{26}N_6OS$: C, 68.75; H, 5.17; N, 16.59; Found C, 68.72; H, 5.20; N, 16.63.

*5.1.10.6. N-[4-(4-Chlorophenyl)thiazol-2-yl]-2-[4-(4-phenylphthalazin-1-yl)piperazin-1-yl]acetamide **(21f)**:*

Crystallized from ethanol (yield 63 %); m.p.173-175 $^0$C; IR (KBr, ν cm $^{-1}$): 3367 (NH), 3053 (aromatic CH), 1697 (C=O); $^1$H NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.91 (s, br, 4H, $2CH_2$

piperazine), 3.50 (s, 2H, $COCH_2$), 3.54 (s, br, 4H, $2CH_2$ piperazine), 7.47 (d, 2H, H-3 and H-5 of 4-Cl-$C_5H_4$, *J*= 8.1 Hz), 7.56 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.67 – 7.70 (m, 3H, H-2, H-6 of $C_6H_5$ and H-5 thiazole), 7.89 – 8.10 (m, 5H, H-5, H-6, H-7 phthalazine and H-2, H-6 of 4-Cl-$C_5H_4$), 8.17 ( d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 12.04 (s, 1H, NH, $D_2O$ exchangable); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ: 51.26 ($2CH_2$ piperazine), 52.33 ($2CH_2$ piperazine), 60.59 ($CO\underline{CH_2}$), 109.30, 121.22, 125.00, 126.62, 126.95, 127.85, 128.89, 129.17, 129.26, 130.14, 132.17, 132.53, 132.72, 133.59, 136.85, 148.09, 156.13, 158.19, 159.34, 169.22 (C=O); MS, *m/z* (%): $M^+$ 540 (4.2), $M^+$+2 542 (1.4); Anal. Calcd. for $C_{29}H_{25}ClN_6OS$: C, 64.37; H, 4.66; N, 15.53; Found C, 64.41; H, 4.65; N, 15.50.

*5.1.10.7. N-[4-(4-Methoxyphenyl)thiazol-2-yl]-2-[4-(4-phenylphthalazin-1-yl) piperazin-1-yl]acetamide **(21g)**:*

Crystallized from ethanol (yield 55 %); m.p.163-165 °C; IR (KBr, ν $cm^{-1}$): 3292 (NH), 3088 (aromatic CH), 1691 (C=O); $^1H$ NMR (DMSO-$d_6$, 300 MHz) δ *ppm*: 2.92 (s, br, 4H, $2CH_2$ piperazine), 3.49 (s, 2H, $COCH_2$), 3.55 (s, br, 4H, $2CH_2$ piperazine), 3.74 (s, 3H, $OCH_3$), 6.98 (d, 2H, H-3 and H-5 of 4-$OCH_3$-$C_5H_4$, *J*= 8.7 Hz), 7.44 (s, 1H, H-5 thiazole), 7.56 – 7.62 (m, 3H, H-3, H-4 and H-5 of $C_6H_5$), 7.67 (d, 2H, H-2 and H-6 of $C_6H_5$, *J*= 7.8 Hz), 7.83 (d, 2H, H-2 and H-6 of 4-$OCH_3$-$C_5H_4$, *J*= 8.1 Hz), 7.87 (d, 1H, H-5 phthalazine *J*= 7.8 Hz), 7.95 – 8.00 (m, 2H, H-6 and H-7 phthalazine), 8.17 (d, 1H, H-8 phthalazine, *J*= 7.8 Hz), 11.91 (s, 1H, NH, $D_2O$ exchangable); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ: 51.26 ($2CH_2$ piperazine), 52.85 ($2CH_2$ piperazine), 55.59 ($OCH_3$), 60.59 ($CO\underline{CH_2}$), 106.55, 114.54, 121.22, 125.04, 126.64, 126.96, 127.50, 127.58, 128.91, 129.28, 130.15, 132.22, 132.58, 136.85, 149.23, 156.15, 157.81, 159.36, 159.45, 169.04 (C=O); MS, *m/z* (%): $M^+$ 536 (1.6); Anal. Calcd. for $C_{30}H_{28}N_6O_2S$: C, 67.14; H, 5.26; N, 15.66; Found C, 67.20; H, 5.28; N, 15.75.

*5.2 Biological evaluation*

*5.2.1. Measurement of inhibitory activity against VEGFR-2*

The kinase activity of VEGFR-2 was measured by use of a phosphotyrosine antibody with the Alpha Screen system (PerkinElmer, USA) according to manufacturer's instructions. Enzyme reactions were performed in 50 mM Tris–HCl pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.01% Tween-20 and 2 mM DTT, containing 10 µM ATP, 0.1 µg/mL biotinylated poly-GluTyr (4:1) and 0.1 nM of VEGFR-2 (Millipore, UK). Prior to catalytic initiation with ATP, the tested compounds at final concentrations ranging from 0-100 µg/mL and enzyme were incubated for 5 min at room temperature. The reactions were quenched by the addition of 25 µL of 100 mM EDTA, 10 µg/mL

Alpha Screen streptavidine donor beads and 10 µg/mL acceptor beads in 62.5 mM HEPES pH 7.4, 250 mM NaCl, and 0.1% BSA. Plate was incubated in the dark overnight and then read by ELISA Reader (PerkinElmer, USA). Wells containing the substrate and the enzyme without compounds were used as reaction control. Wells containing biotinylated poly-GluTyr (4:1) and enzyme without ATP were used as basal control. Percent inhibition was calculated by the comparison of compounds treated to control incubations. The concentration of the test compound causing 50% inhibition ($IC_{50}$) was calculated from the concentration–inhibition response curve (triplicate determinations) and the data were compared with Sorafenib as standard VEGFR-2 inhibitor.

*5.2.2. In vitro cytotoxic activity*

The cytotoxicity assays were performed at National Cancer Institute (NCI), Bethesda, USA (against 56 cell lines). The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µ at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 $^{o}$C, 5% $CO_2$, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition ($T_z$). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Triplicate wells were prepared for each individual dose. Following drug addition, the plates were incubated for an additional 48 h at 37 $^{o}$C, 5% $CO_2$, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 $^{o}$C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the

same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero (*Tz*), control growth (*C*), and test growth in the presence of drug at the five concentration levels ($T_i$)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$[(T_i - T_z) / (C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$

$[(T_i - T_z) / T_z] \times 100$ for concentrations for which $T_i < T_z$

Three dose response parameters were calculated for each experimental compound: Growth inhibition of 50% ($GI_{50}$) was calculated when $[(T_i - T_z) / (C - T_z)] \times 100 = 50$. The compound concentration resulting in total growth inhibition (TGI) was calculated when $T_i = T_z$. The $LC_{50}$ indicating a net loss of cells following treatment was calculated when $[(T_i - T_z) / T_z] \times 100 = -50$.

