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LETTER ARTICLE

Synthesis of Piperidine Conjugated Dihydroquinazolin-4(1*H*)-ones and their Antiproliferative Activity, Molecular Docking Studies and DFT Calculations

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Abstract: ***Background*:** Xanthatin, fluoropyrimidine and thienopyrimidine, pyrazolopyrimidine, pyrimidine carboxamides, and SKLB1002 are reported as VEGFR2 tyrosine kinase inhibitors. Recently, many studies related to different heterocycles conjugated with dihydroquinazolinones are known to have very good biological activities. In this study, we are intended to explore the cytotoxic studies of piperidine conjugated dihydroquinazolinones against colorectal/colon cancer cell lines and along with molecular docking studies and DFT calculations.

***Methods*:** The colorectal/colon cell lines HCT116 and A549 cell lines were treated with these compounds and cytotoxic activities were evaluated by MTT dye uptake method. We performed molecular modelling for compound **3d** using the Auto Dock software. The binding of compound **3d** with target proteins was studied with the collection of experimentally determined PDB database. Optimized geometry by DFT calculations was performed with B3LYP/6-31G (d) basis set.

***Results*:** Piperidine-conjugated dihydroquinazolinone analogues displayed anticancer activity. Particularly, the compound **3d** with electron-withdrawing substituents on a phenyl ring showed significant cytotoxicity against HCT116 and A549 cell lines. Molecular docking studies proved that the compound **3d** has good fitting by forming hydrogen bonds with amino acid residues at the active sites of VEGFR2. The HOMO, LUMO, their energies and UV visible spectrum were predicted using DFT calculations.

***Conclusion*:** Four piperidine-conjugated dihydroquinazolinones were synthesized and evaluated against colorectal and colon cancer cell lines. Compound **3d** significantly inhibited the growth of HCT116 and A549. Molecular docking studies displayed good fitting of compound **3d** by forming different H-bonds with the amino acid at the active sites of the VEGFR2 target. Using a theoretical approach, we optimized HOMO and LUMO plots for the compound **3d**.

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1. INTRODUCTION

Dihydroquinazolinones are nitrogen-comprising heterocycles, this heterocyclic moiety is present in natural products, and these alkaloids display decent biological activity [1]. Due to their application in biology [2], they attracted the interest of many researchers. Many protocols related to the synthesis of dihydroquinazolinones [3], with great biological significance, are reported [4]. For instance, substituted dihydroquinazolinones are reported as cell multiplication inhibitors [5], anti-fibrillatory and choleretic agents [6], anti-inflammatory agents [7], gastrointestinal motility improving and antiemetic agents, [8] IMPDH inhibitors [9], having H1/H2-antihistaminic actions [10], as PARP-1 inhibitors [11] and having anticancer activity [12, 13]. Recently, 4-aminopiperidine- substituted dihydroquinazolinones [14] are reported as inhibitors of both TNF-α and p38 MAP, coumarin-dihydroquinazolinone [15] conjugates as agonists of GPR109a receptors, morpholine conjugated tetrahydro quinazoline carboxamides [16] as antibacterial agents, morpholine and piperazine conjugated dihydroquin-

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azolinones [17] as antioxidants, isoxazolidine conjugated quinazolinones [18] as antivirals and cytostatic agents. Besides, new tetrazole conjugated quinazolin-4-ones [19] are AT_1 receptor antagonists, azomethine-dihydroquinazolinones [20] are established as anti-cholinesterase and anti-cancer agents. Thus, the literature survey marked that these dihydroquinazolinones derivatives showed diverse biological activities. Based on these reports, we intended to synthesize piperidine conjugated dihydroquinazolinones and evaluate their antiproliferative activity against colorectal or colon cancer cell lines.

VEGFR2 plays a crucial role in blood vessel development and in life menacing diseases such as intermediary of cardiovascular disorders and cancer angiogenesis [21]. Besides, it is a well-known part of a membrane receptor that triggers numerous signaling pathways [22]. Notably, inhibition of VEGFR signaling pathway is a desirable therapeutic target for the inhibition of tumor angiogenesis in two ways [23]; first, by hindering ligand attachment to the extracellular sphere of the kinase receptor with monoclonal antibodies, for example, Bevacizumab [24] while the second method is to block the VEGF pathway by avoiding the stimulation of VEGFR-2 sites using tyrosine kinase inhibitors [25], for example, Sunitinib [26]. Recently, furopyrimidine, thienopyridine, pyrazolo[1,5-a] pyrimidine (DMH4) scaffolds [27], quinazolinones [28, 29], xanthatin derivatives [30], SKLB1002 [31], pyrimidine carboxamides [32] were reported as potent VEGFR-2 inhibitors.

In an extension of our studies on the development of new anti-cancer agents [33], we have synthesized a series of piperidine conjugated dihydroquinazolinones which are characterized by NMR and HRMS, and tested for antiproliferative activity against HCT116 and A549 colon cancer cell lines. We also conducted molecular docking analysis to explore the binding mode of the most active compound to the target enzyme along with DFT calculations. These results are presented in this article.

2. EXPERIMENTAL

2.1. Chemistry

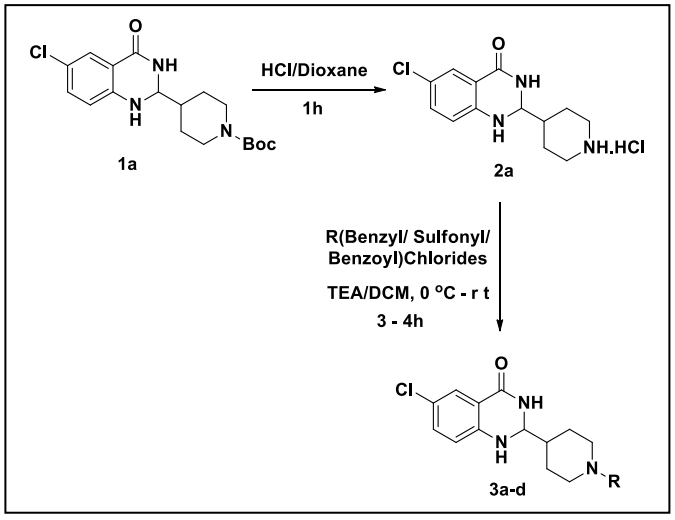
Reactions were monitored by pre-coated TLC plates (Merck 60F254) and were visualized under UV light. Melting points were determined on Selaco melting point device. The ^1H and ^{13}C NMR spectra were recorded using Agilent NMR spectrometer operated at 400 and 100 MHz, respectively with the residual solvent peak as a reference to $SiMe_4$. HRMS were recorded by a JEOL JMS-AX505HA mass spectrometer. IR spectra were taken on Shimadzu FT-IR 8300 spectrometer. Substituted sulfonyl chlorides, benzyl chlorides and benzoyl chlorides were procured from Sigma Aldrich India.

