Regular Article

Novel Sulfonamide Derivatives Carrying a Biologically Active 3,4-Dimethoxyphenyl Moiety as VEGFR-2 Inhibitors

Mostafa Mohammed Ghorab,*[a,b] Mansour Sulaiman Alsaid,[a] Yassin Mohammed Nissan,[c] Abdelkader Elbadawy Ashour,[d] Abdullah Abdulalrahman Al-Mishari,[e] Ashok Kumar,[f] and Sheikh Fayaz Ahmed[d]

[a] *Department of Pharmacognosy, College of Pharmacy, King Saud University; P.O. Box 2457, Riyadh 11451, Saudi Arabia:* [b] *Department of Drug Radiation Research, National Center for Radiation Research and Technology; Nasr City, P.O.Box 29, Cairo 11371, Egypt:* [c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University; Cairo, Egypt, El-Kasr El-Eini Street, Cairo 11562, Egypt:* [d] *Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University; P.O. Box 2457, Riyadh 11451, Saudi Arabia:* [e] *Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University; P.O. Box 2457, Riyadh 11451, Saudi Arabia: and* [f] *Vitiligo Research Chair, College of Medicine, King Saud University; Riyadh 11451, Saudi Arabia.*

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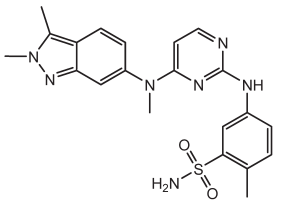
Novel sulfonamides 3–19 with a biologically active 3,4-dimethoxyphenyl moiety were designed and synthesized. The structures of the synthesized compounds were established using elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR spectral data and mass spectroscopy. All the synthesized compounds were evaluated for their *in vitro* anticancer activity against four cancer cell lines, namely human hepatocellular carcinoma (HepG2), human medulloblastoma (Daoy), human cervical cancer (HeLa), and human colon cancer (HT-29), by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and dasatinib as the reference drug. Among the tested derivatives, compounds 4, 10, 16, and 19 showed good activity as cytotoxic agents. The most active derivatives were evaluated for their ability to inhibit vascular endothelial growth factor receptor (VEGFR)-2. Compounds *Z*-4-(3-(3,4-dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-benzenesulfonamide 10 and *Z*-4-(3-(3,4-dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(1*H*-indazol-6-yl)-benzenesulfonamide 19 were more active as VEGFR-2 inhibitors than dasatinib. Molecular docking of the most active derivatives on the active site of VEGFR-2 revealed that compound 19 exhibited favorable and promising results.

Key words growth factor receptor; benzenesulfonamide; 3,4-dimethoxyphenyl; anticancer

Cancer is considered the second common cause of death after cardiovascular diseases, and is expected to become first in the next few years.[1,2] Chemotherapeutic agents used for therapy have two drawbacks: lack of selectivity as most used drugs act on normal as well as tumor cells, leading to serious side effects[3] and refractoriness of tumor cells to traditional anticancer agents. Although the latter may be overcome by using several drug combinations, it will however lead to higher cost and longer treatment durations.[4] Vascular endothelial growth factor receptor (VEGFR) inhibitors have received great attention over the last decade as novel anticancer agents.[5–12] VEGF is a key angiogenic stimulator secreted by tumor cells to switch on the angiogenic phenotype.[13] Moreover, through circulation, angiogenic capillaries stimulate tumor cells to migrate to other sites (metastasis), leading to an increased mortality rate.[13] Through binding to a number of receptors, VEGF has become one of the most important regulators of angiogenesis.[14,15] This process is mainly facilitated through a specific VEGF receptor, the kinase insert domain-containing receptor (KDR or VEGFR-2),[16] which is mostly expressed on vascular endothelial cells, and is up-regulated in angiogenic blood vessels.[17] Inhibition of angiogenesis may result in the arrest of tumor growth, a theory that was first proposed by Folkman in 1971,[18] and has now become a reality, since several VEGFR-2 inhibitors are currently used in clinics as anticancer agents.[19–21] Pazopanib **I** is a VEGFR-2 inhibitor that has been recently approved for the treatment of advanced metastatic renal carcinoma and soft tissue sarcoma, and contains an active benzenesulfonamide moiety in its chemical structure.[22–24] Many sulfonamides exhibited favorable anticancer activity through numerous mechanisms including cell cycle arrest in the G1 phase[25] and inhibition of carbonic anhydrase (CA),[26] histone deacetylases (HDACs),[27] methionine aminopeptidases (MetAPs),[28] matrix metalloproteinase (MMPs),[29] nicotinamide adenine dinucleotide (NADH) oxidase,[30] cyclin-dependent kinase (CDK).[31] Moreover, by binding to β-tubulin, they can disrupt the microtubule assembly.[32] A different agent, KRN 633 **II**, is under clinical trials as a VEGFR-2 inhibitor.[33] It contains the active moiety 3,4-dimethoxyphenyl. Based on the aforementioned facts and as a continuation of our research on novel anticancer agents,[34–40] the current study focuses on the synthesis of novel benzenesulfonamide derivatives bearing the 3,4-dimethoxyphenyl moiety, as VEGFR-2 inhibitors. The most active cytotoxic compounds were evaluated for their VEGFR-2 inhibition activity; moreover, docking of the VEGFR-2 active site was performed to explore the binding mode of the inhibitors. It's worth mentioning that similar work was performed on 4′,7-dicholorquinoline moiety with the substitution of different sulfonamides and good cytotoxic activity was observed to several derivatives against four different cell lines.[41]

* To whom correspondence should be addressed. e-mail: mmsghorab@yahoo.com



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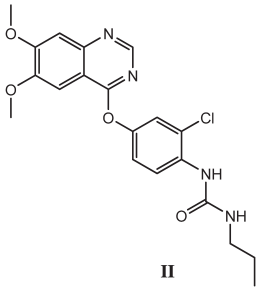


