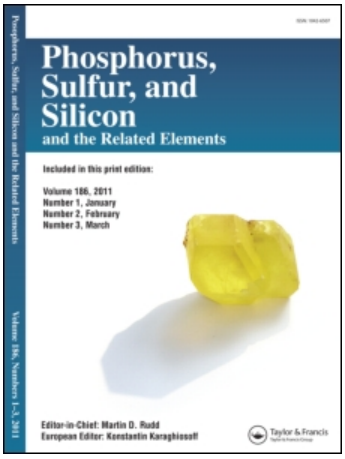

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Computer-Based Ligand Design and Synthesis of Some New Sulfonamides Bearing Pyrrole or Pyrrolopyrimidine Moieties Having Potential Antitumor and Radioprotective Activities

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*A series of new Pyrrole and pyrrolo[2,3-d]pyrimidine derivatives (**5–15**) was designed, synthesized, and biologically evaluated for their in vitro cytotoxic activity. The design of these compounds was based upon the molecular modeling simulation of the fitting values and conformational energy values of the best-fitted conformers to VEGFRTK inhibitors hypothesis. This hypothesis was generated from its corresponding lead compounds using CATALYST software. Some of the newly synthesized compounds **8**, **11**, **12**, and **13** showed interesting cytotoxic activity compared with Doxorubicin as a reference drug. These results are nearly consistent with the molecular modeling studies. Moreover, Compound **7** showed significant radioprotective activity.*

Keywords Pyrrole; pyrrolo[2,3-d]pyrimidine; molecular modeling studies; synthesis; tyrosine kinase inhibitors; antitumor and radioprotective activities

INTRODUCTION

In current cancer chemotherapy, several agents with DNA-cleavage properties, antimitotics, antimetabolites, inhibitors of topoisomerases, and most recently, signal transduction inhibitors are used as drugs.[1] The confluence of two distinct but related activities in the past 10 years

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has dramatically accelerated efforts towards the discovery and development of novel drugs to treat cancer. The first is a rapidly emerging understanding that a number of distinct tyrosine kinases play important roles in tumor progression (growth, survival, metastasis, and angiogenesis). The second is the discovery that small molecule compounds have the capacity to potently and selectively inhibit the biochemical function of tyrosine kinases by competing for ATP binding at the enzyme catalytic site.

In 1970, Folkman and Kerbel proposed that inhibition of angiogenesis can prevent tumor growth[2,3] Subsequently, it was also recognized, that metastasis can be affected by angiogenesis. For these reasons, inhibitors of angiogenesis are expected to be valuable drugs for cancer therapy. The cancer cell is characterized by oncogene-derived tumor expression of pro-angiogenic proteins, such as vascular endothelial growth factor (VEGF), placenta-like growth factor, basic fibroblast growth factor (FGF), platelet-derived endothelial growth factor (PDGF), angiopoietin-2 (Ang-2), hepatocyte growth factor and insulin-like growth factor (ICF).[4] The mentioned pro-angiogenic growth factors bind to specific receptors that possess receptor tyrosine kinase (RTK) activity.

It has been reported that sulfonamides constitute an important class of drugs with several pharmacological activities including antibacterial[5] and antitumor activities.[6,7] Also, different series of pyrroles[8] and pyrrolo[2,3-d]pyrimidines were proved to have antitumor activity via tyrosine kinase inhibition.[9–11]

On the other hand, the importance of sulfur-containing molecules for radioprotection has attracted a considerable attention.[12] Also, pyrrole and pyrrolopyrimidine derivatives were known recently to have significant antioxidant properties and hence a radioprotective activity.[9,13]

In this investigation, ligand design—based on molecular modeling studies for the development of new hits acting as predicted anti-angiogenesis—is performed. All these previous findings encourage us for further exploration of novel antitumor agents. This article aims to design and synthesize a series of sulfonamides bearing pyrrole and pyrrolo[2,3-d]pyrimidine moieties to be evaluated as antitumor and radioprotector hit molecules.

MATERIALS AND METHODS

General Considerations

Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart scientific, Redhill, UK). Elemental analyses (C, H, N) were performed on Perkin-Elmer 2400 analyzer

(Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical Unit of Cairo University. All compounds were within ±0.4% of the theoretical values. IR spectra (KBr) were measured on (Pye Unicam SP1000 IR spectrophotometer, Thermoelectron, Egelbach, Germany). ^{1}H-NMR spectra were obtained on a Bruker 300 MHZ NMR spectrophotometer (Bruker, Munich, Germany) in DMSO-d_6 as a solvent, using tetramethyl-silane (TMS) as internal standard. Mass spectra were run on Varian MAT 311-A70ev (Varian, Fort Collins, USA).