2.1.1. Synthetic Procedure for 2a

We prepared **1a** using a previously reported method [34]. To a solution of **1a (**5 mmol**)** in dioxane (5 mL), a solution of HCl in dioxane (10 mL) was added at 0°C and stirred at room temperature for 1h [35]. The hydrochloride salt of **2a** was obtained in 90% yield.

2.1.1.1. 6-chloro-2-(piperidin-4-yl)-2,3-dihydroquinazolin-4(1H)-one (2a)

White solid, Mp 164- 166 ^oC; IR (KBr) ν_{max} 3294.5, 3195.2, 3077, 2874.56, 1666.84, 985.41 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.21 (S, 1H),7.45-7.44 (d, J= 2.8Hz, 1H), 7.22-7.19 (m, 1H), 7.06 (s, 1H), 6.78-6.76 (d, J= 8.8Hz, 1H), 4.44-3.55 (m, 2H), 2.64-2.55 (m, 2H), 1.82-1.64 (m, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.10, 146.82, 133.38, 126.69, 120.43, 116.57, 116.08, 67.70, 43.70, 40.70, 24.83 ppm. HRMS (ESI): calcd for $[C_{13}H_{16}ClN_3O+H^+]$: 266.1209, found 266.1212, 268.1173 [36].



Scheme 1. Synthesis of compounds **3a-d.**

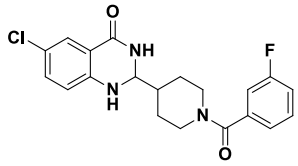
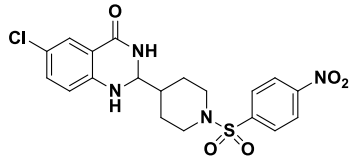
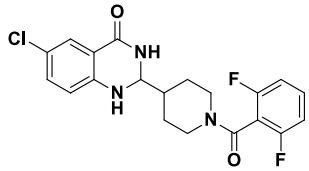
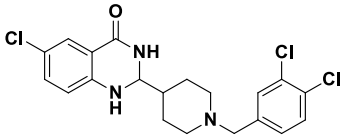
2.1.2. General procedure for the synthesis of 3a-d

The requisite title compounds **3a-d** were synthesized by treating **2a** (1 mmol) with substituted sulfonyl chlorides/ benzoyl chlorides/benzyl chlorides (1 mmol) in the presence of trimethylamine (TEA) (1 mmol) in dichloromethane (DCM) (5 mL) at room temperature for 3-4h [37]. The reaction was examined by TLC. Water (20 mL) was added to the reaction mass, followed by the addition of (20 mL). The organic phase was separated, washed with brine (20 mL) and dried over anhydrous sodium sulphate. The crude products obtained after the removal of the solvent under reduced pressure were purified by column chromatography using silica gel (mesh 60-120) with 30% EtOAc in hexane as eluent, (Scheme **1**) (Table **1**).

2.1.2.1. 6-Chloro-2-(1-(3-fluorobenzoyl)piperidin-4-yl)-2,3-dihydroquinazolin-4(1H)-one (3a)

(0.11g 90% yield) White solid, Mp 209-211^oC; IR (KBr) ν_{max} 3676.08, 3294.5, 3194.25, 3077.05, 2874.56, 2951.54, 1666.84, 879.63 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.17 (s, 1H), 7.50-7.45 (m, 2H), 7.29-7.17 (m, 4H), 6.86 (s, 1H), 6.76- 6.74 (d, J= 8.8 Hz, 1H), 4.3 (m, 2H), 3.52 (m 1H), 2.97 (m, 1H), 2.65-2.64 (m, 1H), 1.86 (m, 1H), 1.71 (m, 1H), 1.55 (m, 1H), 1.36 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.52, 164.18, 163.63, 147.07, 140.86, 130.69, 129.55, 126.78, 122.53, 116.63, 115.08, 114.83,

Table 1. Piperidine conjugated 2,3-dihydroquinazolin-4(1*H*)-ones.

Entry	Product	Yield%
1	 3a	90
2	 3b	86
3	 3c	88
4	 3d	78

66.92, 46.58, 45.65, 22.11 ppm. HRMS (ESI): calcd for $[C_{20}H_{19}ClFN_3O_2+H^+]$: 388.1442, found 388.1445, (m+2) 390.1393 [36].

2.1.2.2. 6-chloro-2-(1-((4-nitrophenyl)sulfonyl)piperidin-4-yl)-2,3-dihydroquinazolin 4(1H)-one (3b)

(0.128g 86% yield) Light yellow solid Mp 212-214 ^oC; IR (KBr) ν_{max} 3293.5, 3195.23, 2874.48, 1666.68, 1079.66, 879.80 cm^{-1}. ^1H NMR (400 MHz, DMSOd_6): δ 8.41 (s, 1H), 8.14-8.12 (d, J= 8Hz, 2H), 7.97-7.95 (d, J= 8Hz, 2H), 7.82 (s, 1H), 7.43-7.41 (d, J=7.6 Hz, 1H), 7.22-7.20 (d, J=7.6Hz, 1H), 6.85 (s, 1H), 6.74 (s, 1H), 2.25 (m, 2H), 1.67-1.54 (m,2H), 1.37-1.24 (m, 4H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.17, 152.23, 147.42, 146.40, 129.60, 128.82, 126.42, 124.0, 121.7, 116.8, 115.7, 66.54, 46.84, 45.22, 22.61 ppm. HRMS (ESI): calcd for $[C_{19}H_{19}ClF_2N_4O_5S+H^+]$: 451.1031, found 451.1033, (m+2) 453.1042 [36].

2.1.2.3. 6-chloro-2-(1-(2,6-difluorobenzoyl)piperidin-4-yl)-2,3-dihydroquinazolin-4(1H)-one (3c)

(0.117g 88% yield) White solid, Mp 224-226 ^oC; IR (KBr) ν_{max} 3674.94, 3293.26, 2963.84, 2874.88, 1665.8, 1078.2, 879.6 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.19 (d, 1H), 7.53-7.48 (m, 1H), 7.46-7.45 (d, J= 2.4 Hz, 1H), 7.23-7.15 (m, 2H), 6.94 (s, 1H), 6.85 (s, 1H), 6.77-6.74 (dd, J=4.4 Hz, 1H), 4.58-4.48 (m, 2H), 3.41- 3.37 (m, 1H), 3.01-2.98 (m,1H), 2.71-2.68 (m, 1H), 1.85 (m, 1H), 1.73-1.71 (m, 1H), 1.63-1.60 (m,1H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ173.38, 164.06, 161.03, 159.82, 148.54, 130.92, 130.47, 126.73, 123.53, 116.57, 115.08, 66.85, 46.71, 45.60, 23.64 ppm; HRMS (ESI): calcd for $[C_{20}H_{18}ClF_2N_3O_2+H^+]$: 406.1315, found 406.1313, (m+2) 408.1275 [36].