II

Results and Discussion

Chemistry The goal of this study was to synthesize a novel series of benzenesulfonamides with a biologically active 3,4-dimethoxyphenyl moiety and to assess their anticancer activity. Thus, reaction of 3,4-dimethoxyacetophenone **1** with dimethylformamide-dimethylacetal in dry xylene yielded the starting material *E*-1-(3,4-dimethoxyphenyl)-3-(dimethylamino)prop-2-en-1-one **2**. Enaminone **2** was allocated an *E*-configuration based on its ^{1}H-NMR spectrum, which indicated that the coupling constant of the doublet signals for olefinic protons was equivalent to 12.3 Hz and associated to *E*-isomers. Reaction of enaminone **2** with sulfa drugs in absolute ethanol and glacial acetic acid (2 : 1) resulted in benzenesulfonamide derivatives **3**–**19** at a reasonable yield (Chart 1). The structures of the obtained products were established using microanalysis, IR, ^{1}H-NMR, and ^{13}C-NMR spectral data. The ^{1}H-NMR spectra for compounds **3**–**19** revealed that these structures were in the *Z*-, and not in the *E*-form, while the coupling constant of the doublet signals for olefinic protons was 8.5–8.6 Hz. Moreover, the *Z*-form is stabilized by intramolecular hydrogen bonds (Chart 1). Similar reaction of 2-enaminone derivative in *E*-configuration with several primary amines revealed *Z*-configuration products were reported in literature.[42] The ^{1}H-NMR spectra of **3** shows a singlet at 10.03 ppm for SO_2NH_2 that was exchangeable with D_2O. The ^{1}H-NMR spectra of **4** revealed a singlet at 1.92 ppm for the $COCH_3$ group whereas that of **6** showed a singlet at 2.30 ppm for the CH_3 group and a singlet at 6.25 ppm for the isoxazole CH. The ^{1}H-NMR spectra of **7** revealed two singlets at 2.07 and 2.09 ppm for the $2CH_3$ groups whereas that of **8** showed a doublet signal at 5.90 ppm for the 2CH groups of pyrazole. The ^{1}H-NMR spectra of **10** exhibited a singlet at 2.44 ppm for the CH_3 group whereas that of **13** presented a singlet at 2.30 ppm for the CH_3 group and that of **14** revealed a singlet at 2.24 ppm for $2CH_3$ groups and a singlet at 5.96 ppm for the pyrimidine CH. The ^{1}H-NMR spectra of **15** displayed a singlet at 2.51 ppm for $2CH_3$ groups whereas that of **17** indicated the presence of two singlets at 3.78 and 3.79 ppm for $2OCH_3$ groups, and a singlet at 5.96 ppm for the pyrimidine CH. The ^{1}H-NMR spectra of **18** showed two singlets at 3.70 and 3.90 ppm for $2OCH_3$ groups, and a singlet at 8.12 ppm for the pyrimidine CH whereas that of **19** showed a singlet signal at 8.13 ppm for the pyrazole CH.

In Vitro **Anticancer Activity** The synthesized compounds were evaluated for their cytotoxic activity against the human cervical cancer (HeLa), human hepatocellular carcinoma (HepG2), human medulloblastoma (Daoy), and human colon cancer (HT-29) cells by using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and dasatinib as the reference drug. Percentages of growth inhibition for all compounds as well as the IC_{50} for the most active compounds are listed in Table 1. Compound **1** inhibited HepG2 cell growth by 58.36% with an IC_{50} of 14.17 μg/mL. However, the percentage of inhibition for the remaining cell lines was below 50. In contrast, compounds **4, 10** and **19** showed over 50% growth inhibition of HeLa, HepG2, and Daoy cells, but below 50% for HT-29 cells. Compound **16** was the only compound that showed over 50% growth inhibition of all the cancer cell lines tested. Compound **4** was the most active against HeLa cells with a remarkable IC_{50} of 1.78 μg/mL, an order of magnitude more potent than dasatinib (IC_{50}: 16.22 μg/mL). Compound **19** was the most active against HepG2 cells, with an IC_{50} of 1.6 μg/mL, and was more potent than dasatinib (IC_{50}: 6.91 μg/mL). The same compound was also the most active against Daoy cells with an IC_{50} of 5.58 μg/mL, almost twice as potent as dasatinib (IC_{50}: 9.2 μg/mL).

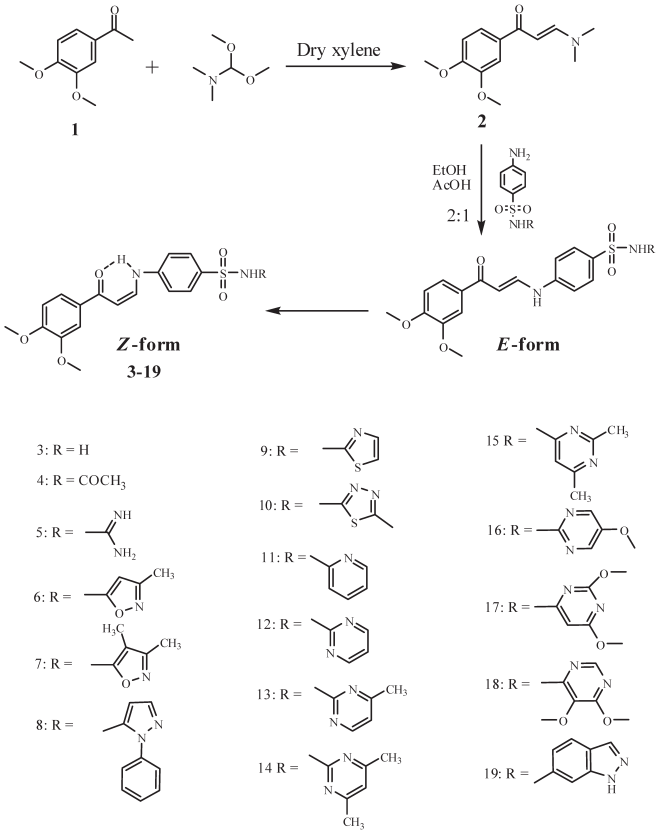


Chart 1. Synthetic Pathways for Compounds **3**–**19**

Structure–Activity Relationship

It appeared obvious that the substitution of sulfonamide derivatives used in the synthesis of compounds **3**–**19** has a remarkable effect on their cytotoxic activity. The acetyl derivative **4** showed favorable activity against HepG2 cells, whereas both the unsubstituted sulfonamide derivative **3** and the guanido derivative **5** exhibited low activity. Among the various heterocyclic sulfonamide derivatives **6**–**19**, the methyl thidiazole derivative **10**, the 5-methoxy pyrimidine derivative **16**, and the benzopyrazole derivative **19** displayed an interesting activity against HepG2 and Daoy cells.