Catalyst Molecular Modeling Experiments

All molecular modeling work was performed on Silicon Graphic (SGI), Fuel workstation (500 MHz, R 14000 A^{TM} processor, 512 MB memory) using the catalyst package of Molecular Simulation (version 4.8), under an IRIX 6.8 operating system, at the Faculty of Pharmacy, Ain Shams University. A generalized vizualizer, confirm, info, HipHop, compare/fit, force field was used throughout.

Training sets, lead compounds **I–VII**, were selected .Molecules were built within the catalyst and conformational models for each compound were generated automatically using the poling algorithm. This emphasizes representative coverage over a 20 Kcal mol^{-1} energy range above the estimated global energy minimum and the best searching procedure was chosen. The training molecules with their associated conformational models were submitted to catalysis by using default common features hypothesis generation by using HipHop commands. The chemical function groups (features) used in this generation step included hydrophobic aromatic and hydrogen bonding acceptors.

Synthesis

4-(N-(3-methyl-isoxazol-5-yl)-4-(2-oxo-2-phenyl-ethylamino) benzenesulfonamide (2)

A mixture of sulfamethoxazol **1** (2.5 g, 0.01 mol) and phenacyl bromide (2 g, 0.01 mol) was refluxed in dimethylformamide (30 mL) in presence of triethylamine (3 drops) for 3 h. The solid obtained was filtered and recrystallized from appropriate solvent (Table I).[7] IR (KBr, cm^{-1}): 3210 (NH), 1676 (C=O), 1340, 1170 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 4.8 (d, 2H, CH_2), 6.1 (s, 1H, isoxazol-H), 6.7–8.10 (m, 9H, Ar-H), 11.00 (s, 2H, 2NH).

4-(2-Amino-3-cyano-4-phenyl-pyrrol-1-yl)-N-(3-methyl-isoxazol-5-yl)-benzenesulfonamide (4)

A mixture of compound **2** (3.7 g, 0.01 mol) and malononitrile (0.7 g, 0.01 mol) was refluxed in ethanol (30 mL) containing sodium ethoxide

TABLE I Physical Data of the Synthesized Compounds (2–15)

Compd. No.	Solvent (crystallization)	M.P. (°C)	Yield (%)	Molecular formula (molecular weight)
2	Ethanol	248–250 (as reported[7])	80	$C_{18}H_{17}N_3O_4S$ (371)
4	Ethanol	183–184 (as reported[7])	75	$C_{21}H_{17}N_5O_3S$ (419)
5	Dioxane	242–244	81	$C_{22}H_{18}N_6O_3S$ (446)
6	Dioxane	262–264	73	$C_{22}H_{17}N_5O_4S$ (447)
7	Methanol	130–132	75	$C_{23}H_{19}N_5O_4S$ (461)
8	Ethanol	190–192	70	$C_{25}H_{21}N_5O_5S$ (503)
10	Ethanol	210–212	65	$C_{27}H_{21}N_5O_5S_2$ (559)
11	Ethanol	100–102	72	$C_{28}H_{23}N_5O_5S_2$ (573)
12	Ethanol	218–219	80	$C_{28}H_{21}N_5O_3S$ (507)
13	Dioxane	125–126	68	$C_{22}H_{18}N_6O_4S$ (462)
14	Ethanol	>300	85	$C_{22}H_{18}N_6O_4S$ (462)
15	Ethanol	>300	69	$C_{22}H_{18}N_6O_3\ S_2$ (478)

(0.5 g) for 3 h. The reaction mixture was acidified with diluted HCl. The solid obtained was recrystallized from appropriate solvent (Table I).[7] IR (KBr,cm^{-1}): 3400, 3260, 3180 (NH, NH_2), 2200 (C≡N), 1350, 1170 (SO_2).

4-(4-Amino-5-phenyl-pyrrolo [2,3-d]pyrimidin-7-yl)-N-(3-methyl-isoxazol-5-yl)-benzenesulfonamide (5)

A solution of compound 4 (4.2 g, 0.01 mol) in formamide (20 mL) was refluxed for 5 h; the reaction mixture was cooled, and then poured onto ice cooled water. The formed solid was recrystallized from appropriate solvent (Table I). IR (KBr, cm^{-1}): 3350, 3230, 3190 (NH ,NH_2), 1580 (C=N), 1340, 1155 (SO_2). MS (m/z): 446 (M^+, 2.85%), 77 (100%).