*5.3. Molecular docking*

The molecular docking studies were carried out using Molecular Operating Environment (MOE) software version 2010.10 [67]. The two compounds, **16k** and **21d**, were drawn on MOE. The structures were subjected to energy minimization using Hamiltonian-Force Field-MMFF94x and the force field partial charges were calculated for each molecule. Stochastic conformational analysis was run for each molecule using default settings and the four most stable conformers for each compound were retained. The X-ray crystal structure of VEGFR-2 in complex with sorafenib was downloaded from http://www.rscb.org/pdb (PDB ID: 4ASD) [68]. The protein-ligand complex obtained from the protein data bank was prepared for docking as follows: 1-The enzyme was 3D protonated, where hydrogen atoms were added at their standard geometry, the partial charges were computed and the system was optimized. 2-Deletion of chain B of the protein together with co-crystallized water molecules was performed. 3-The binding pocket has been defined, isolated and then the back bone was hidden.

Flexible ligand-rigid receptor docking of the most stable conformers was done with MOEDOCK using Alpha triangle placement method and London dG as a scoring function. The obtained poses were subjected to force field refinement using the same scoring function. Ten of the most stable docking models for each ligand were retained with the best scored conformation. In order to validate the docking procedure, sorafenib was re-docked into the active site of 4ASD. The docking results showed a near perfect alignment with the original ligand and displayed the same binding interactions as obtained from the X-ray crystallography pdb file.

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## FIGURE CAPTIONS

**Figure 1.** Structures of VEGFR-2 inhibitors approved for clinical use.

**Figure 2**. Structures of compounds **8-11** and the target 1-piperazinyl-4-phenylphthalazine derivatives **16a-k**, **18a-e** and **21a-g**.

**Figure 3**. Mean % growth inhibitions of the tested compounds over NCI 56 cell line panel.

**Figure 4**. % Growth inhibition exerted by compounds **16k** and **21d** at 10 μM concentration over NCI 56 cell line panel.

**Figure 5**. The best scored docking model of (A) Compound **16k** (in yellow) in 3D style overlaid with sorafenib (in orange), (B) Compound **16k** in 2D style, (C) Compound **21d** (in yellow) in 3D style overlaid with sorafenib (in orange) and (D) Compound **21d** in 2D style; most hydrogen atoms and some residues were omitted for clarity

## TABLE CAPTIONS

**Table 1**. VEGFR-2 kinase inhibitory activity of the target 1-piperazinyl-4-phenylphthalazines.

**Table 2.** NCI *in vitro* testing result of $GI_{50}$ of compounds **16k** (NSC: D-771612/1) and **21d** (NSC: D-771617/1) and sorafenib at five dose level.[a,b]

**Table 3.** TGI values of compounds **16k** and **21d** and sorafenib over the most sensitive cell lines.[a]

**Table 4.** Median growth inhibitory concentrations [a] ($GI_{50}$, μM) of *in vitro* subpanel tumor cell lines for compounds **16k** and **21d** and sorafenib.

## SCHEME CAPTIONS

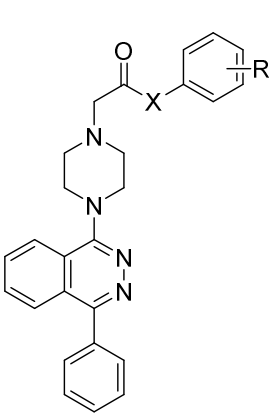
**Scheme 1**. Synthesis of the key intermediate **14**. Reagents and conditions: (i) $NH_2NH_2.H_2SO_4$ / NaOH / reflux 1h / 80%; (ii) $POCl_3$ / heat 85 °C 4h / 73%; (iii) anhydrous piperazine / isopropyl alcohol / heat 75 °C 4h / 85%.

**Scheme 2.** Synthesis of target phthalazines **16a-k.** Reagents and conditions: (i) dioxane / r.t. 2h / 75-93%; (ii) compound **14** / dry acetone / $K_2CO_3$ / reflux 5h / 59-75%.

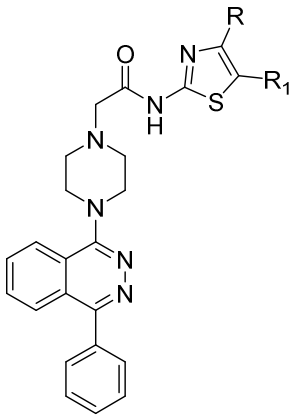
**Scheme 3.** Synthesis of target phthalazines **18a-e.** Reagents and conditions: (i) $CuBr_2$ / $CH_3COOEt$ and $CHCl_3$/ reflux 2-4h / 77-85%; (ii) compound **14** / dry acetone / $K_2CO_3$ / r.t. 3h / 58-81%.

**Scheme 4.** Synthesis of target phthalazines **21a-g.** Reagents and conditions: (i) dry toluene / $K_2CO_3$ / reflux 3h / 65-79%; (ii) compound **14** / dry DMF / $K_2CO_3$ / heating 3h / 55-75%.

**Table 1**.VEGFR-2 kinase inhibitory activity of the target 1-piperazinyl-4-phenylphthalazines.



16a-k / 18a-e



21-a-g

| Compound | X | R | $R_1$ | $IC_{50}$ (μM)[a] |
|---|---|---|---|---|
| **16a** | NH | H | - | 4.68±0.47 |
| **16b** | NH | 4-$CH_3$ | - | 4.27±0.43 |
| **16c** | NH | 3-$CF_3$ | - | 3.66±0.37 |
| **16d** | NH | 4-CN | - | 4.35±0.45 |
| **16e** | NH | 2-Cl | - | 3.95±0.40 |
| **16f** | NH | 3-Cl | - | 4.07±0.37 |
| **16g** | NH | 4-Cl | - | 3.84±0.39 |
| **16h** | NH | 2,6-$Cl_2$ | - | 3.39±0.35 |
| **16i** | NH | 3-$OCH_3$ | - | 1.56±0.16 |
| **16j** | NH | 4-$OCH_3$ | - | 1.35±0.14 |
| **16k** | NH | 4-$COOC_2H_5$ | - | 0.35±0.03 |
| **18a** | - | H | - | 4.54±0.36 |
| **18b** | - | 4-$CH_3$ | - | 4.26±0.44 |
| **18c** | - | 4-Cl | - | 4.29±0.43 |
| **18d** | - | 3-$OCH_3$ | - | 3.42±0.36 |
| **18e** | - | 4-$OCH_3$ | - | 2.25±0.22 |
| **21a** | - | H | H | 4.16±0.35 |
| **21b** | - | $CH_3$ | COCH3 | 3.51±0.30 |
| **21c** | - | $CH_3$ | $COOC_2H_5$ | 3.20±0.29 |
| **21d** | - | $CH_3$ | $CONHC_6H_5$ | 0.40±0.04 |
| **21e** | - | $C_6H_5$ | H | 2.38±0.24 |
| **21f** | - | 4-$ClC_6H_4$ | H | 3.11±0.31 |
| **21g** | - | 4-$OCH_3C_6H_4$ | H | 1.44±0.14 |
| **Sorafenib**[69] | - | - | - | 0.10±0.01/(0.09) [b] |
| **Vatalanib**[15] | - | - | - | 0.18±0.02/(0.043)[b] |
| **Sunitinib**[8] | - | - | - | 0.08[b] |
| **Pazopanib**[9] | - | - | - | 0.03[b] |
| **Vandetanib**[10] | - | - | - | 0.04[b] |

[a] Data were expressed as Mean ± Standard error (S.E.) of three independent experiments.
[b] Reported $IC_{50}$ values.