VEGFR-2 Inhibition Activity The most active compounds (**4**, **10**, **16**, **19**) were evaluated for their *in vitro* VEGFR-2 inhibitory activity in the HeLa cell line, using da-

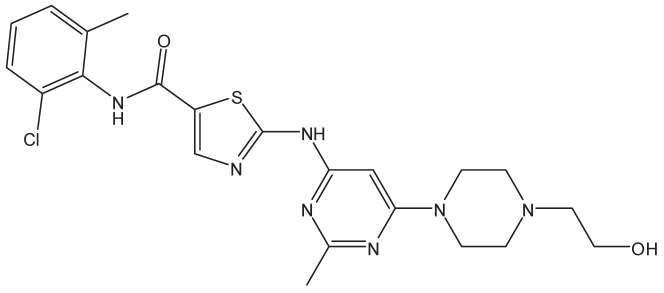
Table 1. *In Vitro* Cytotoxicity of New Compounds against Cancer Cell Lines

Compd.	Hela		HepG-2		Daoy		HT-29	
	% Inhibition*	IC_{50}**	% Inhibition*	IC_{50}**	% Inhibition*	IC_{50}**	% Inhibition*	IC_{50}**
1	45.9	nt#	58.36	14.17±0.83	41.37	nt#	34.83	nt#
2	32.53	nt#	33.76	nt#	29.78	nt#	35.82	nt#
3	30.91	nt#	34.36	nt#	3.34	nt#	0	nt#
4	58.26	1.78±0.08	63.41	2.63±0.13	55.3	7.74±0.26	46.05	nt#
5	37.48	nt#	31.32	nt#	23.67	nt#	28.2	nt#
6	9.36	nt#	5.91	nt#	41.49	nt#	0	nt#
7	36.72	nt#	36.04	nt#	30.3	nt#	0	nt#
8	46.1	nt#	46.69	nt#	35.59	nt#	36.39	nt#
9	29.32	nt#	30.21	nt#	36.82	nt#	19.44	nt#
10	57.51	20.45±0.15	56.5	20.78±0.68	51.32	19.9±1.6	33.7	nt#
11	26.72	nt#	21.65	nt#	24.86	nt#	0	nt#
12	17.12	nt#	33.03	nt#	17.12	nt#	3.7	nt#
13	22.6	nt#	5.5	nt#	22.6	nt#	0	nt#
14	6.04	nt#	0	nt#	6.04	nt#	0	nt#
15	44.07	nt#	44.66	nt#	44.7	nt#	3.65	nt#
16	57.27	22.5±0.9	61	20.74±0.48	53.02	18.44±0.84	52.03	24.46±1.46
17	39.24	nt#	41.17	nt#	35.1	nt#	15.26	nt#
18	24.79	nt#	24.45	nt#	17.59	nt#	0	nt#
19	60.6	2.69±0.19	68.49	1.6±0.2	56.12	5.58±0.42	39.21	nt#
Dasatinib	56.4	16.22±0.78	73.98	6.91±0.31	90.36	9.2±0.2	39.48	25.56±1.44

*Percent of growth inhibition of cells at a concentration of 25 μg/mL, relative to control. **Dose–response curves were used to calculate IC_{50} values (μg/mL), expressed as the mean±S.D. #nt: Not tested.

Table 2. *In Vitro* Inhibition of VEGFR-2 for the Most Active Compounds

Compounds	M_w (g/mol)	IC_{50} values (μg/mL)	VEGFR residual (conc. pg/mL)	VEGFR % inhibition
4	404	1.78	686.491	72.8
10	460	20.45	740.914	80.6
16	470	22.5	775.862	74.7
19	478	2.69	590.541	84.2
Dasatinib	488	0.8	978.443	79.5



Dasatinib

satinib as the reference drug. All four compounds showed a good inhibition (72.8–84.2% of control). Compounds **10** and **19** were more active than the reference drug (79.5%) with an inhibition of 80.6 and 84.2%, respectively. VEGFR-2 residual concentrations as well as VEGFR-2 inhibition percentages for the tested compounds are listed in Table 2. These interesting results urge to synthesize and evaluate VEGFR-2 inhibition activity of more derivatives using other sulfonamide moieties in the future.

Molecular Docking In order to explore the potential binding mode of the most active compounds to VEGFR-2, compounds **4**, **10**, **16**, and **19** were docked on the receptor active site. The protein databank file (PDB ID) 4AGD was selected for this purpose. The file contains data on VEGFR-2 co-crystallized with sunitinib, a type I VEGFR-2 inhibitor. Type I inhibitors bind only to the ATP binding site of kinases with the hinge region with no interactions with allosteric pocket. All docking procedures were achieved by MOE (Molecular Operating Environment) software 10.2008 provided by the chemical computing group, Canada. Docking on the VEGFR-2 active site was performed for the sulfonamide derivatives **4**, **10**, **16**, and **19**. The docking protocol was verified by re-docking the co-crystallized ligand near the active site with an energy score (S) of −22.9143 kcal/mol and a root mean standard deviation (RMSD) of 1.5923. The co-crystalized inhibitor interacts with the active site of VEGFR-2 *via* two hydrogen bonds: the first, between Glu917 and the nitrogen of the pyrrole moiety, and the second, between Cys919 and the carbonyl group (Fig. 1).

The four compounds, **4**, **10**, **16**, and **19** were fit in the active site of VEGFR-2 with energy scores ranging between −11.9827 to −19.6112 kcal/mol. Molecular docking for pazopanib, KNR 633 and dasatinib was also performed. The energy scores of the docked compounds and the amino acid interactions are listed in Table 3.

The optimal docking energy score among the synthesized compounds was displayed by compound **19** with a value of −19.6112 kcal/mol supporting its excellent activity as a

Table 3. Docking Score (S) and Amino Acid Interactions for the Most Active Compounds on the VEGFR-2 Active Site

Compounds	(S) (kcal/mol)	Amino acid	Interacting group	Type of interaction	H-Bond length (Å)
4	−13.5853	Lys838	C=O	H bond (acceptor)	2.49
		Cys919	C=O	H bond (acceptor)	2.70
10	−11.9827	Cys919	SO_2	H bond (acceptor)	2.99
16	−13.9947	Cys919	OCH_3	H bond (acceptor)	2.99
19	−19.6112	Lys838	C=O	H bond (acceptor)	2.48
		Glu917	NH pyrazole	H bond (donor)	2.02
		Cys919	SO_2	H bond (acceptor)	3.03
Pazopanib	−20.6243	Cys919	N pyrazole	H bond (acceptor)	3.18
KRN 633	−17.8653	Cys919	N pyrimidin	H bond (acceptor)	2.89
Dasatinib	−8.2171	Cys919	C=O	H bond (acceptor)	3.05
Sunitinib	−22.9143	Glu917	N pyrrole	H bond (donor)	2.93
		Cys919	C=O	H bond (acceptor)	2.92