N-(3-Methyl-isoxazol-5-yl)-4-(4-oxo-5-phenyl-3,4-dihydro-pyrrolo[2,3-d]pyrimidin-7-yl) benzenesulfonamide (6)

A solution of compound **4** (4.2 g, 0.01 mol) in formic acid (30 mL) was refluxed for 5 h, the reaction mixture was cooled, poured onto ice water, the separated solid was recrystallized from appropriate solvent (Table I). IR (KBr, cm^{-1}): 3190, 3120 (NH), 1650 (C=O), 1580 (C=N) and 1350, 1160 (SO_2). MS (m/z): 447 (M^+,7.75%), 69 (100%).

N-(3-Cyano-1-(4-(N-(3-methylisoxazol-5-yl)sulfamoyl)phenyl)-4-phenyl-1H-pyrrol-2-yl)acetamide (7)

A solution of **4** (4.2 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 1 h, the reaction mixture was then concentrated, the solid separated was recrystallized from appropriate solvent (Table I). IR (KBr, cm^{-1}): 3230, 3190 (2NH), 3100 (CH arom.), 2210 (C≡N), 1730 (C=O), 1350, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 1.8 [s, 3H, CH_3], 2.5 [s, 3H,

$COCH_3$], 6.8 [s, 2H, CH pyrrole+CH isoxazole], 7.2–8.7 [m, 11H, Ar-H+2NH].

N-Acetyl-N-(3-cyano-1-(4-(N-(3-methylisoxazol-5-yl)sulfamoyl)phenyl)-4-phenyl-1H-pyrrol-2-yl) acetamide (8)

A solution of **4** (4.2 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 10 h, the reaction mixture was then concentrated, the solid separated was recrystallized from appropriate solvent (Table I). IR (KBr, cm^{-1}): 3190 (NH), 2200 (C≡N), 1780, 1750 (2C=O), 1350, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 1.9 [s, 3H, CH_3], 2.6 [s, 6H, $2COCH_3$], 6.8 [s, 1H, CH isoxazole], 7.2–8.6 [m, 11H, Ar-H+CH pyrrole +NH].

4-(3-Cyano-4-phenyl-2-(phenylsulfonamido)-1H-pyrrol-1-yl)-N-(3-methylisoxazol-5-yl)benzenesulfonamide (10) and N-(3-Cyano-1-(4-(N-(3-methylisoxazol-5-yl)sulfamoyl)phenyl)-4-phenyl-1H-pyrrol-2-yl)-4-methylbenzenesulfonamide (11)

A mixture of compound **4** (4.2 g, 0.01 mol) and benzenesulfonylchloride (1.9 g, 0.01 mol) or toluenesulfonyl chloride (2 g, 0.01 mol) in benzene (20 mL) containing 3 drops of pyridine was refluxed for 8 h. The reaction mixture was concentrated and then acidified with diluted HCl. The solid obtained was crystallized from appropriate solvent to give **10** and **11**, respectively (Table I). IR (KBr, cm^{-1}) **10**: 3442 (2NH), 2206 (C≡N), 1332, 1162 (SO_2). ^{1}H-NMR (DMSO-d_6) **10**: 2.4 [s, 3H, CH_3], 6.3 [s, 2H, CH isoxazole+CH pyrrole], 6.9–8.3 [m, 14H, Ar-H], 11.2 [s, 2H, 2NH]. MS (m/z) **10**: 558 (M-1,15.1%), 57 (100%), 77 (69.8%). IR (KBr,cm^{-1}) **11**: 3426 (2NH), 2220 (C≡N), 1374, 1162 (SO_2). ^{1}H-NMR (DMSO-d_6) 11: 2.5 [s, 6H, $2CH_3$], 6.3 [s, 1H, CH pyrrole], 7.0–8.2 [m, 14H, Ar-H+CH isoxazole], 9.1 [s, 2H, 2NH].

4-(2-(Benzylideneamino)-3-cyano-4-phenyl-1H-pyrrol-1-yl)-N-(3-methylisoxazol-5-yl)benzenesulfonamide (12)

A mixture of **4** (4.2 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol) was refluxed in ethanol (20 ml) for 3 h, the reaction mixture was concentrated. The solid separated was recrystallized from appropriate solvent (Table I). IR (KBr, cm^{-1}): 3230 (NH), 2200 (C≡N), 1600 (C=N), 1330, 1162 (SO_2). MS (m/z): 507 (M^+, 20.4%) , 82 (100%), 77 (28.6%).

