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Targeting VEGF to design pyrimidines against breast cancer and diabetic retinopathy

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Abstract

A library of 900 pyrimidine derivatives was screened virtually on vascular endothelial growth factor (VEGF) using Vlife MDS 4.1 software to identify potential candidates with anticancer and anticataract activity. A series of 2,4,6-substituted pyrimidine derivatives were synthesized in good yields from chalcones, where chalcones have been prepared according to claisen - schimidt condensation by condensing various ketones with aromatic aldehyde in presence of ethanol and sodium or potassium hydroxide. Their structures were confirmed by IR, ^{1}H NMR and mass spectra. Biological screening of the potential candidates was done for anticancer and anticataract activity. The present study reveals that a derivative of pyrimidine shows activity against breast cancer and diabetic retinopathy through inhibition of VEGF.

Keywords: *Pyrimidines; VEGF inhibitor; Molecular docking; Anticancer, anticataract activity.*

1.Introduction

Cancer is a group of disease in which there is uncontrolled multiplication and spread within the body of the body's own cells [1]. If the spread is not controlled, it can result in death. Worldwide, one in seven deaths is due to cancer; cancer causes more deaths than AIDS, tuberculosis, and malaria combined [2]. Diabetes is justly recognized as an emerging global epidemic, representing one of the leading causes of morbidity and mortality worldwide. Hyperglycemia, the common characteristic of both type 1 diabetes mellitus and type 2 diabetes mellitus, has the potential to cause serious complications due to its insidious and chronic nature [3]. Diabetes is one of the most common causes of blindness among the people of working groups. It causes cataract, glaucoma and damage of the blood vessels inside the eye, such condition is called "Diabetic Retinopathy'. Diabetic retinopathy is an acute retinal disorder which causes manifestation of diabetes on the retina. About 210 million people all over the world have diabetes mellitus; among which 10-18% of people are suffering from diabetic retinopathy. So for the prevention of diabetic retinopathy and gradual vision loss, early detection and diagnosis of diabetic retinopathy is required [4].

In case of cancer as well as diabetic retinopathy, vascular endothelial growth factor (VEGF) plays important role. VEGF plays key role in stimulating the process cancer cell use to create new blood vessel known as a angiogenesis. Angiogenesis is the formation of new blood vessels, which is essential for the development, progression, and metastasis of malignant tumors [5]. In the absence of angiogenesis, tumors cannot grow beyond 1–2 mm in size [6]. VEGF is a primary stimulus of angiogenesis in tumors and functions through binding VEGFR1 and VEGFR2 expressed on endothelial cells [7]. VEGF contribute to the development of DME by affecting the tight junction of endothelial cell in the retina by increasing the leukocyte count, these leukocytes are known to cause leakage, and this cause the breakdown of blood retinal barrier and the accumulation of fluid in the macula and that resulted into the distorted vision. The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking. Molecular docking is an efficient tool for investigating receptor-ligand interactions and for virtual screening, which plays a key role in rational drug design, especially when the crystal structure of a receptor or enzyme is available [8]. The 2, 4, 6-substituted pyrimidines have been reported to have powerful activities such as anticancer [9], antiinflammatory [10], analgesic [11], antihistaminic [12], antimalarial [13], antimicrobial [14], and antioxidant [15]. In the present work, we propose to design and synthesize a series of 2, 4, 6- substituted pyrimidine derivatives against breast cancer and diabetic retinopathy through inhibition of VEGF.

2. Materials and methods

2.1. Docking studies [16]

The library of 900 pyrimidine analogues were virtually screened by batch docking on VEGF receptor using VLife MDS 4.1.The crystal structure (Figure 1) of VEGF (PDB CODE- 2vpf) was obtained from protein data bank, opened in MDS sheet, saved by removing water molecule and used further for docking purpose. The 2D structures of the compounds were built and then converted into the 3D with the help of VLife MDS 4.1 software. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using Merck Molecular Force Field (MMFF).

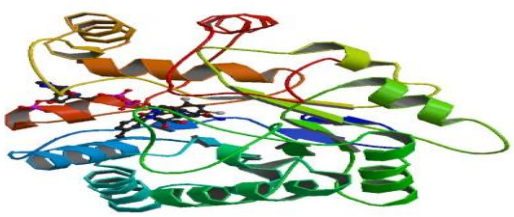


Figure 1. structure of receptor VEGF (pdb code: 2vpf).