**Table 2.** NCI *in vitro* testing result of $GI_{50}$ of compounds **16k** (NSC: D-771612/1) and **21d** (NSC: D-771617/1) and sorafenib at five dose level.[a,b]

| Subpanel /tumor cell lines | Compound | | |
|---|---|---|---|
| | **16k** | **21d** | **Sorafenib** |
| | $GI_{50}$(μM) | $GI_{50}$(μM) | $GI_{50}$(μM) |
| **Leukemia** | | | |
| CCRF-CEM | 2.82 | 4.52 | 2.00 |
| HL60(TB) | 2.01 | 2.61 | 1.58 |
| K-562 | 3.26 | 4.44 | 3.16 |
| MOLT-4 | **2.00** | **2.86** | 3.16 |
| RPMI-8226 | 2.42 | 2.74 | 1.58 |
| SR | **1.75** | 3.93 | 3.16 |
| **Non-Small Cell Lung Cancer** | | | |
| A549/ATCC | **2.44** | 3.61 | 3.16 |
| HOP-62 | 6.51 | > 100 | 2.00 |
| NCI-H226 | 3.33 | 7.21 | 2.00 |
| NCI-H23 | 4.29 | 7.20 | 2.00 |
| NCI-H322M | NT | 6.58 | 2.51 |
| NCI-H460 | 3.90 | 7.81 | 2.51 |
| NCI-H522 | **1.41** | 3.53 | 2.00 |
| **Colon Cancer** | | | |
| COLO 205 | 2.87 | 3.26 | 2.00 |
| HCT-116 | 3.75 | 4.36 | 1.58 |
| HCT-15 | 3.04 | 6.45 | 2.51 |
| HT29 | 3.29 | 8.11 | 2.00 |
| KM12 | 3.67 | 5.12 | 1.58 |
| SW-620 | 4.50 | > 100 | 2.51 |
| **CNS Cancer** | | | |
| SF-268 | 5.56 | NT | 2.51 |
| SF-295 | 3.64 | 4.95 | 1.58 |
| SF-539 | 3.53 | > 100 | 1.58 |
| SNB-19 | **2.33** | > 100 | 3.16 |
| SNB-75 | **2.84** | NT | 3.16 |
| U251 | 4.39 | 8.64 | 2.00 |
| **Melanoma** | | | |
| LOX IMVI | 2.62 | 7.18 | 1.58 |
| M14 | 4.13 | 8.94 | 2.00 |
| MDA-MB-435 | 3.68 | 6.38 | 1.58 |
| SK-MEL-2 | 2.42 | 72.20 | 1.58 |
| SK-MEL-28 | 3.59 | > 100 | 2.00 |
| SK-MEL-5 | **1.36** | 2.16 | 1.58 |
| UACC-257 | 3.05 | 5.01 | 2.00 |
| **Ovarian Cancer** | | | |
| IGROV1 | 9.19 | > 100 | 2.51 |
| OVCAR-3 | 4.81 | 6.52 | 3.16 |
| OVCAR-4 | **2.62** | **2.96** | 3.16 |
| OVCAR-5 | NT | 91.00 | 3.16 |
| OVCAR-8 | 3.46 | 3.60 | 2.51 |
| NCI/ADR-RES | 3.46 | 5.04 | 2.51 |
| SK-OV-3 | 9.28 | > 100 | 2.51 |
| **Renal Cancer** | | | |
| 786-0 | 4.06 | > 100 | 3.16 |

| | | | |
|---|---|---|---|
| A498 | NT | NT | 2.51 |
| ACHN | 4.52 | > 100 | 3.16 |
| CAKI-1 | 7.05 | > 100 | 3.16 |
| SN12C | 3.41 | > 100 | 2.51 |
| UO-31 | 3.07 | NT | 2.51 |
| **Prostate Cancer** | | | |
| PC-3 | 2.32 | 3.19 | 2.00 |
| DU-145 | 5.61 | 7.12 | 3.16 |
| **Breast Cancer** | | | |
| MCF7 | 2.98 | 4.75 | 2.51 |
| MDA-MB-231/ATCC | 2.19 | 1.65 | 1.26 |
| HS 578T | 4.17 | > 100 | 2.51 |
| BT-549 | **2.63** | 4.83 | 3.16 |
| T-47D | **1.29** | **<u>0.60</u>** | 1.58 |
| MDA-MB-468 | 2.01 | 2.70 | 2.00 |

[a]Bold figures indicate superior potency than sorafenib, bold underlined figures refer to submicromolar $GI_{50}$ values.
[b]NT = not tested

**Table 3.** TGI values of compounds **16k** and **21d** and sorafenib over the most sensitive cell lines.[a]

| Subpanel /tumor cell lines | Compound | | |
|---|---|---|---|
| | **16k** | **21d** | **Sorafenib** |
| | TGI(μM) | TGI(μM) | TGI(μM) |
| **Non-Small Cell Lung Cancer** | | | |
| A549/ATCC | 42.30 | > 100 | 7.36 |
| HOP-62 | 27.20 | > 100 | 23.55 |
| NCI-H226 | 18.60 | > 100 | 14.42 |
| NCI-H522 | **5.03** | > 100 | 14.82 |
| **CNS Cancer** | | | |
| SNB-75 | 17.70 | > 100 | 10.42 |
| **Melanoma** | | | |
| SK-MEL-2 | **6.13** | > 100 | 14.85 |
| SK-MEL-28 | 36.90 | > 100 | 13.39 |
| SK-MEL-5 | **2.76** | > 100 | 31.26 |
| UACC-257 | 19.30 | > 100 | 17.33 |
| **Ovarian Cancer** | | | |
| OVCAR-3 | 48.50 | > 100 | 10.32 |
| OVCAR-4 | 20.30 | > 100 | 4.31 |
| OVCAR-8 | 22.70 | > 100 | 4.73 |
| SK-OV-3 | 30.00 | > 100 | 16.36 |
| **Renal Cancer** | | | |
| 786-0 | 39.90 | > 100 | 7.62 |
| **Breast Cancer** | | | |
| MCF7 | 12.50 | > 100 | 9.97 |
| MDA-MB231/ATCC | **12.50** | **15.50** | 23.93 |
| T-47D | **7.82** | **8.64** | 14.38 |
| MDA-MB-468 | **12.30** | > 100 | 13.09 |