4-(3-Cyano-4-phenyl-2-ureido-pyrrol-1-yl)-N-(3-methyl-isoxazol-5-yl)-benzenesulfonamide (13)

A mixture of 4 (4.2 g, 0.01 mol) and urea (0.6 g, 0.01 mol) in ethanol (30 mL) containing sodium ethoxide (0.23 g, 0.01 mol) was refluxed for 3 h. The reaction mixture was cooled and acidified with diluted HCl. The separated crystals were recrystallized from appropriate solvent

(Table I). IR (KBr, cm^{-1}): 3352, 3226, 3195 (NH, NH_2), 2204 (C≡N), 1690 (C=O), 1310, 1120 (SO_2). MS (m/z): 461 (M-1, 5.59%), 64 (100%).

4-(4-Amino-2-oxo-5-phenyl-1,2-dihydro-pyrrolo[2,3-d]pyrimidin-7-yl)-N-(3-methyl-isoxazol-5-yl)-benzenesulfonamide (14) and 4-(4-Amino-5-phenyl-2-thioxo-1,2-dihydro-pyrrolo[2,3-d]-pyrimidin-7-yl)-N-(3-methyl-isoxazol-5-yl)-benzenesulfonamide (15)

A mixture of **4** (4.2 g, 0.01 mol), urea (0.9 g, 0.015 mol) or thiourea (1.14 g, 0.015 mol) was fused together at 250°C in an oil bath for 20 min; the formed mass was then triturated with ethanol, and the separated crystals were recrystallized from appropriate solvent give **10** and **11**, respectively. (Table I).

IR (KBr,cm^{-1}) **14**: 3480, 3370, 3200 (NH, NH_2), 1650 (C=O), 1610 (C=N), 1340, 1170 (SO_2). MS (m/z) **14**: 462 (M^+, 30%), 92 (100%). IR (KBr, cm^{-1}) **15**: 3450, 3410, 3380, 3230(NH, NH_2), 1600 (C=N), 1300 (C=S), 1320, 1150 (SO_2). MS (m/z) **15**: 478 (M^+, 2.9%), 55 (100%).

Biological Testing

Antitumor Activity

Ehrlich Ascites Carcinoma cells (EAC) were obtained by needle aspiration of ascitic fluid from the preinoculated mice under aseptic conditions. Tumor cells suspension (2.5×10^6 per ml) was prepared in saline. Tested compounds were prepared by dissolving 10 μmol of the tested compounds in a mixture of 7 ml DMSO and 3 ml Saline.

In a set of sterile test tubes (200, 100, 75, 50, 50, and 25 μL) of each tested compound were mixed with 100 μL of tumor cell suspension , then completed to 1 mL with saline to obtain a solution of (200, 100, 75, 50, 50, and 25 μL, respectively) for each tested compound. The test tubes were incubated at 37^5C for 2 h. Trypan blue exclusion test was carried out to calculate the percentage of non-viable cells.[14] The results of in vitro cytotoxic activity experiments are presented in Table II.

Radioprotective Activity

This study was conducted to evaluate the potency of compound (**7**) as protective agent against γ-irradiation-induced toxicity. Female Swiss albino mice were injected intraperitoneally with suspension of the tested compound in carboxy methylcellulose once every other day for a total of 3 injections during 7 days. Each injection was given 30 min prior to exposure to a single dose of whole body γ- irradiation at a dose

TABLE II Fit and Energy Values and In Vitro Cytotoxic Activity of Some Newly Synthesized Compounds

			Non viable cells (%)					
			Concentration (μMol/mL)					
Compound No.	Fit value	Energy K/cal^{-1}	200	100	75	50	25	IC50* (nMol /ml)
5	2.99	7.43	0	0	0	0	0	>200
6	2.98	14.91	0	0	0	0	0	>200
7	3.65	13.98	0	0	0	0	0	>200
8	3.61	10.37	100	100	60	20	0	67.5
10	3.59	3.09	0	0	0	0	0	>200
11	4.31	17.09	100	100	70	30	0	64.2
12	3.67	4.34	100	80	20	5	0	75.78
13	3.68	14.19	100	70	25	5	0	88.5
14	2.92	10.73	0	0	0	0	0	>200
15	2.98	4.46	0	0	0	0	0	>200
Vatalanib	5.00	0.13	—	—	—	—	—	—
Doxorubicin	—	—	100	75	64	55	20	81.5

*$\mathbf{IC_{50}}$**value** which corresponds to the compound concentration causing 50% mortality in net cells.

level of 6 Gy. Lipid peroxide level (LPx), and Glutathione content (GSH) were estimated in blood of animals at the end of the experiment.

Chemicals and facilities. All chemicals and reagents were of the highest grade commercially available. Facilities including animal house and biochemical equipments have been made available by the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Animals. Female Swiss albino mice weighing 25–30 g were used in this study. Mice were housed at a constant temperature ($24 \pm 2C^{\circ}$) with alternating 12 h light and dark cycles and fed standard laboratory food and water.