2.1.2.4. 6-chloro-2-(1-(3,4-dichlorobenzyl)piperidin-4-yl)-2,3-dihydroquinazolin-4(1H)-one (3d)

(0.109g 78% yield) White solid, Mp 160-162 ^oC; IR (KBr) ν_{max} 3295.28, 3076.72, 1665.48, 1589.00, 890.2 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.09 (s,1H), 7.54-7.45 (m,3H), 7.25-7.19 (m, 2H), 6.81 (s, 1H), 6.77- 6.75 (d, J= 8.8 Hz, 1H), 4.51 (s, 1H), 3.04 (s, 2H), 2.80-2.78 (m, 2H), 1.86-1.83 (m, 2H), 1.88-1.80 (m, 2H), 1.39-1.33 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.85, 148.61, 136.24, 132.26, 130.25, 129.83, 129.60, 126.34, 123.74, 116.80, 115.46, 66.88, 64.74, 55.42, 44.63, 22.36 ppm. HRMS (ESI): calcd for $[C_{20}H_{20}Cl_3N_3O+H^+]$: 424.0922, found 424.0927, 426. 0872, 428.0865, 429.0925 [36].

2.2. Biology

2.2.1. Cell lines and Culture

The human colorectal carcinoma cells (HCT116) and colon adenocarcinoma (A549 cells) were used for the cytotoxic assessment of synthesized compounds. To test the anti-proliferation, we used MTT assay. Human cell lines HCT116 and A549 cells were bought from the National

Center for Cell Science, Pune, India. Cells were developed in DMEM accompanied with 10% heat-inactivated Fetal Bovine Serum (FBS), 100 U/mL of penicillin, and 100 mg of streptomycin/mL and incubated at 37°C in a humidified atmosphere comprising 5% CO_2.

2.2.2. MTT Assay

The cytotoxic effect of synthesized compounds against HCT116 and A549 cell lines was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromi de] dye uptake method as described previously [38]. Briefly, the cells ($2.5x10^4$/ml) were seeded and incubated in triplicate in a 96-well plate in the presence of different concentrations of test compounds for 72 h at 37°C. Thereafter, 20 µL MTT solution (5 mg/mL) was added and incubated for 2 h at 37°C. The lysis buffer (20% SDS-sodium dodecyl sulphate), 50% dimethylformamide) was added and incubation was continued for 4 to 5 hours. We measured the Optical Density (OD) at 570 nm using the Varioskan plate reader.

2.3. Docking Studies

We performed molecular modeling of compound (**3d**) using the open source software Auto Dock. We selected target protein from the collection of experimentally determined 3D-structures of biological macromolecules database PDB. The docking poses in Auto Dock are ranked according to their docking scores and the lowest binding energy of macromolecule-ligand complex is considered being the best. Finally, both the ranked list of docked ligand and their corresponding binding poses were analyzed. The docking study with VEGFR2 target using Auto Dock 4.2 proved H-bond interaction and strong binding affinity.

2.4. DFT Calculations

The computational calculations were performed using Gauss View molecular visualization program [39] and Gaussian 09 program package [40]. The plots of HOMO and LUMO of the compound **3d** were optimized using the theoretical approach. The density functional theory (DFT/B3LYP) method with the 6-31G (d) basis set was used.

3. RESULTS

3.1. Anti-proliferative Activity

The proliferation rate of HCT116 and A549 cells in the presence of a different concentration of compound **2a**, **3a-d** was tested at 48 h time point using MTT assay. Results showed the significant antiproliferative effect of piperidine conjugated dihydroquinazolinones derivatives on HCT116 and A549 cells. Importantly, compound **3d** showed significant antiproliferative action with an IC_{50} value of 7.56 µM and 13.56 µM on HCT116 and A549 cells respectively. We used paclitaxel as the positive control for *in vitro* cytotoxicity assay (Table **2**) (Supplementary Material).

Table 2. Antiproliferative activity of 2,3-dihydroquinazolinones

Compound	Name of the Cell Line (IC_{50} values µM)	
	HCT116	A549
2a	20.47	29.57
3a	19.59	28.67
3b	19.44	30.56
3c	16.78	26.45
3d	7.56	13.56

3.2. Molecular Docking Studies

Molecular modeling studies were performed to explore the binding mode of the most active compounds to the target enzymes. A Lamarckian genetic algorithm method [41] implemented in the program suite was employed to identify appropriate binding modes and confirmation of the ligand molecules [42]. The compound **3d** found to have a binding energy of -9.96 kJ/mol with VEGFR2 target (PDB Code: 2QU5) with ligand efficiency of -0.37 (Fig. **1**). In addition, compound **3d** was found to show hydrogen bond interaction with active site amino acid residues Cys 919 at a distance of 1.698 Å (Fig. **2**) (Table **3**).

3.3. HOMO-LUMO Analysis Compound 3d

The geometry of **3d** was optimized by DFT/B3LYP/6-31G(d) [39] basis set and is given in Fig. (**3**). For compound **3d**, HOMO confined mainly on quinazolinone ring with the calculated energy E_{HOMO}=6.0649 eV. Instead, LUMO was contributed by only benzo- and a carbonyl group in quinazolinone ring with energy E_{LUMO}=1.2553 eV. Thus, the energy gap was observed to be 4.8096 eV between HOMO and LUMO (Fig. **4**). Finally, the UV Visible spectrum was predicted by DFT calculations which exhibited maximum absorption of a wavelength at 160 and 167 nm with force constants f = 0.3576 and f = 1.4199 respectively (Fig. **5**).

4. DISCUSSION

Four dihydroquinazolinone analogues bearing piperidine were designed, and the prepared compounds were assessed

Table 3. The dock score results of the compound 3d.