[a]Bold figures refer to superior efficacies than sorafenib regarding TGI values

**Table 4.** Median growth inhibitory concentrations[a] ($GI_{50}$, μM) of *in vitro* subpanel tumor cell lines for compounds **16k** and **21d** and sorafenib.

| Subpanel tumor cell line | 16k | | 21d | | Sorafenib | |
|---|---|---|---|---|---|---|
| | **MG-MID** | **Selectivity index** | **MG-MID** | **Selectivity index** | **MG-MID** | **Selectivity index** |
| Leukemia | 2.37 | 1.52 | 3.51 | 9.31 | **2.44** | 0.97 |
| NSCL Cancer | 3.64 | 0.99 | >19.42 | <1.68 | 2.31 | 1.02 |
| Colon Cancer | 3.52 | 1.02 | >21.21 | <1.54 | 2.03 | 1.16 |
| CNS Cancer | 3.71 | 0.97 | >53.39 | <0.61 | 2.33 | 1.02 |
| Melanoma | 2.97 | 1.21 | >28.38 | <1.15 | 1.76 | 1.34 |
| Ovarian Cancer | 5.47 | 0.66 | >44.16 | <0.74 | 2.78 | 0.85 |
| Renal Cancer | 4.42 | 0.81 | >100 | <0.32 | 2.84 | 0.83 |
| Prostate Cancer | 3.96 | 0.91 | 5.15 | 6.34 | 2.58 | 0.91 |
| Breast Cancer | 2.54 | 1.42 | >19.08 | <1.71 | **2.17** | 1.09 |
| Full panel MG-MID[b] | 3.62 | | >32.70 | | 2.36 | |

[a] Median value calculated according to the data obtained from NCI's *in vitro* disease-oriented human tumor cell screen.

[b] $GI_{50}$ (μM) full panel mean-graph midpoint (MG-MID) = the average sensitivity of all cell lines toward the test agents.