Irradiation. Whole body gamma irradiation was performed at the NCRRT, Cairo, Egypt, using Gamma cell-40 (Caesium-137 source). Animals were irradiated at 3 doses, every other day 30 min after injection with compound **7** at dose level of 6 Gy delivered at a dose rate of 0.86 Gy/min.

Compound **7** *dosing.* Tested compound was suspended in caboxymethylcellulose (CMC) and given to mice by intraperitoneal injection (i.p.) of the maximum tolerated dose (150 mg /kg body weight) once every other day for a total of 3 injections during 7 days.

Experimental design. From the beginning of the experiment, mice were divided into eight groups. All experimental animals were categorized as follows:

1) **control**: Animals served as untreated control group;
2) **CMC**: Animals were treated by i.p. injection of Carboxymethylcellulose;
3) **Rad.**: Animals were subjected to 3 doses; every other day of whole body γ-irradiation at a dose level of 6 Gy starting from day 10;
4) **compound 7:** Animals were treated by i.p. injection of suspension of compound **7** in Carboxymethylcellulose; and
5) **compound 7 + Rad**: Mice injected i.p. with compound **7** and subjected to whole body γ-irradiation.

Samples collection. Animals were fasted for 16 h prior to each sampling. Samples were collected after 1day post last irradiation dose. Whole blood was collected by heart puncture after light anesthesia using heparinized syringes. One part was used for glutathione (GSH) estimation. The separated plasma from heparinized blood was used for the determination of lipid peroxide as malondialdehyde (MDA).

Analytical procedures. Lipid peroxide (Lpx) level in plasma was ascertained by the formation of MDA and measured as described by Yoshioka et al.[15]GSH content was determined according to Beutler et al.[16]

Statistical analysis. Student's t test[17] was used for the evaluation of the biochemical parameters.

RESULTS

Generation of Vascular Epithelial Growth Factor Receptor Tyrosine Kinase (VEGFRTK) Inhibitor Hypothesis Using CATALYST Software

The lead compounds **I–VII,** which were reported to have selective VEGFRTK inhibitory activity[8] were used to generate common feature hypothesis of VEGFRTK inhibitor (Figure 1).

The set of conformational models of each structure of the lead compounds was performed and was used to generate the common feature hypothesis, where 10 hypotheses were generated. The assessment of the ideal hypothesis among the generated ones indicated that hypothesis ranked number 2 was the ideal one (Figures 2 and 3). Such an ideal hypothesis encompassed five features namely (Figure 2); 3 hydrophobic (HP), two hydrogen bonding acceptors (HBA). The constraint distances

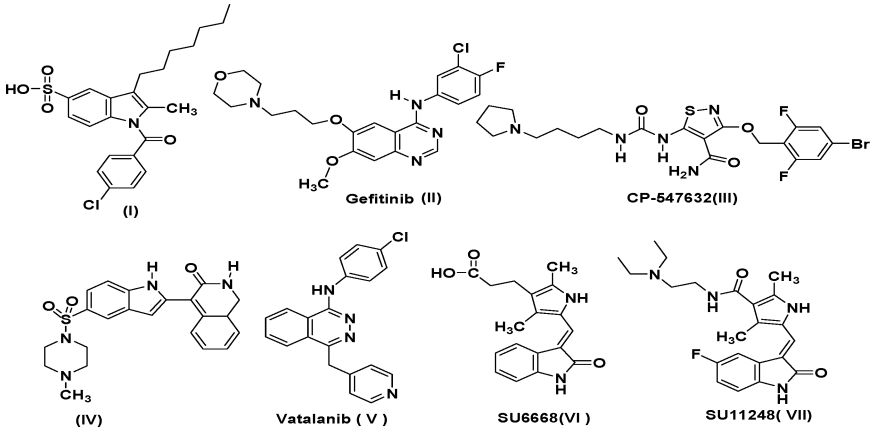


FIGURE 1 Structures of selective VEGFRTK inhibitory lead compounds **I–VII** used for generation ofcommon feature VEGFRTK inhibitor hypothesis.

and torsion angle were measured (Figure 3). The validation of this ideal hypothesis is due to the full mapping of all its five features with the lead compound **V** (**Vatalanib**, Figure 4) and most of the other lead compounds. Molecular modeling simulation studies were then conducted by measuring the compare/fit values, separately, between the conformational models of compounds **5–15** and ideal selective VEGFRTK inhibitor hypothesis (Figure 4). The results of the best fitting values, as well as the conformational energy of the best-fitted conformer with this hypothesis, are given in Table II. The result of simulation studies have revealed that compounds **5–15** would be promising active hit molecules. These findings prompted us to synthesize compounds **5–15** (Schemes 1–2).