Binding Energy (kJ mol^{-1})	Ligand Efficiency	Inhibition Constant	vdW+H-bond+ desolv energy	No. of H- bonds	Bonding Residues	Bond Lengh (Å)
-9.96	-0.37	49.88	-11.01	1	2QU5: A: CYS919:O	1.698

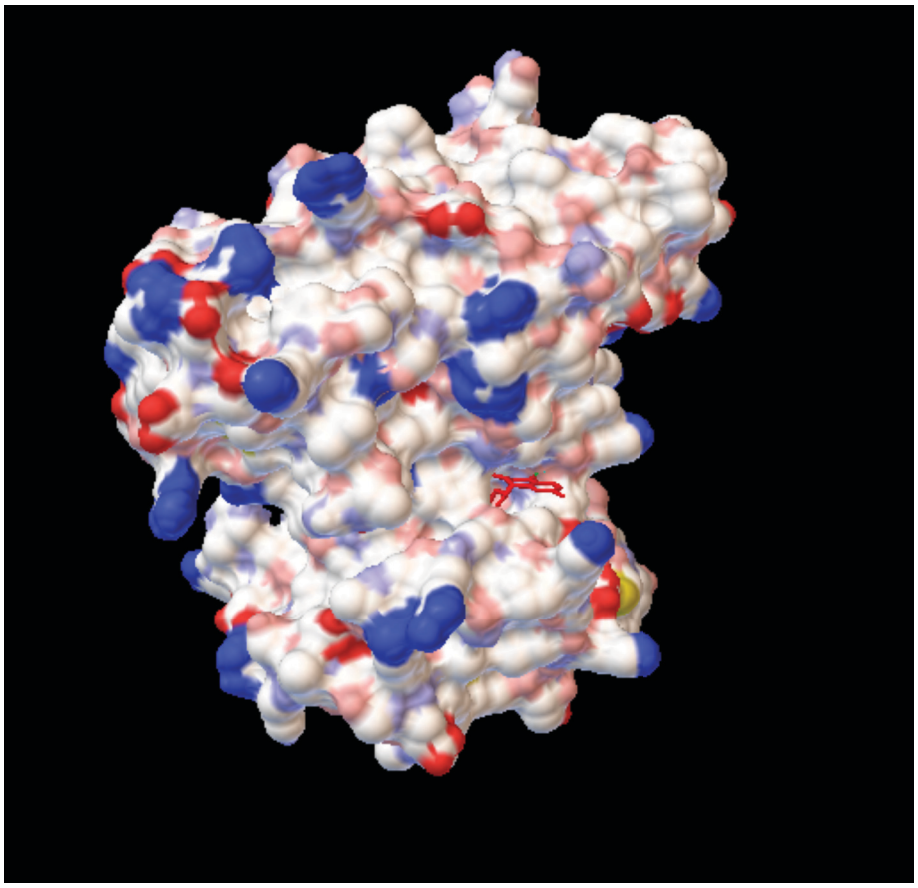


Fig. (1). Docking of molecule (**3d**) in the active site pocket of VEGFR2 target.

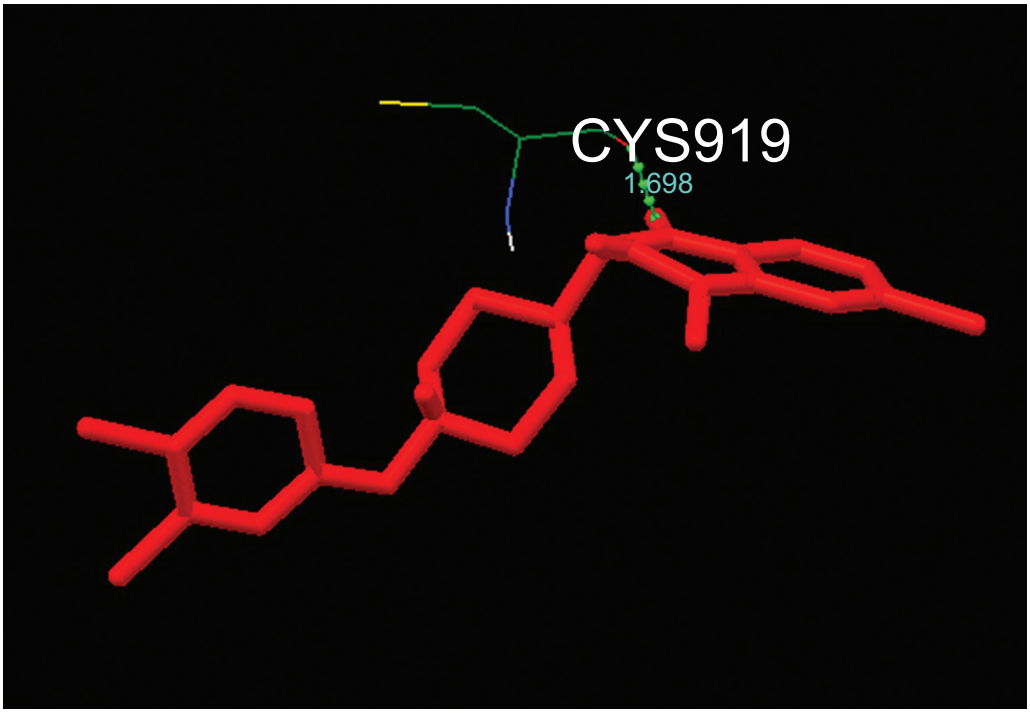


Fig. (2). Hydrogen bond interaction of the molecule (**3d**) in the active site pocket of VEGFR2.

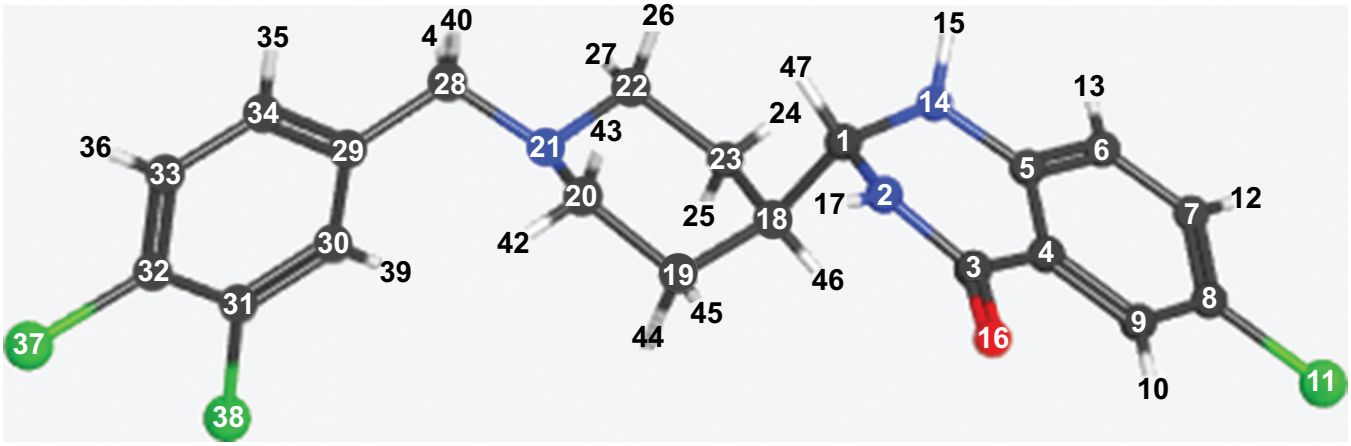


Fig. (3). Optimized geometry of **3d** calculated using DFT/B3LYP/6-31G(d) basis set.