Fig. 1. Co-crystallized Ligand on the Active Site of VEGFR-2

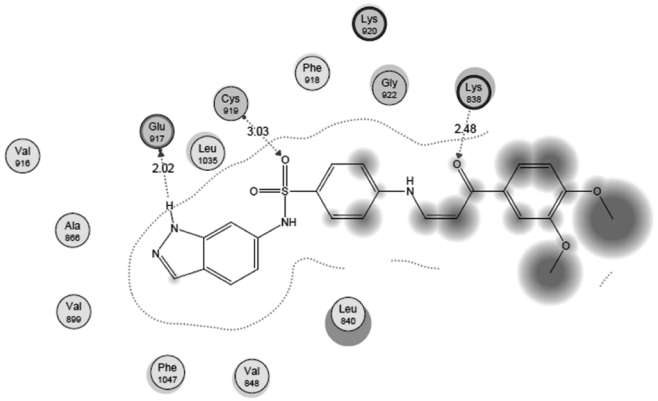


Fig. 2. 2D Image of Compound **19** Docked onto the VEGFR-2 Active Site

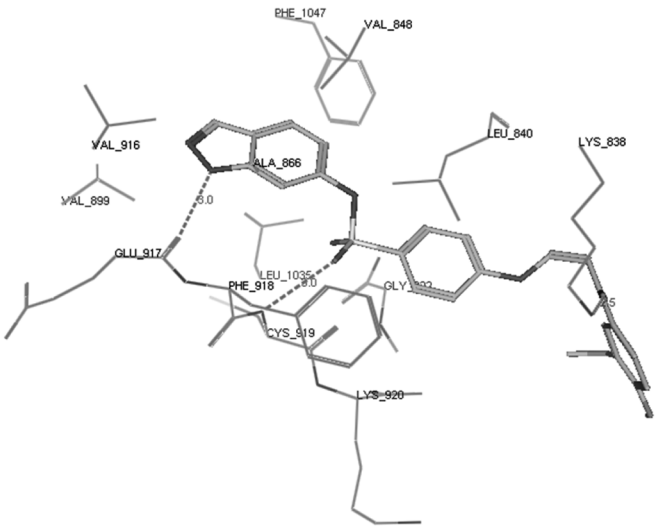


Fig. 3. 3D Image of Compound **19** Docked to the VEGFR-2 Active Site

VEGFR-2 inhibitor (84.2% growth inhibition rate). Compound **19** interacted with the same amino acids (Glu917 and Cys919) in a comparable manner to the co-crystallized ligand did, and had an additional hydrogen bond with Lys838. The amino acid interactions exhibited by compound **19** are illustrated in Figs. 2 and 3. Amino acid interactions of pazopanib, KRN 633 and dasatinib are illustrated in Figs. 4–6, respectively.

Experimental

Chemistry Elemental analyses were performed using a model 2400 CHNS/O analyzer (PerkinElmer, Inc., U.S.A.). All the values were within ±0.4% of the theoretical values; melting points (mp; uncorrected) were determined using an open capillary on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, U.K.). Precoated silica gel plates (Kieselgel 0.25 mm, 60 F254, Merck, Germany) were used for thin layer chromatography. A developing solvent system of chloroform–methanol (8 : 2) was used, and the spots were detected by ultraviolet light. IR spectra (KBr disc) were recorded using a Fourier transform infrared (FT-IR) spectrophotometer (PerkinElmer, Inc., U.S.A.). ^{1}H-NMR spectra were scanned on a NMR spectrophotometer (Bruker AXS Inc., Switzerland), operating at 500 MHz for ^{1}H, and 125.76 MHz for ^{13}C. Chemical shifts are expressed as δ-values (ppm) relative to tetramethylsilane (TMS) as an internal standard, and dimethyl sulfoxide (DMSO)-d_6 as a solvent. All reagents used were of analytical grade. The starting material 3,4-dimethoxyacetophenone **1** was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), and was directly used for the preparation of the target com-

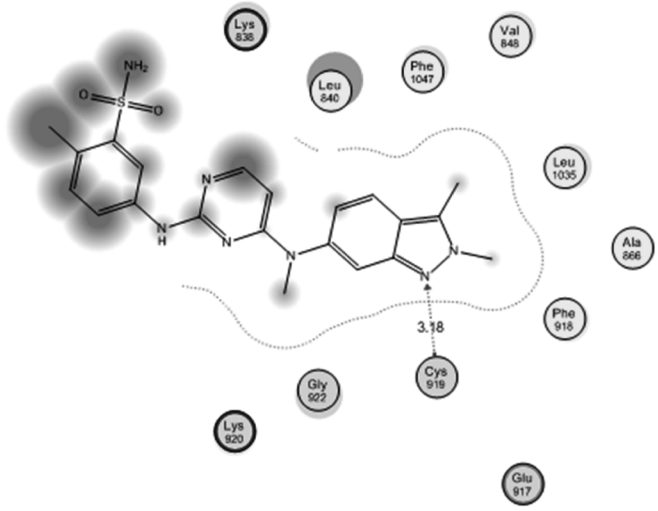


Fig. 4. 2D Image of Pazopanib Docked onto the VEGFR-2 Active Site

Fig. 5. 2D Image of KRN 633 Docked onto the VEGFR-2 Active Site

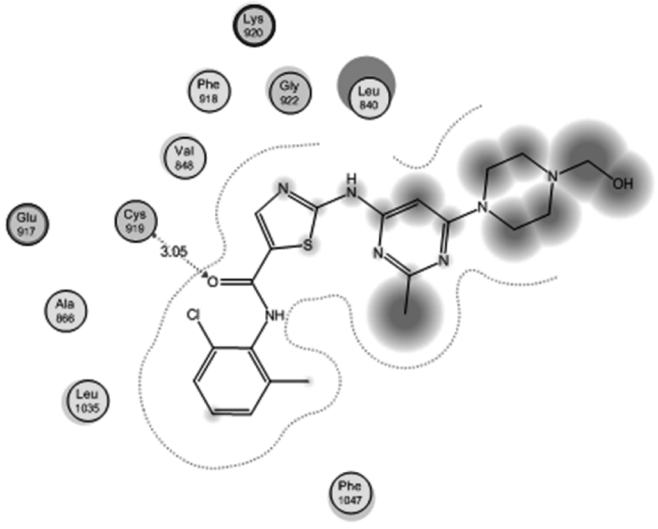


Fig. 6. 2D Image of Dasatinib Docked onto the VEGFR-2 Active Site

pounds.