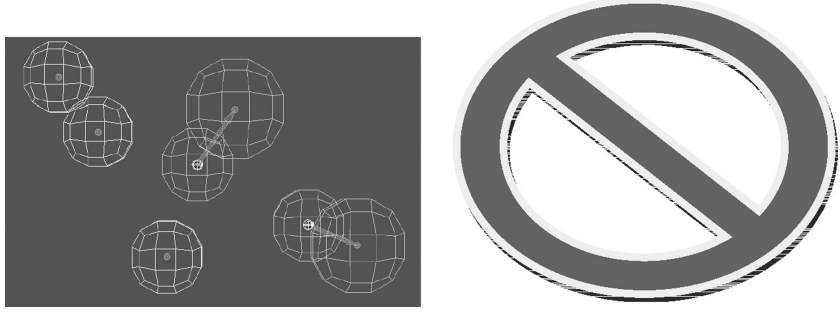


FIGURE 2 5 features [3 HP and 2 HBA].

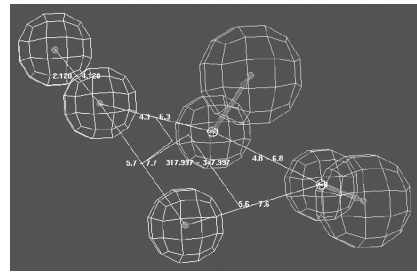


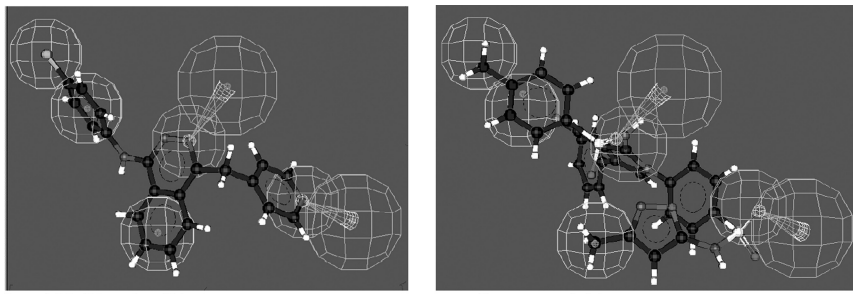
FIGURE 3 Constraint distances and torsion angle of VEGFRTK inhibitor hypothesis.

Chemistry

In this investigation, a series of new compounds incorporating the Pyrrole or pyrrolo[2,3-d]pyrimidine moieties attached to sulfonamide moiety (**5–15**) was designed, synthesized (Schemes 1–3), and biologically evaluated for their in vitro cytotoxic and radioprotective activities.

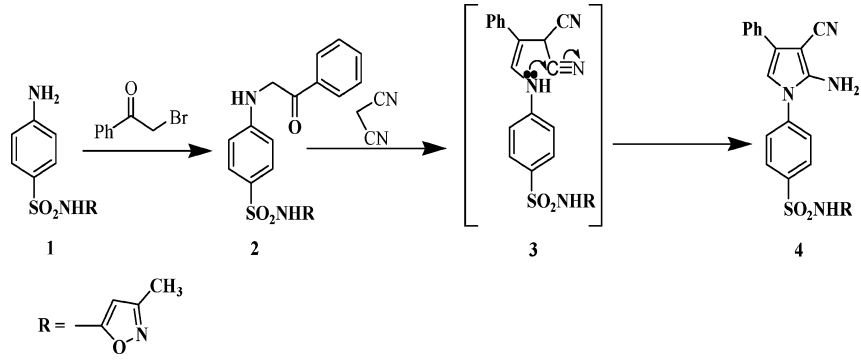
Thus, Interaction of sulfamethoxazole **1** with phenacyl bromide furnished 4-(2-oxo-2-phenyl-ethylamino)-benzenesulfonamide **2**, which upon reaction with malononitrile in ethanol in presence of sodium ethoxide yielded the pyrrole derivative **4**[7] (Scheme 1).

The pyrrolopyrimidine derivative **5** was obtained by the reaction of **4** with formamide. The structure of **5** was established through microanalysis and its IR spectrum, which showed the absence of (C≡N) band and presence of (NH_2) at 3350, 3230 cm^{-1}. The mass spectrum of compound **5** exhibited a molecular ion peak m/z at 446 (M^+, 2.85%) with a base peak at 77 (Scheme 2).