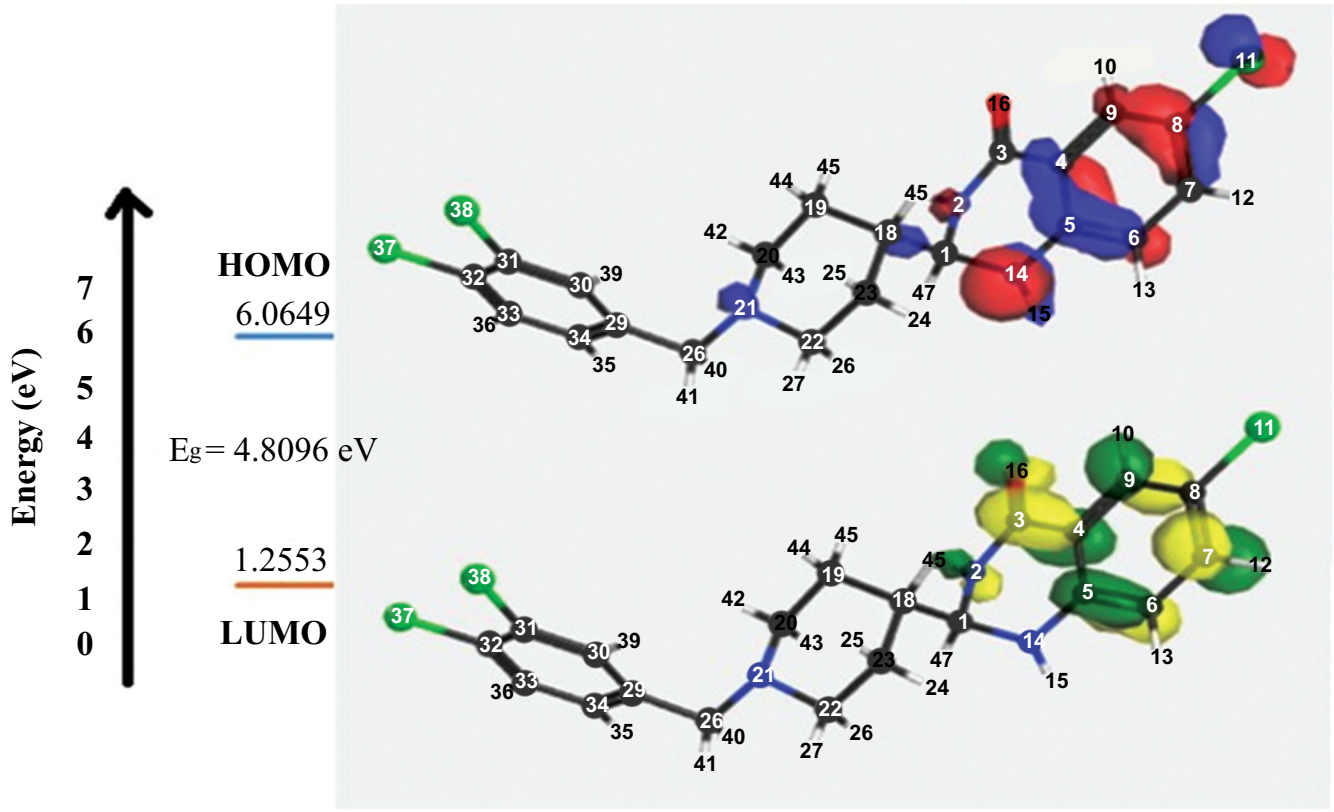


Fig. (4). HOMO, LUMO and energy gap between them in **3d.**

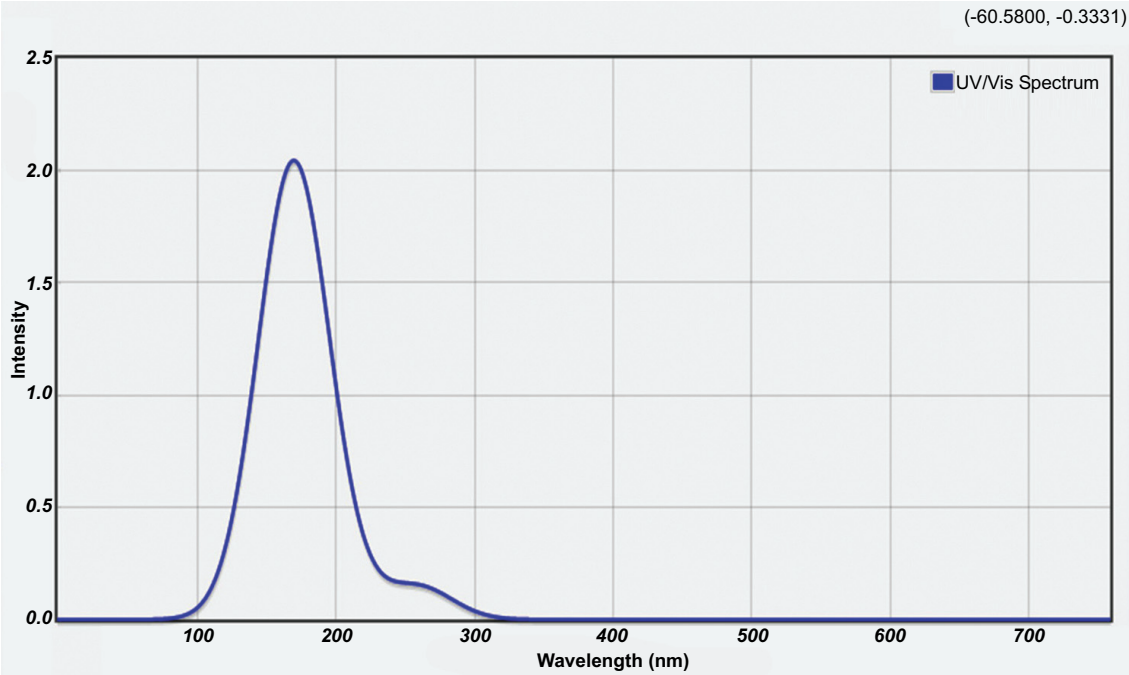


Fig. (5). Calculated UV Visible spectrum of **3d** using DFT.

against colorectal and colon cancer cell lines. Most compounds owned good anti-proliferative activities against HCT116 and A549 cells, compound **3d** showed a substantial inhibition. VEGFR2 receptor plays an important role in cardiovascular disorders and cancer angiogenesis. Therefore, the inhibition of VEGFR2 receptor has implications in cancer progression inhibition. In the present study, we attempted to find new VEGFR2 receptor modulators using selective antagonist. The best-fit docked conformations of these compounds **3a-d** were further selected for the estimation of binding free energy. Binding free energy calculations inferred that the compound **3d**, displayed the binding energy of -9.96 kJ/mol, respectively. Compound **3d** maintained a crucial interaction with CYS919 and therefore, exhibited good binding affinity. The molecular orbital analyses show the distribution of HOMO and LUMO molecular orbitals over the atoms of the molecule. The plots of HOMO and LUMO of the potent compound **3d** were fully optimized using the theoretical approach.