Z-1-(3,4-Dimethxoyphenyl)-3-(dimethylamino)prop-2-en-1-one (**2**)

A solution of 3,4-dimethoxyacetophenone (1.8 g, 0.01 mol) in dimethylformamide-dimethylacetal (1.19 g, 0.01 mol) was refluxed in dry xylene (20 mL) for 24 h. The obtained solid was collected by filtration, while still hot, and recrystallized from dioxane to yield compound **2**.

Yield, 66%; mp 123.7°C. IR: 3080 (arom.), 2997, 2931, 2839 (aliph.), 1635 (CO). ^{1}H-NMR: 3.11 (s, 6H, $2NCH_3$), 3.80 (s, 6H, $2OCH_3$), 5.82, 6.98 (2d, 2H, CH=CH, J=12.3 Hz), 7.47–7.69 (m, 3H, Ar-H). ^{13}C-NMR: 44.8(2), 56.0(2), 91.1, 110.8, 111.1, 121.2, 133.4, 148.7, 151.7, 154.1, 185.3. MS *m/z* (%): 235 (M^+) (12.73), 136 (100). *Anal.* Calcd for $C_{13}H_{17}NO_3$ (235): C, 66.36; H, 7.28; N, 5.95. Found: C, 66.07; H, 7.54; N, 6.23.

Synthesis of Benzenesulfonamide Derivatives (**3**–**19**)

General Procedure

A mixture of **2** (2.35 g, 0.01 mol) and sulfa drugs (0.012 mol) in absolute ethanol (10 mL) and glacial acetic acid (5 mL) was refluxed for 22h, and subsequently left to cool. The solid product formed was collected by filtration and recrystallized from acetic acid to give compounds **3**–**19.**

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-benzenesulfonamide (**3**)

Yield, 89%; mp 276.4°C. IR: 3329, 3236 (NH_2, NH), 3005 (arom.), 2974, 2939, 2839 (aliph.), 1635 (CO), 1375, 1188 (SO_2). ^{1}H-NMR: 3.83 (s, 6H, $2OCH_3$), 6.23, 6.60 (2d, 2H, CH=CH, J=8.5 Hz), 7.03–8.15 (m, 7H, Ar-H), 10.30 (s, 2H, SO_2NH_2), 12.04 (s, 1H, NH). ^{13}C-NMR: 55.9, 56.1, 95.2, 110.5, 111.3, 111.4, 115.2, 121.7, 127.9, 128.1, 131.5, 132.2, 142.8, 144.5, 149.0, 152.7, 189.6. MS *m/z* (%): 362 (M^+) (6.48), 155 (100). *Anal.* Calcd for $C_{17}H_{18}N_2O_5S$ (362): C, 56.34; H, 5.01; N, 7.73. Found: C, 56.62; H, 5.29; N, 7.46.

Z-*N*-(4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-phenylsulfonyl)acetamide (**4**)

Yield, 87%; mp 215.5°C. IR: 3329, 3236 (NH), 3078 (arom.), 2974, 2939, 2841 (aliph.), 1718, 1635 (2CO), 1373, 1155 (SO_2). ^{1}H-NMR: 1.92 [s, 3H, $COCH_3$], 3.83 [s, 6H, $2OCH_3$], 6.22, 6.61 [2d, 2H, CH=CH, J=8.6 Hz]. 7.02–8.17 [m, 7H, Ar-H], 10.40 [s, 1H, SO_2NH], 12.03 [s, 1H, NH]. ^{13}C-NMR: 23.6, 55.9, 56.0, 95.2, 110.5, 111.3, 115.1, 115.2, 121.7, 122.0, 130.1(2), 131.4, 143.8, 145.1, 152.7, 152.8, 169.2, 189.6. MS *m/z* (%): 404 (M^+) (2.65), 93 (100). *Anal.* Calcd for $C_{19}H_{20}N_2O_6S$ (404): C, 56.42; H, 4.98; N, 6.93. Found: C, 56.16; H, 5.35; N, 7.26.

Z-*N*-Carbamimidoyl-4-(3-(3,4-dimethoxyphenyl)-3-oxoprop-1-enylamino)benzenesulfonamide (**5**)

Yield, 91%; mp 260.1°C. IR: 3435, 3336, 3228 (NH_2, NH), 3100 (arom.), 2976, 2939, 2843 (aliph.), 1635 (CO), 1593 (CN), 1375, 1166 (SO_2). ^{1}H-NMR: 3.83 (s, 6H, $2OCH_3$), 6.20, 6.57 (2d, 2H, CH=CH, J=8.6 Hz), 7.03–7.73 (m, 9H, Ar-H+NH_2), 8.13 (s, 1H, SO_2NH), 10.15 (s, 1H, NH), 12.03 (s, 1H, NH, imino).^{13}C-NMR: 55.9, 56.1, 94.9, 110.4, 111.3, 115.1, 115.2, 121.7, 127.8, 131.6(2), 133.3, 142.9, 144.5, 152.5, 158.4, 158.5, 189.5. MS *m/z* (%): 404 (M^+) (17.22), 77 (100). *Anal.* Calcd for $C_{18}H_{20}N_4O_5S$ (404): C, 53.45; H, 4.98; N, 13.85. Found: C, 53.16; H, 5.31; N, 13.56.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(3-methylisoxaz-ol-5-yl)benzenesulfonamide (**6**)

Yield, 88%; mp 160.3°C. IR: 3161(NH), 3066 (arom.), 2978, 2889, 2839 (aliph.), 1637 (CO), 1589 (CN), 1375, 1161 (SO_2). ^{1}H-NMR: 2.30 (s, 3H, CH_3), 3.83 (s, 6H, $2OCH_3$), 6.14, 6.61 (2d, 2H, CH=CH, J=8.5 Hz), 6.25 (s, 1H, CH isoxazole), 7.03–8.15 (m, 7H, Ar-H), 10.40 (s, 1H, SO_2NH), 12.00 (s, 1H, NH). ^{13}C-NMR: 12.4, 56.1(2), 95.8, 100.7, 110.5, 111.3, 113.0(2), 121.8, 122.0, 129.2, 131.8(2), 142.4, 145.9, 149.1,

152.8, 158.0, 170.8, 189.7. MS *m/z* (%): 443 (M^+) (37.18), 96 (100). *Anal.* Calcd for $C_{21}H_{21}N_3O_6S$ (443): C, 56.87; H, 4.77; N, 9.48. Found: C, 56.52; H, 4.40; N, 9.11.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide (**7**)