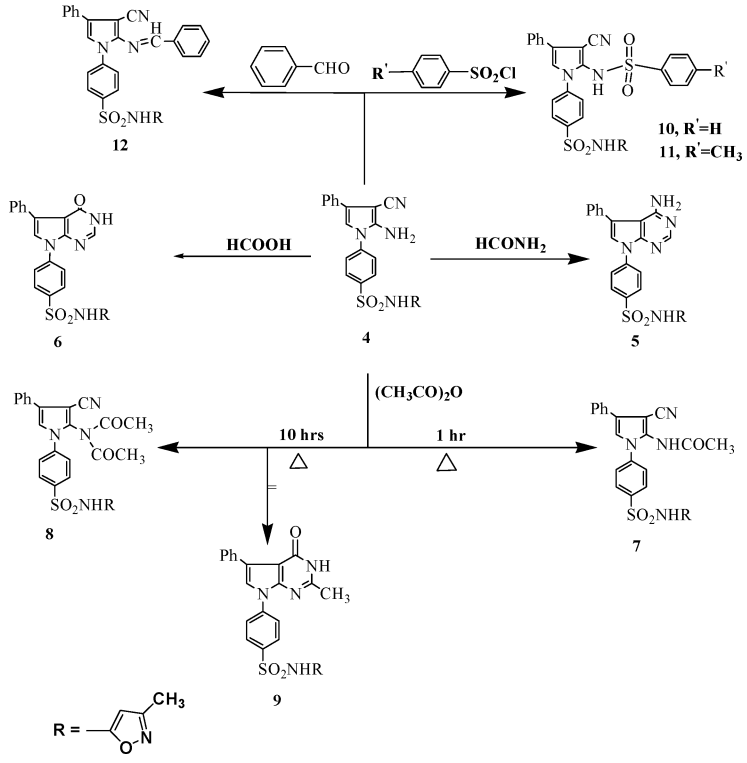
Lead **V** (**Vatalanib**) Compound **11**

FIGURE 4 Mapping of VEGFRTK inhibitor hypothesis with **Vatalanib** and compound **11**.

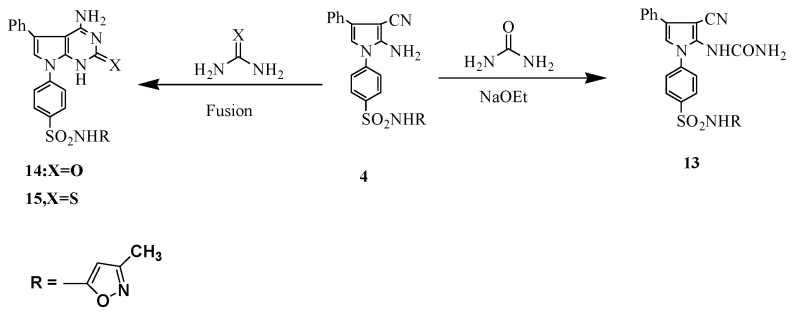


SCHEME 1

The behavior of compound **4** towards acid derivatives was also investigated. Thus, heating compound **4** with formic acid caused cyclization to give pyrrolopyrimidine derivative **6**. The IR spectrum of compound **6** showed the absence of (C≡N) band and presence of band at 1650 cm^{-1}



SCHEME 2



SCHEME 3

(C=O). The mass spectrum of compound **6** exhibited a molecular ion peak m/z at 439 (M^+, 7.75%) with a base peak at 69 (Scheme 2).

Reaction of compound **4** with acetic anhydride for 1 hr yielded the monoacetyl derivative **7** , while 10 hours reaction time, furnished the diacetyl derivative **8** instead of the expected fused pyrrolopyrimidine **9**. The IR spectrum of compound **7** revealed the presence of (C≡N) band at 2210 cm^{-1} and carbonyl group at 1730 cm^{-1}. Also, the IR spectrum of compound **8** showed the presence of (C≡N) band at 2200 cm^{-1} and two carbonyl groups at 1780 and 1750 cm^{-1}. ^{1}H-NMR spectrum of compound **7** exhibited singlet at 2.5 ppm of the acetyl group.

Interaction of compound **4** with benzenesulphonyl chloride or toluenesulphonyl chloride yielded the corresponding pyrrole derivatives **10** and **11**, respectively. The structure of compound **10** was confirmed by its IR spectrum which showed a band at 2206 cm^{-1} corresponds to the (C≡N) group. The mass spectrum of compound **10** exhibited a molecular ion peak m/z at 558 (M-1, 15.1%) with a base peak at 57. Also, the IR spectrum of compound **11** showed the presence of (C≡N) band. ^{1}H-NMR spectrum of compound **11** revealed singlet at 2.5 ppm for two (CH_3).