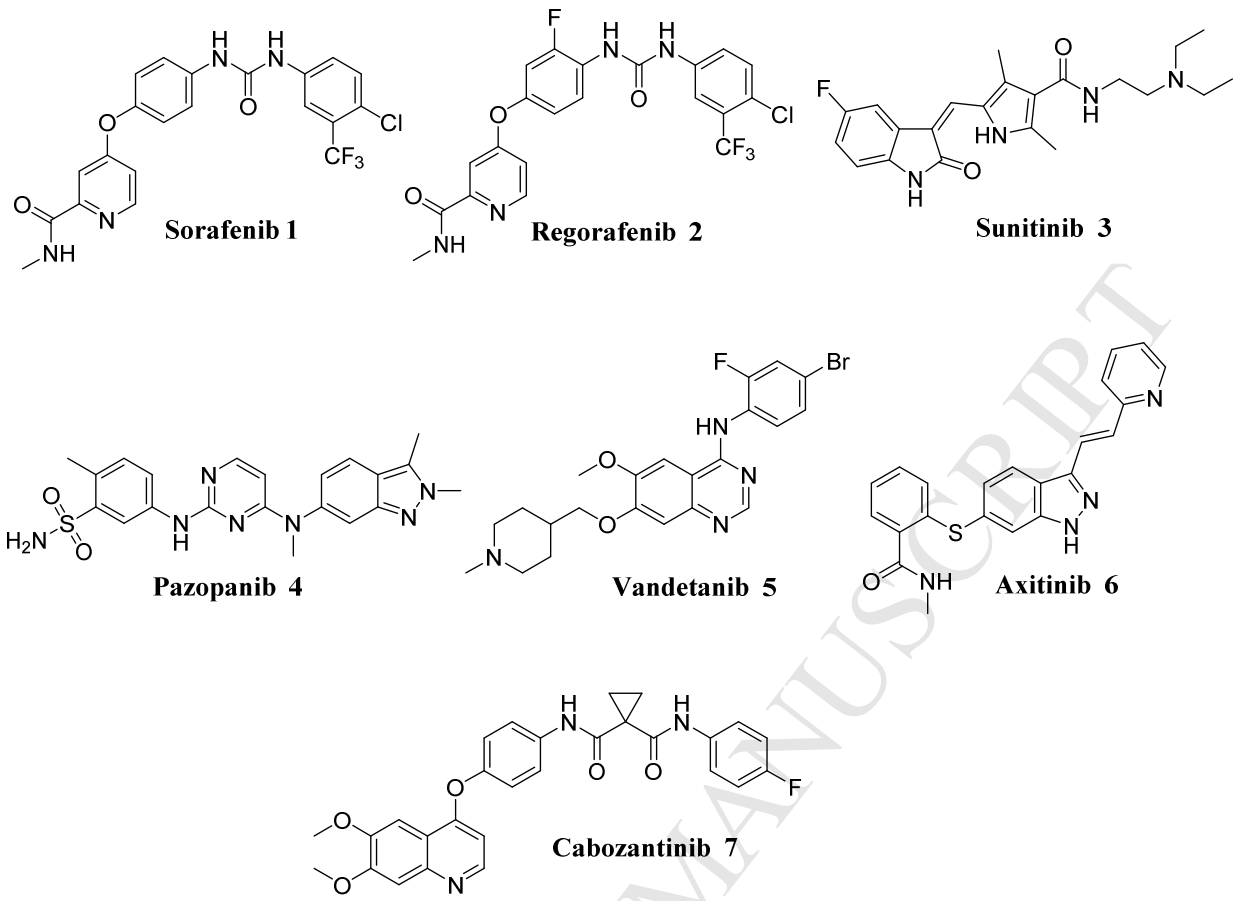



**Figure 1.** Structures of VEGFR-2 inhibitors approved for clinical use.

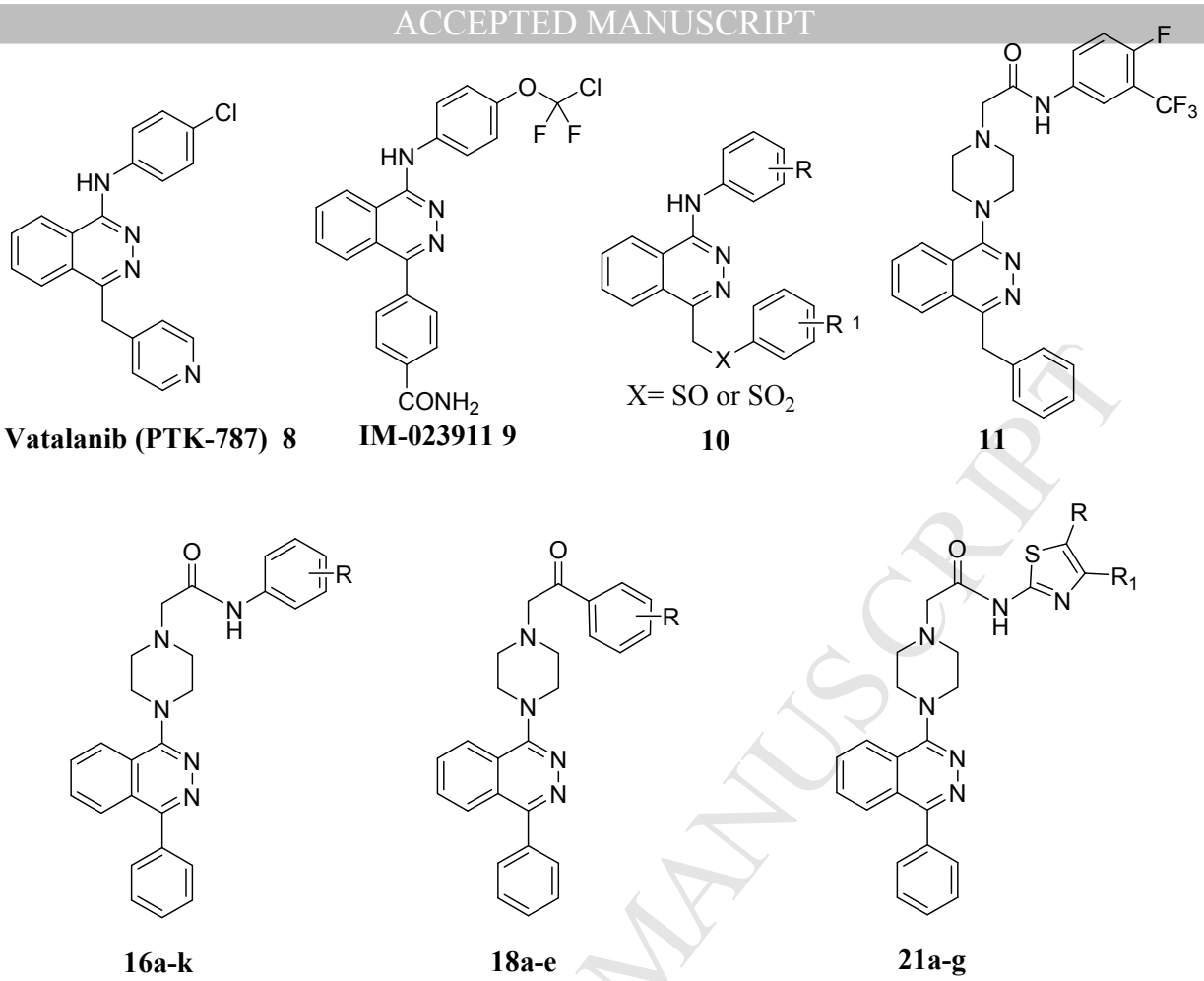



**Figure 2.** Structures of compounds **8-11** and the target 1-piperazinyl-4-phenylphthalazine derivatives **16a-k**, **18a-e** and **21a-g**.