Yield, 86%; mp 238.2°C. IR: 3334 (NH), 3089 (arom.), 2960, 2835 (aliph.), 1635 (CO), 1589 (CN), 1346, 1159 (SO_2). ^{1}H-NMR: 2.07, 2.09 (2s, 3H, $2CH_3$), 3.83 (s, 6H, $2OCH_3$), 6.26, 6.63 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.03–8.16 (m, 7H, Ar-H), 10.42 (s, 1H, SO_2NH)], 12.03 (s, 1H, NH). ^{13}C-NMR: 6.3, 10.7, 56.10, 56.12, 95.8, 100.7, 105.5, 110.6, 111.4(2), 121.8, 122.0, 129.1, 129.2(2), 143.8, 145.9, 149.1, 152.8, 156.1, 161.9, 189.7. MS *m/z* (%): 457 (M^+) (31.52), 135 (100). *Anal.* Calcd for $C_{22}H_{23}N_3O_6S$ (457): C, 57.76; H, 5.07; N, 9.18. Found: C, 57.98; H, 5.36; N, 9.39.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(1-phenyl-1*H*-pyrazol-5-yl)-benzenesulfonamide (**8**)

Yield, 80%; mp 236.1°C. IR: 3415(NH), 3049 (arom.), 2966, 2835, 2802 (aliph.), 1653 (CO), 1570 (CN), 1338, 1159 (SO_2). ^{1}H-NMR: 3.84 (s, 6H, $2OCH_3$), 5.90 (d, 2H, 2CH pyrazole, *J*=7.1 Hz), 6.28, 6.63 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.05–8.13 (m, 12H, Ar-H), 10.41 (s, 1H, SO_2NH), 12.00 (s, 1H, NH). ^{13}C-NMR: 56.13, 56.16, 95.7, 104.3, 110.5, 110.6, 111.4(2), 121.8, 122.0, 124.7(2), 127.9, 129.3(2), 131.5, 132.1(2), 135.0, 138.7, 142.5, 144.6, 145.7, 149.1, 152.8, 189.7. MS *m/z* (%): 504 (M^+) (16.38), 169 (100). *Anal.* Calcd for $C_{26}H_{24}N_4O_5S$ (504): C, 61.89; H, 4.79; N, 11.10. Found: C, 61.56; H, 5.28; N, 11.41.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(thiazol-2-yl)-benzenesulfonamide (**9**)

Yield, 83%; mp 260.3°C. IR: 3151 (NH), 3101 (arom.), 2971, 2929, 2860 (aliph.), 1635 (CO), 1589 (CN), 1375, 1168 (SO_2). ^{1}H-NMR: 3.83 (s, 6H, $2OCH_3$), 6.22, 6.58 (2d, 2H, CH=CH, *J*=8.6 Hz), 6.80–8.14 (m, 9H, Ar-H+2CH thiazole), 10.30 (s, 1H, SO_2NH), 12.02 (s, 1H, NH). ^{13}C-NMR: 56.11, 56.13, 95.2, 108.5, 110.5, 110.6, 111.3, 111.4, 121.9, 124.9, 128.2, 131.5(2), 136.4, 142.7, 144.7, 149.0, 152.7, 169.1, 189.5. MS *m/z* (%): 445 (M^+) (29.11), 93 (100). *Anal.* Calcd for $C_{20}H_{19}N_3O_5S_2$ (445): C, 53.92; H, 4.30; N, 9.43. Found: C, 53.59; H, 3.95; N, 9.14.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (**10**)

Yield, 80%; mp 186.9°C. IR: 3153 (NH), 3055 (arom.), 2926, 2839, (aliph.), 1635 (CO), 1589 (CN), 1375, 1141 (SO_2). ^{1}H-NMR: 2.44 (s, 3H, CH_3), 3.83 (s, 6H, $2OCH_3$), 6.24, 6.59 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.04–8.13 (m, 7H, Ar-H), 10.31 (s, 1H, SO_2NH), 12.02 (s, 1H, NH). ^{13}C-NMR: 16.5, 55.1(2), 95.4, 110.5, 110.6, 111.3, 111.4, 121.7, 121.9, 128.1, 131.5(2), 144.1, 145.1, 149.1, 152.5, 154.8, 168.2, 186.7. MS *m/z* (%): 460 (M^+) (8.75), 124 (100). *Anal.* Calcd for $C_{20}H_{20}N_4O_5S_2$ (460.53): C, 52.16; H, 4.38; N, 12.17. Found: C, 52.42; H, 4.09; N, 12.39.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (**11**)

Yield, 79%; mp 240.4°C. IR: 3431 (NH), 3101 (arom.), 2931, 2835 (aliph.), 1635 (CO), 1589 (CN), 1394, 1151 (SO_2). ^{1}H-NMR: 3.83 (s, 6H, $2OCH_3$), 6.22, 6.58 (2d, 2H, CH=CH, *J*=8.5 Hz), 6.87–8.13 (m, 11H, Ar-H), 10.30 (s, 1H, SO_2NH), 12.00 (s, 1H, NH). ^{13}C-NMR: 56.1(2), 95.4, 110.5, 110.6, 111.3, 113.8, 113.9, 115.2, 121.8, 121.9, 129.2, 129.3(2), 135.6, 140.6, 144.1, 149.0, 152.5, 152.7, 152.7, 189.6. MS *m/z* (%): 439 (M^+) (23.19), 77 (100). *Anal.* Calcd for $C_{22}H_{21}N_3O_5S$ (439): C, 60.12; H, 4.82; N, 9.56. Found: C, 60.39; H, 4.49; N, 9.21.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(pyrimidin-2-yl)benzenesulfonamide (**12**)

Yield, 83%; mp 272.5°C. IR: 3221 (NH), 3088 (arom.), 2939, 2875, 2823 (aliph.), 1653 (CO), 1577 (CN), 1375, 1184 (SO_2). ^{1}H-NMR: 3.83 (s, 6H, $2OCH_3$), 6.24, 6.59 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.04–8.51 (m, 10H, Ar-H), 10.34 (s, 1H, SO_2NH), 12.00 (s, 1H, NH). ^{13}C-NMR: 56.5(2), 95.6, 110.4, 110.6, 111.3, 111.4, 115.0, 121.8, 121.9, 130.0, 131.4(2), 142.5, 144.5, 149.0, 152.5, 157.4, 158.8, 172.5, 189.6. MS *m/z* (%): 440 (M^+) (1.85), 79 (100). *Anal.* Calcd for $C_{21}H_{20}N_4O_5S$ (440): C, 57.26; H, 4.58; N, 12.72. Found: C, 56.88; H, 4.22; N, 12.44.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide (**13**)