By using cavity determination option of software, cavities of enzyme were determined. The cavities in the receptor were mapped to assign an appropriate active site, the basic feature used to map the cavities was the surface mapping of the receptor and identifying the geometric voids as well as scaling the void for its hydrophobic characteristics. Hence, all the cavities that are present in receptor are identified and ranked based on their size and hydrophobic surface area. Cavity no. 1 is selected for docking. The active site for docking was defined as all atoms within 5 A ° radius. Using biopredicta tool of software, open docking and then batch docking was performed. Batch docking shows browsing of receptor, ligand (molecule), and the result generated was saved in output file. Molecules saved in output file as a docked ligand format with proper conformation and further used to check binding interactions. Results generated were saved as log file in output folder. For checking binding interaction, first receptor structure was opened in MDS followed by compound which was saved as ligand dock file. From tool option clicked on merge molecule so that compound and receptor is merged together. From biopredicta tool selected ligand and receptor structure to check their interactions. Melting points were determined in open capillaries and are uncorrected. All compounds were characterized by IR, ^{1}H NMR and mass spectra. The IR spectra were recorded on a JASCO FT-IR 4100 spectrometer, using KBr discs. The ^{1}H NMR spectra were obtained on a Varian-NMR-mercury 300 spectrometer in DMSO-d6 as a solvent and TMS as internal standard, chemical shifts are given in ppm, where mass spectroscopy done by HR-MS.

2.2. General procedure for the synthesis of compounds (RBK-1to RBK-16) [17]

Step I: Synthesis of chalcones

Chalcones were synthesized by reaction of appropriately substituted acetophenones and aldehydes. A mixture of aldehyde derivatives (0.01 mol) and acetophenone derivatives (0.01 mol) was dissolved in 30 ml ethanol in a 250 ml round-bottomed flask equipped with a magnetic stirrer. Then 10 ml NaOH solution (1g in 10 ml H_2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution became turbid. The reaction temperature was maintained between 20-25˚C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 h the reaction mixture was neutralized by 0.1 - 0.2 N hydrochloric acid where the precipitation occurred. On filtering the crude chalcones were dried in air and recrystallized by rectified ethanol.

Step II: Synthesis of pyrimidine derivatives

A mixture (0.01 mol) of chalcone derivative and guanidine nitrate, urea or thiourea in alkaline medium in potassium hydroxide (0.003 mol) in the presence of ethanol (10 ml). The entire reaction mixture was microwave irradiated at 180 watts for 2-16 minutes and then kept aside for 2-3 h to ontain pyrimidine derivatives. The completion of reaction was monitored by thin layer chromatography.

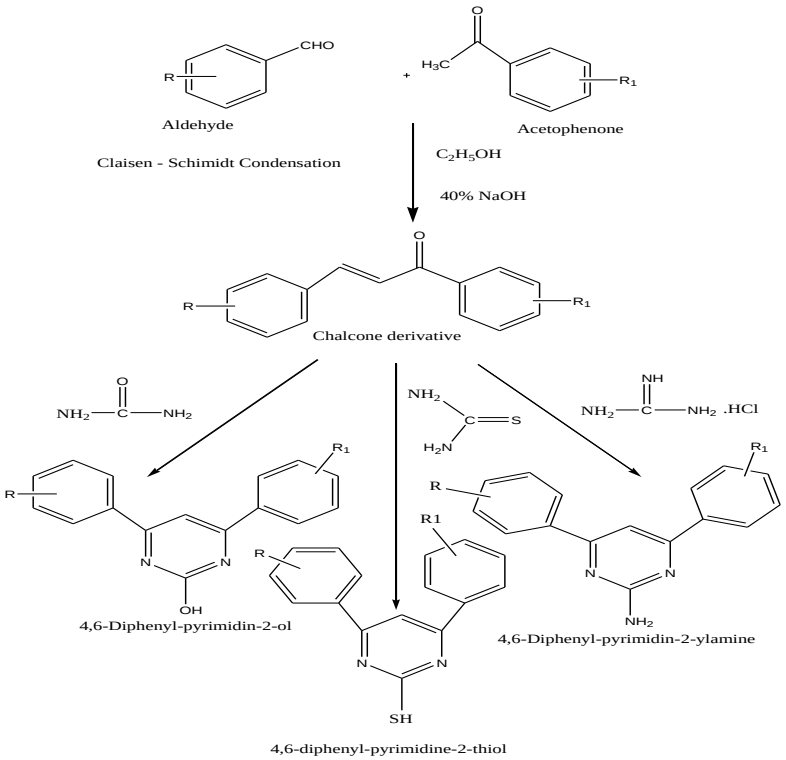


Figure 2. scheme of synthesis.