The benzlidine derivative **12** was obtained in a good yield via the reaction of compound **4** with benzaldehyde. The IR spectrum showed the presence of bands at 2200 and 1600 cm^{-1} corresponds to (C≡N) and (C=N) groups, respectively. Also, mass spectrum of compound **12** exhibited a molecular ion peak m/z at 507 (M^+, 20.4%), with a base peak at 82.

When compound **4** was allowed to react with urea in ethanol in the presence of sodium ethoxide yielded compound **13** via elimination of ammonia. This was proved by IR spectrum which showed bands at 3352, 3226, 3195 cm^{-1} (NH, NH_2), 2204 cm^{-1} (C≡N) and 1690 cm^{-1} (C=O). The mass spectrum of compound **13** exhibited a molecular ion peak m/z at 461 (M-1, 5.59%), with a base peak at 64.

Finally, fusion of compound **4** with urea and/or thiourea gave the condensed pyrimidine derivatives **14** and **15**, respectively. IR spectrum

of compounds **14** and **15** revealed the disappearance of the carbonitrile function group and presence of band at 1650 cm^{-1} (C=O) for compound **14** and 1250 cm^{-1} (C=S) for compound **15**.

Antitumor Activity

Results presented in Table II showed some promising compounds as antitumor agents. Compounds **8**, **11** were the most potent, while compound **12** showed moderate activity, and compound **13** seems to be the least active one. The presence of a cyano group in all these compounds enhances their antitumor activity since the cyano group has been reported to exhibit antitumor activity.[18,19] Compounds **8** and **11** containing diacetyl group or tolyl group respectively showed the highest in-vitro cytotoxic activity. While the incorporation of a benzilidine moiety in case of compound **12** and ureido group in compound **13** did not greatly enhance the antitumor activity.

Radioprotective Activity

Radiation exposure significantly increases lipid peroxidation (LPx), such increase seems to be due to the result of inactivation of scavenger enzymes activities induced by reactive oxygen species (ROS). Oxidative stress occurs in living organisms when the production of ROS exceeds the ability of organisms to prevent their accumulation.[20,21] Such elevation of LPx is accompanied by decline in GSH content and in the activity of related antioxidant enzyme SOD. Additionally LPx can be initiated by hydrogen abstraction from lipid molecules by lipid radiolytic products. This leads to permeability changes, secondary alteration in membrane proteins and other sequences.[22,23]

Effect of Compound 7 and/ or γ-Irradiation on Lipid Peroxidation and Antioxidant Status

Glutathione Level in Blood (GSH)

As summarized in Table III, a significant depletion in GSH level was observed in blood of irradiated mice compared to their corresponding controls. While mice exposed to γ-irradiation and treated with compound **7** showed significant increase of GSH content in blood.

Lipid Peroxidation Content (MDA) in Plasma

As shown in Table III, mice exposed to γ-irradiation showed significant elevation in Malonaldialdehyde (MDA) level in plasma compared with the control values. Treatment with compound **7** prior irradiation

TABLE III Effect of Compound Administration on Blood Glutathione (GSH) Content and Plasma Lipid Peroxide Concentrations (LPx) of Normal and Irradiated Mice

Groups		GSH mg/dl	LPx μMol/ml
Control	Mean ± SE	78.59 ± 5.45	78.05 ± 0.79
	% change	(100%)	(100%)
CMC	Mean ± SE#	75.45 ± 0.28	80 ± 1.18
		(96.15%)	(103.9%)
Rad.	Mean ± SE #	38.98 ± 1.03***	101.29 ± 0.11***
		(49.59%)	(129.78%)
Compd. 7	Mean ± SE #	89.3 ± 9.73	52.57 ± 4.86
		(113.62%)	(67.35%)
Compd. 7+Rad	Mean ± SE #	260.5 ± 1.7***	74.86 ± 1.41
	Mean ± SE #	(331.47%)	(95.9%)

Each value is the mean of six mice ±SE. # : percentage of change from control group; *significant difference from control at $P < 0.05$; **high significance at $P < 0.01$; ***very high significance at $P < 0.001$.; CMC: carboxy methyl cellulose.

exhibited a higher reduction in MDA levels in blood as compared with irradiated group.

CONCLUSION

The present data showed that some compounds combining both pyrrole or pyrrolo[2,3-d]pyrimidine and benzenesulfonamide moieties exhibited promising in-vitro cytotoxic activity against (EAC) cell line, Compound **11** showed the highest fit value and highest in vitro cytotoxic activity when compared with other tested compounds and Doxorubicin as a reference drug. Additionally, compound **7** has a radical scavenging effect and can prevent radical generation in mice immediately after irradiation, probably by inhibiting the chain reaction of membrane lipid peroxidation, which follows thereafter.

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