Yield, 90%; mp 245.2°C. IR: 3410 (NH), 3100 (arom.), 2958, 2923, 2836 (aliph.), 1635 (CO), 1589 (CN), 1338, 1151 (SO_2). ^{1}H-NMR: 2.30 (s, 3H, CH_3), 3.83 (s, 6H, $2OCH_3$), 6.23, 6.59 (2d, 2H, CH=CH, *J*=8.6 Hz), 6.89–8.32 (m, 9H, Ar-H), 10.33 (s, 1H, SO_2NH), 12.01 (s, 1H, NH). ^{13}C-NMR: 23.7, 56.11, 56.12, 95.5, 110.5, 110.6, 111.3, 112.5(2), 121.8, 121.9, 130.3, 131.5(2), 142.5, 145.4, 149.0, 152.8, 157.0, 157.9, 168.7, 189.6. MS *m/z* (%): 454 (M^+) (24.28), 93 (100). *Anal.* Calcd for $C_{22}H_{22}N_4O_5S$ (454): C, 58.14; H, 4.88; N, 12.33. Found: C, 58.48; H, 4.56; N, 12.03.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**14**)

Yield, 92%; mp 276.5°C. IR: 3220 (NH), 3056 (arom.), 2939, 2843 (aliph.), 1635 (CO), 1587 (CN), 1340, 1149 (SO_2). ^{1}H-NMR: 2.24 (s, 6H, $2CH_3$), 3.83 (s, 6H, $2OCH_3$), 5.96 (s, 1H, CH pyrimidine), 6.55, 6.76 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.04–8.11 (m, 7H, Ar-H), 10.31 (s, 1H, SO_2NH), 12.01 (s, 1H, NH). ^{13}C-NMR: 23.5(2), 56.1(2), 96.4, 108.2, 110.6, 111.4, 112.3(2), 114.6, 115.5, 130.5, 130.7(2), 142.8, 146.7, 149.1, 152.6, 163.8(2), 167.7, 184.2. MS *m/z* (%): 468 (M^+) (22.17), 164 (100). *Anal.* Calcd for $C_{23}H_{24}N_4O_5S$ (468): C, 58.96; H, 5.16; N, 11.96. Found: C, 58.67; H, 5.44; N, 12.33.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(2,6-dimethylpyrimidin-4-yl)benzenesulfonamide (**15**)

Yield, 87%; mp 243.3°C. IR: 3415 (NH), 3072 (arom.), 2935, 2839 (aliph.), 1637 (CO), 1589 (CN), 1348, 1149 (SO_2). ^{1}H-NMR: 2.51 (s, 6H, $2CH_3$), 3.82 (s, 6H, $2OCH_3$), 6.22, 6.59 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.01–8.14 (m, 8H, Ar-H), 10.34 (s, 1H, SO_2NH), 12.00 (s, 1H, NH). ^{13}C-NMR: 19.0, 22.3, 56.1(2), 95.7, 100.6, 110.5, 110.6, 111.3, 111.4, 121.8, 121.9, 127.7, 129.2, 130.3, 142.3, 146.5, 149.0, 152.5, 152.8, 162.6, 163.0, 186.8. MS *m/z* (%): 468 (M^+) (5.63), 89 (100). *Anal.* Calcd for $C_{23}H_{24}N_4O_5S$ (468): C, 58.96; H, 5.16; N, 11.96. Found: C, 59.32; H, 4.87; N, 11.63.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(5-methoxylpyrimidin-2-yl)benzenesulfonamide (**16**)

Yield, 81%; mp 216.7°C. IR: 3373 (NH), 3101 (arom.), 2837, 2735 (aliph.), 1635 (CO), 1591 (CN), 1375, 1153 (SO_2). ^{1}H-NMR: 3.78 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 6.23, 6.59 (2d, 2H, CH=CH, *J*=8.5 Hz), 7.02–8.30 (m, 9H, Ar-H), 10.35 (s, 1H, SO_2NH), 12.02 (s, 1H, NH). ^{13}C-NMR: 55.9, 56.6, 56.7, 95.6, 110.5, 110.6, 111.3, 111.4, 121.8, 125.7, 129.9, 130.1(2), 142.5(2), 144.0, 145.0, 145.4, 149.8, 152.5, 172.5, 186.8. MS *m/z* (%): 470 (M^+) (33.82), 123 (100). *Anal.* Calcd for $C_{22}H_{22}N_4O_6S$ (470): C, 56.16; H, 4.71; N, 11.91. Found: C, 55.79; H, 4.47; N, 12.25.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(2,6-dimethoxylpyrimidin-4-yl)benzenesulfonamide (**17**)

Yield, 86%; mp 201.3°C. IR: 3134 (NH), 3061 (arom.), 2902, 2835 (aliph.), 1637 (CO), 1589 (CN), 1363, 1151 (SO_2). ^{1}H-NMR: 3.78, 3.79 (2s, 6H, 2OCH_3 pyrimidine), 3.83 (s, 6H, 2OCH_3), 5.96 (s, 1H, CH pyrimidine), 6.24, 6.58 (2d, 2H, CH=CH, J=8.6 Hz), 7.02–8.12 (m, 7H, Ar-H), 10.37 (s, 1H, SO_2NH), 12.01 (s, 1H, NH). ^{13}C-NMR: 54.2, 54.9, 55.8, 55.9, 84.9, 95.8, 110.5, 110.6, 111.4(2), 121.8, 121.9, 129.6, 129.8(2), 142.4, 145.8, 149.1, 152.8, 160.3, 164.7, 172.5, 189.6. MS *m*/*z* (%): 500 (M^+) (19.34), 152 (100). *Anal.* Calcd for $C_{23}H_{24}N_4O_7S$ (500): C, 55.19; H, 4.83; N, 11.19. Found: C, 55.52; H, 4.57; N, 10.90.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(5,6-dimethoxylpyrimidin-4-yl)benzenesulfonamide (**18**)

Yield, 80%; mp 244.1°C. IR: 3189 (NH), 3082 (arom.), 2941, 2839 (aliph.), 1631 (CO), 1589 (CN), 1375, 1161 (SO_2). ^{1}H-NMR: 3.70, 3. 90 (2s, 6H, 2OCH_3 pyrimidine), 3.83 (s, 6H, 2OCH_3), 6.20, 6.60 (2d, 2H, CH=CH, J=8.5 Hz), 7.06–7.94 (m, 7H, Ar-H), 8.12 (s, 1H, CH pyrimidine), 10.30 (s, 1H, SO_2NH), 12.00 (s, 1H, NH). ^{13}C-NMR: 54.5, 55.9, 56.1, 56.5, 95.6, 110.5, 110.6, 111.4(2), 121.8, 121.9, 127.6, 130.2(2), 131.5, 142.5, 144.0, 149.1, 150.9, 152.6(2), 162.0, 189.6. MS *m*/*z* (%): 500 (M^+) (5.84), 151 (100). *Anal.* Calcd for $C_{23}H_{24}N_4O_7S$ (500): C, 55.19; H, 4.83; N, 11.19. Found: C, 54.92; H, 4.50; N, 11.48.