CONCLUSION

The title compounds **3a-d** were prepared and analyzed by spectral methods. We used cytotoxicity analyses for initial investigation of piperidine-conjugated dihydroquinazolinones versus HCT116 and A549. Among all the screened derivatives, compound **3d** was found to be the most potent against HCT116 and A549 at lower concentrations. Molecular docking studies to analyze hydrogen bond interaction of the molecule with an active site and the DFT calculations to optimize the structure of the compound **3d** were conducted.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the manuscript and its supplementary material.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

[1] **(a)** Ferrando, C.; Foy, J.M.; Pratt, C.N.F.W.; Purvis, J.R. On the pharmacological actions of a diuretic, fenquizone, with particular reference to its site of action. *J. Pharm. Pharmacol.,* **1981**, *33*(4), 219-222. [http://dx.doi.org/10.1111/j.2042-7158.1981.tb13761.x] [PMID: 6115903] **(b)** Wang, G-J.; Wu, X-C.; Chen, C-F.; Lin, L-C.; Huang, Y-T.; Shan, J.; Pang, P.K.T. Vasorelaxing action of rutaecarpine: Effects of rutaecarpine on calcium channel activities in vascular endothelial and smooth muscle cells. *J. Pharmacol. Exp. Ther.,* **1999**, *289*(3), 1237-1244. [PMID: 10336511]

[2] Shi, C-S.; Li, J-M.; Chin, C-C.; Kuo, Y-H.; Lee, Y-R.; Huang, Y-C. Evodiamine induces cell growth arrest, apoptosis and suppresses tumorigenesis in human urothelial cell carcinoma cells. *Anticancer Res.,* **2017**, *37*(3), 1149-1159. [http://dx.doi.org/10.21873/anticanres.11428] [PMID: 28314276]

[3] **(a)** Wang, Z-X.; Xiang, J-C.; Wang, M.; Ma, J-T.; Wu, Y-D.; Wu, A-X. One-pot total synthesis of evodiamine and its analogues through a continuous biscyclization reaction. *Org. Lett.,* **2018**, *20*(20), 6380-6383. [http://dx.doi.org/10.1021/acs.orglett.8b02667] [PMID: 30284836] **(b)** Rueping, M.; Antonchick, A.P.; Sugiono, E.; Grenader, K. Asymmetric Brønsted acid catalysis: Catalytic enantioselective synthesis of highly biologically active dihydroquinazolinones. *Angew. Chem. Int. Ed. Engl.,* **2009**, *48*(5), 908-910. [http://dx.doi.org/10.1002/anie.200804770] [PMID: 19101974] **(c)** Narasimhamurthy, K.H.; Chandrappa, S.; Sharath Kumar, K.S.; Harsha, K.B.; Ananda, H.; Rangappa, K.S. Easy access for the synthesis of 2-aryl 2,3-dihydroquinazolin-4(1H)-ones using gem-dibromomethylarenes as synthetic aldehyde equivalent. *RSC Advances,* **2014**, *4*(65), 34479-34486. [http://dx.doi.org/10.1039/C4RA02312A]

[4] Rajaka, L.; Penumati, N.; Nagaiah, K. Y, P.; Kumar C, G., Convenient and scalable synthesis of 2,3-dihydroquinazoli4(1H)-one derivatives and their anticancer activities. *Synth. Commun.,* **2015**, *45*(15), 1893-1901 [http://dx.doi.org/10.1080/00397911.2015.1046555]

[5] Yale, H.L.; Kalkstein, M. Substituted 2,3-Dihydro-4(1H)-quinazolinones. A new class of inhibitors of cell multiplication. *J. Med. Chem.,* **1967**, *10*(3), 334-336. [http://dx.doi.org/10.1021/jm00315a010] [PMID: 22185126]

[6] Bonola, G.; Da Re, P.; Magistretti, M.J.; Massarani, E.; Setnikar, I. 1-aminoacyl-2,3-dihydro-4(1H)-quinazolinone derivatives with choleretic and antifibrillatory activity. *J. Med. Chem.,* **1968**, *11*(6), 1136-1139. [http://dx.doi.org/10.1021/jm00312a007] [PMID: 5680025]

[7] Khalil, M.A.; Soliman, R.; Farghaly, A.M.; Bekhit, A.A. Non-steroidal anti-inflammatory agents: Novel pyrazolyl-, 1,2-oxazolyl-, and 1,3-diazinyl derivatives of 4(3H)-quinazolinones. *Arch. Pharm. (Weinheim),* **1994**, *327*(1), 27-30. [http://dx.doi.org/10.1002/ardp.19943270105] [PMID: 8117186]

[8] Baldazzi, C.; Barbanti, M.; Basaglia, R.; Benelli, A.; Bertolini, A.; Piani, S. A new series of 6-chloro-2,3-dihydro-4(1H)-quinazolinone derivatives as antiemetic and gastrointestinal motility enhancing agents. *Arzneimittelforschung,* **1996**, *46*(9), 911-918. [PMID: 8876941]

[9] Birch, H.L.; Buckley, G.M.; Davies, N.; Dyke, H.J.; Frost, E.J.; Gilbert, P.J.; Hannah, D.R.; Haughan, A.F.; Madigan, M.J.; Morgan, T.; Pitt, W.R.; Ratcliffe, A.J.; Ray, N.C.; Richard, M.D.; Sharpe, A.; Taylor, A.J.; Whitworth, J.M.; Williams, S.C. Novel 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1H-quinazolin-4-ones as IMPDH inhibitors. *Bioorg. Med. Chem. Lett.,* **2005**, *15*(23), 5335-5339. [http://dx.doi.org/10.1016/j.bmcl.2005.06.108] [PMID: 16202581]

[10] Büyüktimkin, S.; Buschauer, A.; Schunack, W. Quinazolinones. 18. Synthesis and H1/H2-antihistaminic action of omega-[2-aryl-2,3-dihydro-4(1H)-quinazolinone-1-yl]alkyl substituted ureas and cyanoguanidines. *Arch. Pharm. (Weinheim),* **1991**, *324*(5), 291-295. [http://dx.doi.org/10.1002/ardp.19913240507] [PMID: 1679625]