4-(4-Chloro-phenyl)-6-phenyl-pyrimidine-2-thiol (RBK-1)

Melting point: 114-116^0 C; Yield: 62%; IR: 3130 (CH stretch aromatic), 2273.78 (C-SH), 1606.70 (C=C); NMR: 7.46 (m, 4H, aromatic), 7.46 (s, 1H, CH pyrimidine), 7.32 (m, 5H, phenyl), 3.0 (s, 1H, SH); Mass: 298.03 (100%), 300.03 (36.5%), 299.04 (18%)

4,6-Bis-(2-hydroxy-phenyl)-pyrimidine-2-thiol (RBK-2)

Melting point: 136-137^0 C; Yield: 64%; IR : 3150.43 (CH stretch aromatic), 2314.35 (C-SH), 1634.67 (C=C); NMR: 7.05 (m, 8H, CH, benzene), 6.67 (s, 1H, CH pyrimidine), 5.00 (s, 2H, OH benzene), 3.0 (s, 1H, SH, pyrimidine); Mass: 296.06 (100%), 297.07 (18.1%), 298.06 (4.7%)

4-Methyl-6-(3-nitro-phenyl)-pyrimidine-2-thiol (RBK-3)

Melting point: 148-150^0 C; Yield: 78% ; IR:3042.79 (CH stretch aromatic), 2850.79 (CH stretch alkyl), 2354.11 (C-SH), 1617.76 (C=C), 1322.54 (NO_2 stretch); NMR: 8.41 (s, 1H, OH, benzene), 7.87 (d, 3H, CH, benzene), 7.16 (s, 1H, CH, pyrimidine), 3.0 (s, 1H, SH, pyrimidine); Mass: 247.04 (100%), 248.04 (14.1%), 249.04 (4,7%)

4-Methyl-6-styryl-pyrimidine-2-ylamine (RBK-4)

Melting point: 138-139^0 C; Yield: 81%; IR: 3324.11 (NH), 3112.43 (CH stretch aromatic), 2876.00 (CH stretch alkyl), 1626.13 (C=C); NMR: 7.21 (m, 5H, CH, benzene), 6.99 (s, 2H, CH, ethylene), 6.54 (s, 1H, CH, pyrimidine), 4.0 (s, 1H, SH, pyrimidine), 2.35 (s, 3H, CH); Mass: 211.11 (100%), 212.11 (15.6%)

4-(2-Hydroxy-phenyl)-6-phenyl-pyrimidine-2-ol (RBK-5)

Melting point: 128-130^0 C; Yield: 73%**;** IR: 3414.98 (0H), 3182.66 (CH stretch aromatic), 1626.13 (C=C); NMR: 7.33 (d, 4H, CH, benzene), 7.16 (m, 5H, CH, benzene), 6.61 (s, 1H, OH, benzene), 5.0 (s, 1H, OH, pyrimidine); Mass: 264.09 (100%), 265.09 (18.6%)

4-(4-Chloro-phenyl)-6-methyl-pyrimidine-2-ol (RBK-6)

Melting point: 154-156^0 C; Yield: 71% ; IR: 3324.59 (OH), 3222.19 (CH stretch aromatic), 2906.15 (CH stretch alkyl), 1643.44 (C=C), 764.00 (C-Cl); NMR: 7.33 (d, 4H, CH, benzene), 6.37 (s, 1H, CH,pyrimidine), 5.0 (s, 1H, OH, pyrimidine), 2.35 (s, 3H, CH); Mass: 220.04 (100%), 222.04 (18.6%), 221.04 (13%)

4-(4-Bromo-phenyl)-6-(4-Chloro-phenyl)-pyrimidine-2-ylamine (RBK-7)