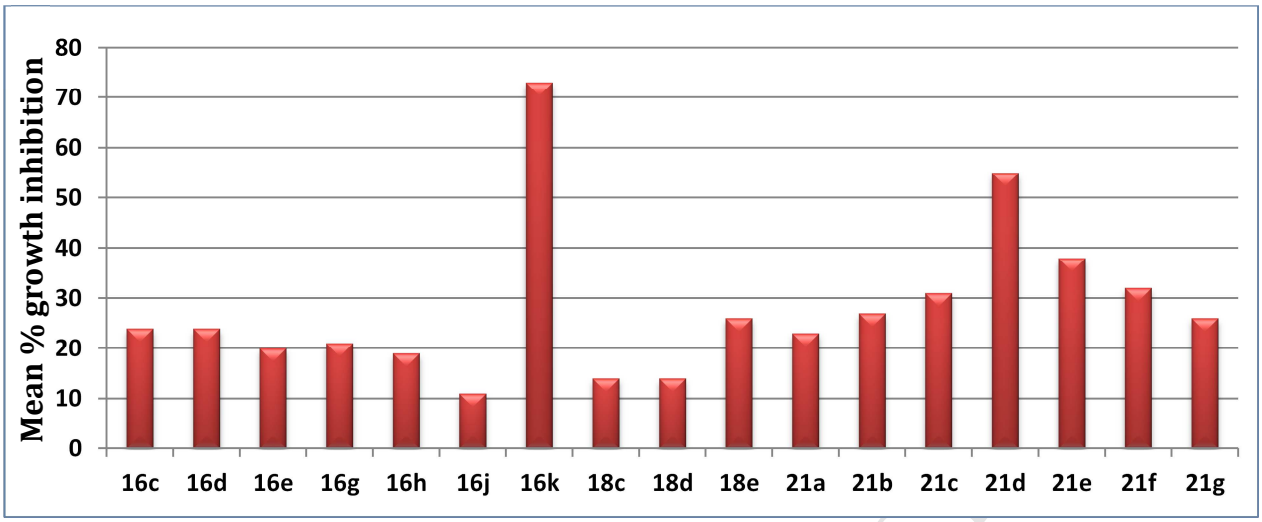



**Figure 3**. Mean % growth inhibitions of the tested compounds over NCI 56 cell line panel.

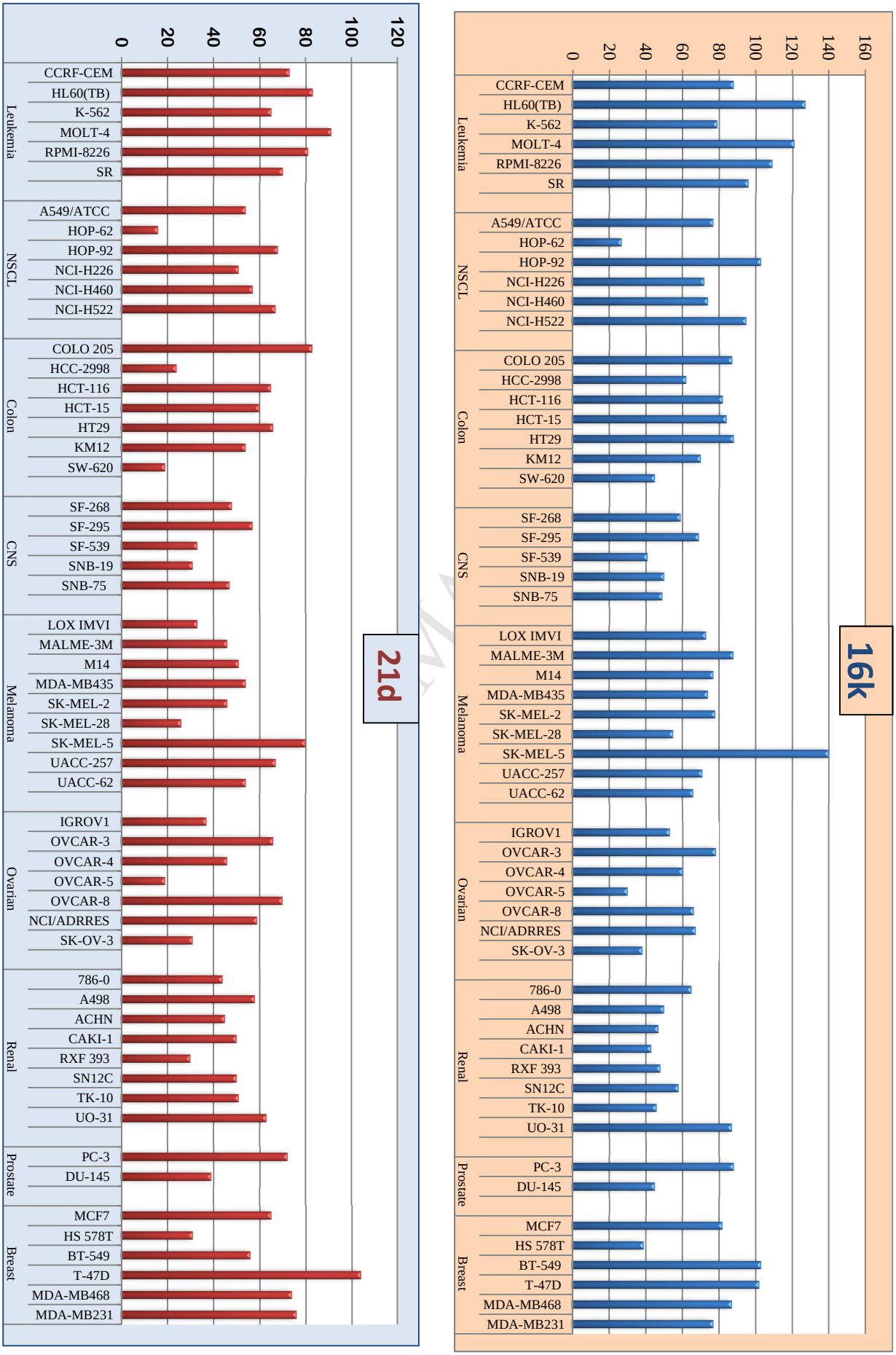
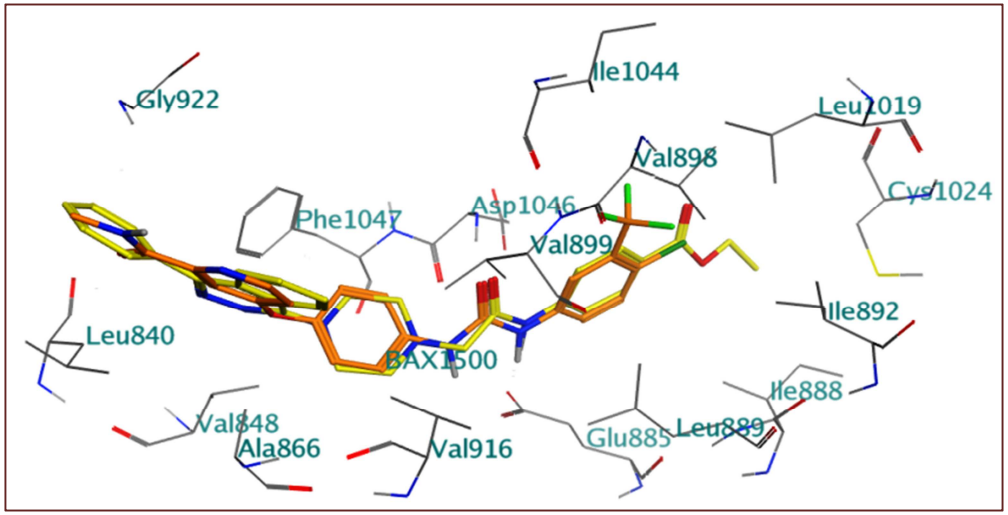
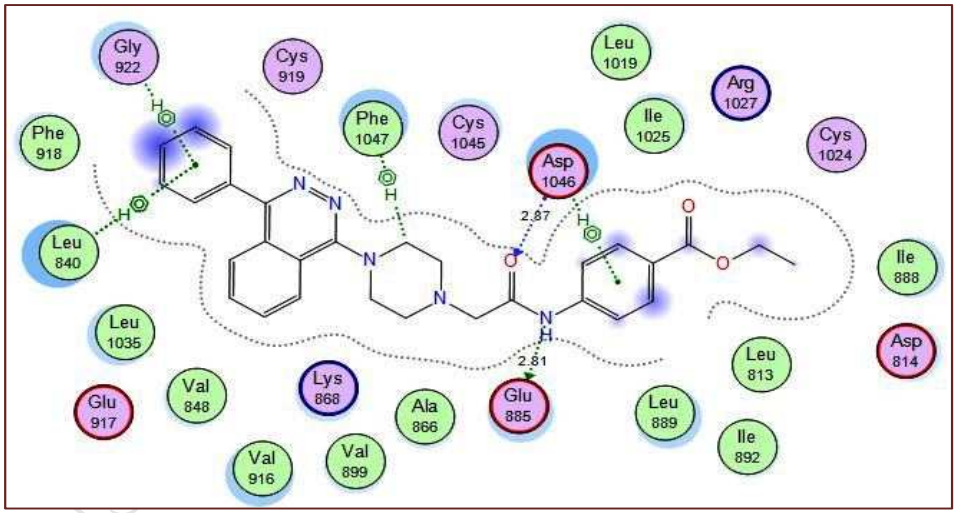