[11] Iwashita, A.; Tojo, N.; Matsuura, S.; Yamazaki, S.; Kamijo, K.; Ishida, J.; Yamamoto, H.; Hattori, K.; Matsuoka, N.; Mutoh, S. A novel and potent poly(ADP-ribose) polymerase-1 inhibitor, FR247304 (5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone), attenuates neuronal damage in *in vitro* and *in vivo* models of cerebral ischemia. *J. Pharmacol. Exp. Ther.,* **2004**, *310*(2), 425-436. [http://dx.doi.org/10.1124/jpet.104.066944] [PMID: 15075382]

[12] (a) Roopan, S.M.; Nawaz Khan, F.; Jin, J.S.; Senthil Kumar, R. An efficient one pot–three component cyclocondensation in the synthesis of 2-(2-chloroquinolin-3-yl)-2,3-dihydroquinazolin-4(1H)-ones: Potential antitumor agents. *Res. Chem. Intermed.,* **2011**, *37*(8), 919-927. [http://dx.doi.org/10.1007/s11164-011-0301-3] (b) Kamal, A.; Bharathi, E.V.; Reddy, J.S.; Ramaiah, M.J.; Dastagiri, D.; Reddy, M.K.; Viswanath, A.; Reddy, T.L.; Shaik, T.B.; Pushpavalli, S.N.C.V.L.; Bhadra, M.P. Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids as anticancer agents. *Eur. J. Med. Chem.,* **2011**, *46*(2), 691-703. [http://dx.doi.org/10.1016/j.ejmech.2010.12.004] [PMID: 21194809]

[13] Narasimhamurthy, K.H.; Ananda, H.; Sharath, K.S.; Rangappa, K.S. Dihydroquinazolinones as potential antiproliferative and tumor inhibiting agents. *OMCIJ,* **2016**, *1*(2)555560

[14] Hunt, J.A.; Kallashi, F.; Ruzek, R.D.; Sinclair, P.J.; Ita, I.; McCormick, S.X.; Pivnichny, J.V.; Hop, C.E.C.A.; Kumar, S.; Wang, Z.; O'Keefe, S.J.; O'Neill, E.A.; Porter, G.; Thompson, J.E.; Woods, A.; Zaller, D.M.; Doherty, J.B. p38 Inhibitors: Piperidine- and 4-aminopiperidine-substituted naphthyridinones, quinolinones, and dihydroquinazolinones. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(3), 467-470. [http://dx.doi.org/10.1016/S0960-894X(02)00990-3] [PMID: 12565952]

[15] Singh, L.R.; Kumar, A.; Upadhyay, A.; Gupta, S.; Palanati, G.R.; Sikka, K.; Siddiqi, M.I.; Yadav, P.N.; Sashidhara, K.V. Discovery of coumarin-dihydroquinazolinone analogs as niacin receptor 1 agonist with in-vivo anti-obesity efficacy. *Eur. J. Med. Chem.,* **2018**, *152*(25), 208-222. [http://dx.doi.org/10.1016/j.ejmech.2018.04.037] [PMID: 29709786]

[16] Selvakumar, B.P.; Elango, K. Synthesis, characterization and *in vitro* antibacterial evaluation of 1-(7,7-dimethyl-2-morpholino-5,6,7,8-tetrahydroquinazolin-4-yl)piperidine-4-carboxamide derivatives. *Res. Chem. Intermed.,* **2017**, *43*, 5535-5546. [http://dx.doi.org/10.1007/s11164-017-2945-0]

[17] Jothieswari, D.; Reddy, K.B. Antioxidant activity of some newer dihydro quinazolin-4-one derivatives. *IJNTPS,* **2015**, *5*(5), 162-171.

[18] Piotrowska, D.G.; Andrei, G.; Schols, D.; Snoeck, R.; Grabkowska-Drużyc, M. New isoxazolidine-conjugates of quinazolinones-synthesis, antiviral and cytostatic activity. *Molecules,* **2016**, *21*(7), 959. [http://dx.doi.org/10.3390/molecules21070959] [PMID: 27455228]

[19] Ismail, M.A.H.; Barker, S.; Abou el-Ella, D.A.; Abouzid, K.A.M.; Toubar, R.A.; Todd, M.H. Design and synthesis of new tetrazolyl- and carboxy-biphenylylmethyl-quinazolin-4-one derivatives as angiotensin II AT1 receptor antagonists. *J. Med. Chem.,* **2006**, *49*(5), 1526-1535. [http://dx.doi.org/10.1021/jm050232e] [PMID: 16509571]

[20] Iqbal, J.; Saeed, A.; Shah, S. J.; al-Rashida, M.; Shams-ul, M. Biological evaluation of azomethine-dihydroquinazolinone conjugates as cancer and cholinesterase inhibitors. *Med. Chem.,* **2016**, *12*(1), 74-82.

[21] Basagiannis, D.; Christoforidis, S. Constitutive endocytosis of VEGFR2 protects the receptor against shedding. *J. Biol. Chem.,* **2016**, *291*(32), 16892-16903. [http://dx.doi.org/10.1074/jbc.M116.730309] [PMID: 27298320]

[22] Domingues, I.; Rino, J.; Demmers, J.A.A.; de Lanerolle, P.; Santos, S.C.R. VEGFR2 translocates to the nucleus to regulate its own transcription. *PLoS One,* **2011**, *6*(9)e25668 [http://dx.doi.org/10.1371/journal.pone.0025668] [PMID: 21980525]

[23] Tugues, S.; Koch, S.; Gualandi, L.; Li, X.; Claesson-Welsh, L. Vascular endothelial growth factors and receptors: Anti-angiogenic therapy in the treatment of cancer. *Mol. Aspects Med.,* **2011**, *32*(2), 88-111. [http://dx.doi.org/10.1016/j.mam.2011.04.004] [PMID: 21565214]

[24] Holmes, K.; Roberts, O.L.; Thomas, A.M.; Cross, M.J. Vascular endothelial growth factor receptor-2: Structure, function, intracellular signalling and therapeutic inhibition. *Cell. Signal.,* **2007**, *19*(10), 2003-2012. [http://dx.doi.org/10.1016/j.cellsig.2007.05.013] [PMID: 17658244]

[25] Gotink, K.J.; Verheul, H.M. Anti-angiogenic tyrosine kinase inhibitors: What is their mechanism of action? *Angiogenesis,* **2010**, *13*(1), 1-14. [http://dx.doi.org/10.1007/s10456-009-9160-6] [PMID: 20012482]

[26] Bang, Y.J.; Kang, Y.K.; Kang, W.K.; Boku, N.; Chung, H.C.; Chen, J.S.; Doi, T.; Sun, Y.; Shen, L.; Qin, S.; Ng, W.T.; Tursi, J.M.; Lechuga, M.J.; Lu, D.R.; Ruiz-Garcia, A.; Sobrero, A. Phase II study of sunitinib as second-line treatment for advanced gastric cancer. *Invest. New Drugs,* **2011**, *29*(6), 1449-1458.