Z-4-(3-(3,4-Dimethoxyphenyl)-3-oxoprop-1-enylamino)-*N*-(1*H*-indazol-6-yl)benzenesulfonamide (**19**)

Yield, 89%; mp 252.4°C. IR: 3346 (NH), 3100 (arom.), 2966, 2933, 2839 (aliph.), 1627 (CO), 1589 (CN), 1350, 1157 (SO_2). ^{1}H-NMR: 3.82 (s, 6H, 2OCH_3), 6.18, 6.60 (2d, 2H, CH=CH, J=8.6 Hz), 6.95–8.08 (m, 10H, Ar-H), 8.13 (s, 1H, CH pyrazole), 10.32 (s, 1H, SO_2NH), 12.00 (s, 1H, NH), 12.8 (s, 1H, NH pyrazole). ^{13}C-NMR: 56.5(2), 95.6, 100.3, 110.4, 110.6, 111.3, 115.3(2), 116.2, 120.2, 121.7, 129.3, 131.4(2), 132.2, 140.7, 142.4, 143.8, 144.4, 149.0, 152.8, 186.8. MS *m*/*z* (%): 478(M^+) (14.84), 116 (100). *Anal.* Calcd for $C_{24}H_{22}N_4O_5S$ (478): C, 60.24; H, 4.63; N, 11.71. Found: C, 60.53; H, 4.31; N, 11.98.

***In Vitro* Anticancer Evaluation**

Chemicals and Supplies

MTT and DMSO were purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM)/high glucose, DMEM/F12, fetal bovine serum (FBS), L-glutamine, and penicillin–streptomycin were obtained from Gibco Inc. (NY, U.S.A.).

Cell Lines

Four tumor cell lines were utilized in this study, namely the HeLa, HepG2, Daoy, and the HT-29 cells. These cell lines were obtained from American Type Culture Collection (Manassas, VA, U.S.A.). HeLa, HepG2, and HT-29 cells were cultured in DMEM/high glucose supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin–streptomycin. Daoy cells were cultured in DMEM/F12 supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin–streptomycin.

Methods

Growth Inhibitory Activity of the New Compounds Screened by MTT Assay

The antiproliferative activity of the new compounds was assessed at the Cell Culture Laboratory, College of Pharmacy, King Saud University, in a primary four cell lines-one concentration (25 μg/mL) anticancer assay by using the aforementioned cell lines. The cytotoxicity of the newly synthesized compounds was determined by testing the capacity of the reducing enzymes present in the viable cells to convert MTT to formazan crystals as previously reported.[43] Briefly, cells were seeded in complete medium, at a density of 2 $\times 10^4$ cells/well, into 96-well microtiter plates and incubated at 37°C in a humidified atmosphere with 5% CO_2, for 24 h. The complete medium was then changed to serum-free medium (SFM), containing the test compounds (25 μg/mL), or an equivalent volume of solvent (DMSO), as control. After incubation, the SFM in the control and test wells was replaced by MTT (100 μL/well; 0.5 mg/mL) in phosphate-buffered saline (PBS) and incubated at 37°C for an additional 3 h. The MTT solution was removed and the purple formazan crystals formed at the bottom of the wells were dissolved using isopropyl alcohol (100 μL/well), and the plate was further incubated for 2 h at room temperature, with constant agitation. The plate was read in a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT, U.S.A.), and the absorbance at 549 nm recorded.

The dose response curves of the compounds exhibiting $\geq$50% inhibition in the aforementioned one-dose prescreening assay for each cell line were established using the following concentrations: 1.56, 3.125, 6.25, 12.5, 25, and 50 μg/mL. The concentration effecting IC_{50} was calculated. The cytotoxic activity of the well-known antitumor drug dasatinib, a potent multi-targeted inhibitor of BCR-ABL and SRC family kinases,[44] was determined at the same concentrations of the tested compounds against the four cell lines, and utilized as a standard for comparative purposes.

***In Vitro* VEGFR-2 Inhibition Assay** HeLa cells (1.2–1.8$\times 10^4$ cells/well) were cultured in a volume of 200 μL/well (100 μL of complete growth medium +100 μL of the tested compounds) in a 96-well plate for 18–24 h before the VEGFR-2 enzyme assay was performed.[45]

Molecular Docking Molecular docking studies were performed on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE, 10.2008) software. All the minimizations were performed with MOE until an RMSD gradient of 0.05 kcal mol^{-1} Å^{-1} with MMFF94X force field, and the partial charges were automatically calculated. The X-ray crystallographic structure of VEGFR-2 complexed with sunitinib was obtained from the protein data bank (PDB ID: 4AGD). The protein was prepared for docking studies in the following way: (i) the co-crystallized ligand molecule was removed from the enzyme active site; (ii) hydrogen atoms were added to the structure with their standard geometry; (iii) MOE Alpha Site Finder was used to search for the active site in the protein structure and to generate dummy atoms from the obtained alpha spheres; (iv) the obtained model was then used in predicting ligand–protein interactions at the active site.

Conclusion

A novel series of benzenesulfonamide derivatives bearing the 3,4-dimethoxyphenyl moiety were synthesized and evaluated for their cytotoxic activity against four different cell lines. Four compounds (**4**, **10**, **16**, **19**) showed good cytotoxic activity when compared to dasatinib (reference drug). The same compounds were evaluated for their *in vitro* ability to inhibit VEGFR-2. Compounds **10** and **19** were more active than dasatinib as VEGFR-2 inhibitors. Structure–activity

relationship favors heterocyclic sulfonamide derivatives and, in particular, methyl thiadiazole derivative **10** and benzopyrazole derivative **19**. Molecular docking on the active site of VEGFR-2 for compound **19** exhibited good fitting and comparable amino acid interactions to those displayed by sunitinib.

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Conflict of Interest The authors declare no conflict of interest

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