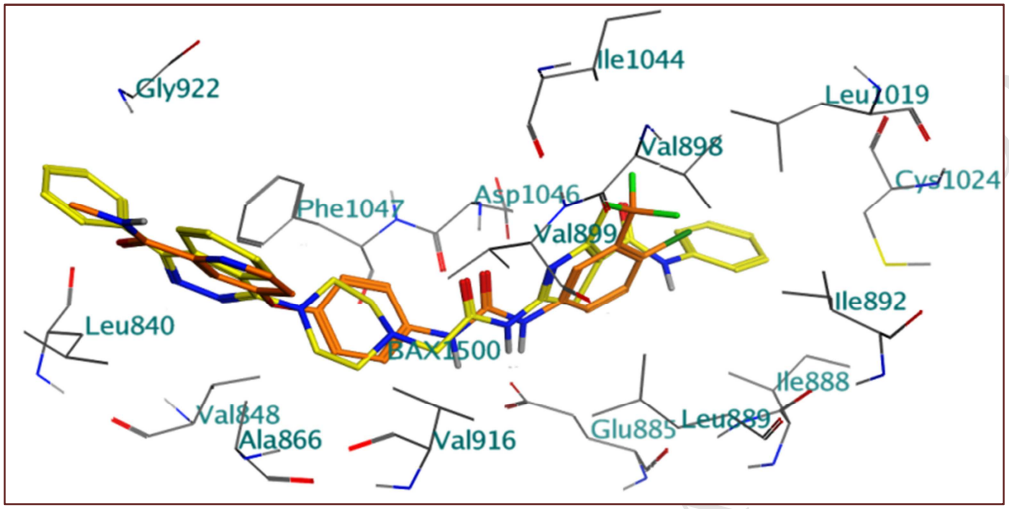

**Figure 4**. % Growth inhibition exerted by compounds **16k** and **21d** at 10 μM concentration over NCI 56 cell line panel.



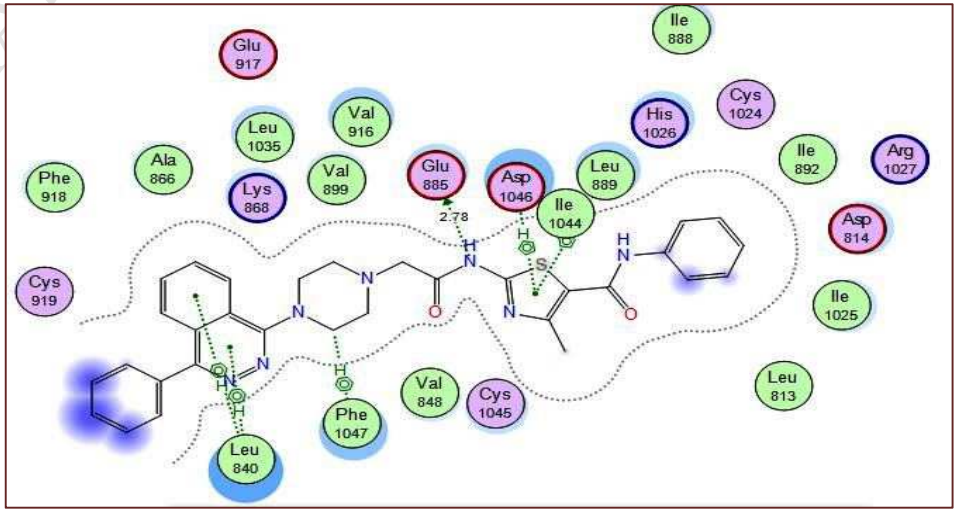

(A)




(B)

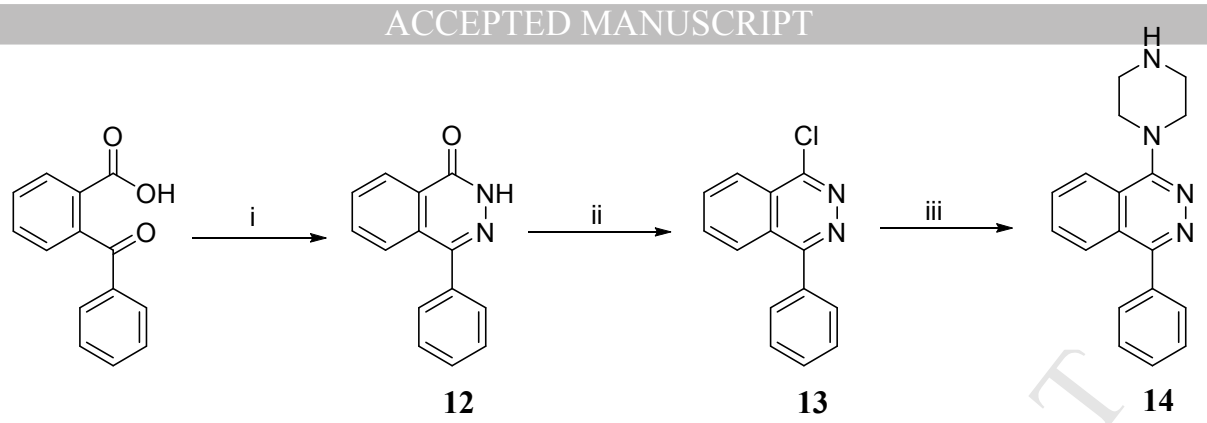



(C)

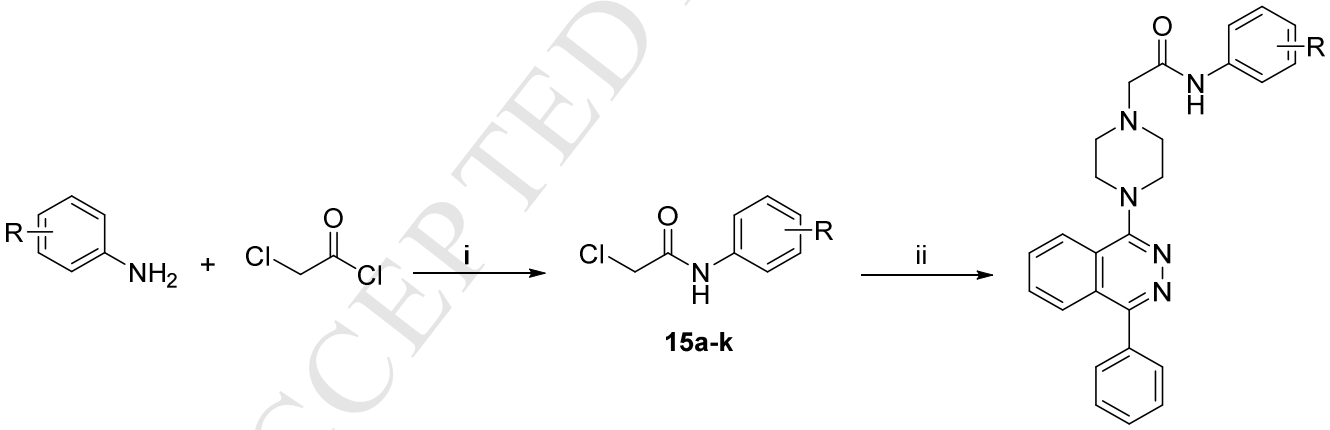



(D)