[http://dx.doi.org/10.1007/s10637-010-9438-y] [PMID: 20461441]

[27] Aziz, M.A.; Serya, R.A.T.; Lasheen, D.S.; Abdel-Aziz, A.K.; Esmat, A.; Mansour, A.M.; Singab, A.N.B.; Abouzid, K.A.M. Discovery of potent VEGFR-2 inhibitors based on furopyrimidine and thienopyrimidne scaffolds as cancer targeting agents. *Sci. Rep.,* **2016**, *6*, 24460. [http://dx.doi.org/10.1038/srep24460] [PMID: 27080011]

[28] Li, H.; Ha, H.L.; Ding, X.; Bae, C.; Gazy, N.; Hao, J.; Zhong, L. DMH4, a VEGFR2 inhibitor, effectively suppresses growth and invasion of lung cancer cells. *J. Appl. Biomed.,* **2018**, *16*(1), 46-50. [http://dx.doi.org/10.1016/j.jab.2017.10.006]

[29] Zhang, L.; Shan, Y.; Ji, X.; Zhu, M.; Li, C.; Sun, Y.; Si, R.; Pan, X.; Wang, J.; Ma, W.; Dai, B.; Wang, B.; Zhang, J. Discovery and evaluation of triple inhibitors of VEGFR-2, TIE-2 and EphB4 as anti-angiogenic and anti-cancer agents. *Oncotarget,* **2017**, *8*(62), 104745-104760. [http://dx.doi.org/10.18632/oncotarget.20065] [PMID: 29285210]

[30] Yu, Y.; Yu, J.; Pei, C.G.; Li, Y.Y.; Tu, P.; Gao, G.P.; Shao, Y. Xanthatin, a novel potent inhibitor of VEGFR2 signaling, inhibits angiogenesis and tumor growth in breast cancer cells. *Int. J. Clin. Exp. Pathol.,* **2015**, *8*(9), 10355-10364. [PMID: 26617743]

[31] Zhang, S.; Cao, Z.; Tian, H.; Shen, G.; Ma, Y.; Xie, H.; Liu, Y.; Zhao, C.; Deng, S.; Yang, Y.; Zheng, R.; Li, W.; Zhang, N.; Liu, S.; Wang, W.; Dai, L.; Shi, S.; Cheng, L.; Pan, Y.; Feng, S.; Zhao, X.; Deng, H.; Yang, S.; Wei, Y. SKLB1002, a novel potent inhibitor of VEGF receptor 2 signaling, inhibits angiogenesis and tumor growth *in vivo*. *Clin. Cancer Res.,* **2011**, *17*(13), 4439-4450. [http://dx.doi.org/10.1158/1078-0432.CCR-10-3109] [PMID: 21622720]

[32] Bold, G.; Schnell, C.; Furet, P.; McSheehy, P.; Brüggen, J.; Mestan, J.; Manley, P.W.; Drückes, P.; Burglin, M.; Dürler, U.; Loretan, J.; Reuter, R.; Wartmann, M.; Theuer, A.; Bauer-Probst, B.; Martiny-Baron, G.; Allegrini, P.; Goepfert, A.; Wood, J.; Littlewood-Evans, A. A novel potent oral series of vegfr2 inhibitors abrogate tumor growth by inhibiting angiogenesis. *J. Med. Chem.,* **2016**, *59*(1), 132-146. [http://dx.doi.org/10.1021/acs.jmedchem.5b01582] [PMID: 26629594]

[33] Chandrappa, S.; Kavitha, C.V.; Shahabuddin, M.S.; Vinaya, K.; Ananda Kumar, C.S.; Ranganatha, S.R.; Raghavan, S.C.; Rangappa, K.S. Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid derivatives and evaluation of their cytotoxicity and induction of apoptosis in human leukemia cells. *Bioorg. Med. Chem.,* **2009**, *17*(6), 2576-2584. [http://dx.doi.org/10.1016/j.bmc.2009.01.016] [PMID: 19243955]

[34] Raghavendra, G.M.; Nandeesha, K.N.; Sandhya, N.C.; Rangappa, K.S.; Mantelingu, K. Transition metal free one-pot synthesis of quinazolinones and their alkaloids. *Int. J. Chem.,* **2014**, *35*(2), 1634-1642.

[35] Han, G.; Tamaki, M.; Hruby, V.J. Fast, efficient and selective deprotection of the tert-butoxycarbonyl (Boc) group using HCl/dioxane (4 m). *J. Pept. Res.,* **2001**, *58*(4), 338-341. [http://dx.doi.org/10.1034/j.1399-3011.2001.00935.x] [PMID: 11606219]

[36] Narasimhamurthy, K.H. Novel approach for the synthesis of nitrogen containing heterocycles and their biological studies. *Ph.D Thesis, University of Mysore,* **2015**.

[37] Balaraman, S.; Vaidyanathan, S.P.; Madhuri S.; Kuppanagounder, P.E.; Synthesis and antiviral activity of 4-(7,7-dimethyl-4-[4-{N-aroyl/benzyl}1-piperazinyl]-5,6,7,8-tetrahydroquinazolin-2-yl)morpholine derivatives. *ARKIVOC,* **2017**, *iv*, 353-364. [http://dx.doi.org/10.24820/ark.5550190.p010.037]

[38] Sharath, K.S.; Hanumappa, A.; Hegde, M.; Narasimhamurthy, K.H.; Raghavan, S.C.; Rangappa, K.S. Synthesis and antiproliferative effect of novel 4-thiazolidinone-, pyridine- and piperazine-based conjugates on human leukemic cells. *Eur. J. Med. Chem.,* **2014**, *81*(0), 341-349. [http://dx.doi.org/10.1016/j.ejmech.2014.05.009] [PMID: 24852281]

[39] Dennington, R., Keith, T. and Millam, J. (**2009**) Gauss View, Version 5. Semichem Inc., Shawnee Mission.

[40] Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H.P.; Izmaylov, A.F.; Bloino, J.; Zheng, G.; Sonnenberg, J.L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J.A. Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; J. E.; Knox, Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; and Fox, D. J., (**2010**) Gaussian 09, Revision B.01. Gaussian Inc., Wallingford.

[41] Jones, G.; Willett, P.; Glen, R.C.; Leach, A.R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.,* **1997**, *267*(3), 727-748. [http://dx.doi.org/10.1006/jmbi.1996.0897] [PMID: 9126849]

[42] Tripathi, A.; Bankaitis, V.A. Molecular docking: From lock and key to combination lock. *J Mol Med Clin Appl,* **2017**, *2*(1). [http://dx.doi.org/10.16966/2575-0305.106] [PMID: 29333532]