**Figure 5**. The best scored docking model of (A) Compound **16k** (in yellow) in 3D style overlaid with sorafenib (in orange), (B) Compound **16k** in 2D style, (C) Compound **21d** (in yellow) in 3D style overlaid with sorafenib (in orange) and (D) Compound **21d** in 2D style; most hydrogen atoms and some residues were omitted for clarity

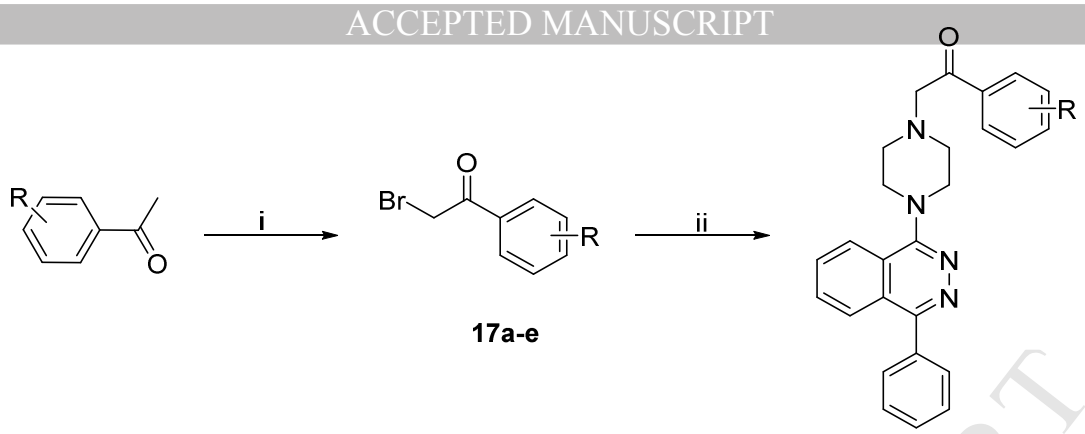



**Scheme 1**. Synthesis of the key intermediate **14**. Reagents and conditions: (i) $NH_2NH_2.H_2SO_4$ / NaOH / reflux 1h / 80%; (ii) $POCl_3$ / heat 85 °C 4h / 73%; (iii) anhydrous piperazine / isopropyl alcohol / heat 75 °C 4h / 85%.



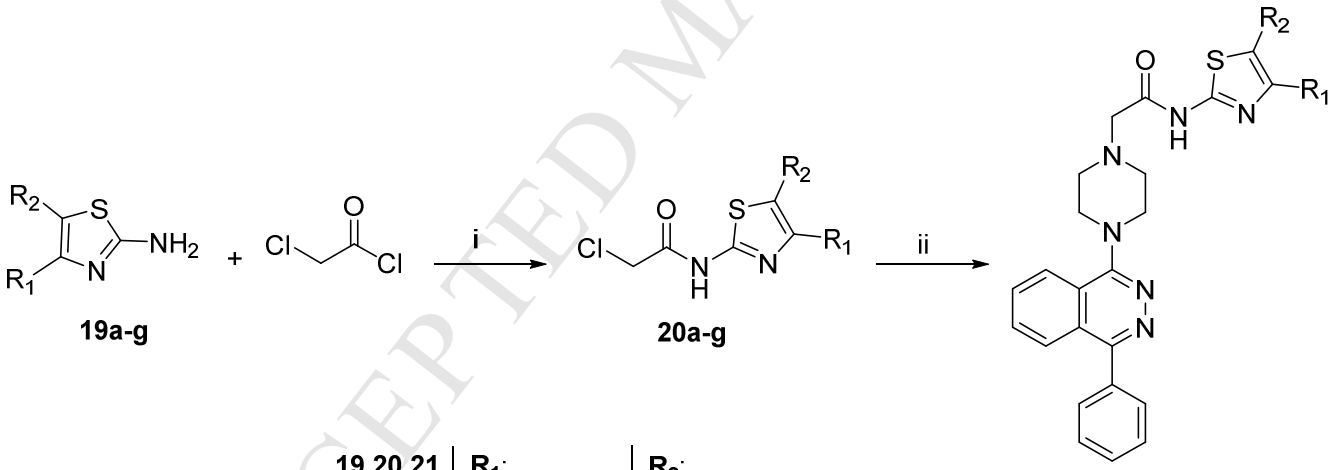

R; **a**: H, **b**: 4-$CH_3$, **c**: 3-$CF_3$, **d**: 4-CN, **e**: 2-Cl, **f**: 3-Cl, **g**: 4-Cl, **h**: 2,6-$Cl_2$, **i**: 3-$OCH_3$, **j**: 4-$OCH_3$, **k**: 4-$COOC_2H_5$.

**Scheme 2.** Synthesis of target phthalazines **16a-k.** Reagents and conditions: (i) dioxane / r.t. 2h / 75-93%; (ii) compound **14** / dry acetone / $K_2CO_3$ / reflux 5h / 59-75%.




R; **a**: H, **b:** 4-$CH_3$, **c:** 4-Cl, **d**: 3-$OCH_3$, **e:** 4-$OCH_3$

**Scheme 3.** Synthesis of target phthalazines **18a-e.** Reagents and conditions: (i) $CuBr_2$ / $CH_3COOEt$ and $CHCl_3$/ reflux 2-4h / 77-85%; (ii) compound **14** / dry acetone / $K_2CO_3$ / r.t. 3h / 58-81%.




| 19,20,21 | $R_1$; | $R_2$; |
|---|---|---|
| **a** | H | H |
| **b** | $CH_3$ | $COCH_3$ |
| **c** | $CH_3$ | $COOC_2H_5$ |
| **d** | $CH_3$ | $CONHC_6H_5$ |
| **e** | $C_6H_5$ | H |
| **f** | 4-$ClC_6H_4$ | H |
| **g** | 4-$OCH_3C_6H_4$ | H |

**Scheme 4.** Synthesis of target phthalazines **21a-g.** Reagents and conditions: (i) dry toluene / $K_2CO_3$ / reflux 3h / 65-79%; (ii) compound **14** / dry DMF / $K_2CO_3$ / heating 3h / 55-75%.

## Highlights

- Three series of 1-piperazinyl-4-phenylphthalazines **16a-k, 18a-e** & **21a-g** were synthesized.

-The prepared phthalazines were evaluated for their inhibitory activity against VEGFR-2.

**-16k** and **21d** inhibited VEGFR-2 with $IC_{50}$ = 0.35±0.03 and 0.40±0.04µM, respectively.

- Anticancer activity of seventeen selected compounds was screened by the NCI USA.

- Compound **16k** exhibited potent broad spectrum anti-proliferative activity in NCI assay.