Melting point: 162-164^0 C; Yield: 64% ; IR: 3224.59 (OH), 3177.62 (CH stretch aromatic), 1611.15 (C=C), 764.00 (C-Cl), 683.29(C-Br); NMR: 7.37 (d, 4H, CH, benzene), 7.33 (d, 4H, CH, benzene), 6.90 (s, 1H, CH pyrimidine), 4.0 (s, 2H, NH_2, pyrimidine); Mass: 360.98 (100%), 359.98 (77.2%), 362.98 (24.2%)

4-(4-Bromo-phenyl)-6-(2-hydroxy-phenyl)-pyrimidine-2-ol (RBK-8)

Melting point: 146-148^{0}C ; Yield: 71% ; IR: 3244.59 (OH), 3162.00 (CH stretch aromatic), 1633.33 (C=C), 699.29 (C-Br); NMR: 7.37 (d, 4H, CH benzene), 7.05 (m, 4H, CH, benzene), 5.0 (s, 1H, OH, pyrimidine), 5.0 (s, 1H, OH, benzene),; Mass: 343.09 (100%), 344.09 (15.6%)

4-Phenyl-6-styryl-pyrimidine-2-thiol (RBK-9)

Melting point: 136-138^0 C; Yield: 53%; IR: 3323.17 (OH), 3162.00 (CH stretch aromatic), 1633.33 (C=C); NMR: 7.41 (s, 1H, CH pyrimidine), 7.32 (m, 5H, CH, benzene), 7.23 (m, 5H, CH, benzene), 6.99 (s, 2H, CH, ethylene), 3.0 (s, 1H, SH, pyrimidine); Mass: 290.09 (100%), 291.09 (21%), 292.08 (4.4%)

2-(2-Amino-6-(3,4-dimethoxy-phenyl)-pyrimidine-2-yl)-phenol (RBK-10)

Melting point: 132-134^0 C; Yield: 47%; IR: 3344.59 (OH), 3324.11 (NH), 3282.15 (CH stretch aromatic), 1613.01 (C=C); NMR: 7.05 (m, 4H, CH, benzene), 6.90 (s, 1H, CH, pyrimidine), 6.88 (s, 1H, CH, benzene), 6.72 (d, 2H, CH, benzene), 5.00 (s, 1H, OH, benzene), 4.0 (s, 1H, SH, pyrimidine), 3.73 (s, 2H, CH, benzene); Mass: 323.13 (100%), 324.13 (20.4%), 325.13 (2.7%)

4-(4-Bromo-phenyl)-6-(3-nitro-phenyl)-pyrimidine-2-ol (RBK-11)

Melting point: 149-151^0 C; Yield: 76%; IR: 3282.15 (CH stretch aromatic), 3278.61 (OH), 1613.01 (C=C), 1487.11 (NO_2), 669.56 (C-Br); NMR: 8.41 (s, 1H, CH, benzene), 7.58 (d, 3H, CH benzene), 7.37 (d, 4H, CH benzene), 6.88 (S, 1H, CH, pyrimidine), 5.0 (s, 1H, OH, pyrimidine); Mass: 370.99 (100%), 372.99 (18.1%), 371.99 (19%)

4-(3-Nitro-phenyl)-6-phenyl-pyrimidine-2-ylamine (RBK-12)

Melting point: 158^0 C; Yield: 69%; IR: 3318.56 (N-H), 3211.34 (CH stretch aromatic), 1623.67 (C=C), 1487.11 (NO_2); NMR: 8.41 (s, 1H, CH, benzene), 7.58 (d, 3H, CH benzene), 7.37 (m, 5H, CH benzene), 4.0 (s, 2H, NH_2, pyrimidine); Mass: 292.10 (100%), 293.10 (18.1%), 294.10 (2.2%)

4,6-Bis-(2-hydroxy-phenyl)-pyrimidine-2-ol (RBK-13)

Melting point: 161-163^0 C; Yield: 82%; IR: 3278.61 (OH), 3213.15 (CH stretch aromatic), 1613.01 (C=C); NMR: 7.05 (m, 8H, CH, benzene), 6.67 (s, 1H, CH pyrimidine), 5.0 (s, 1H, OH, pyrimidine), 5.0 (s, 2H, OH benzene); Mass: 267.08 (100%), 268.08 (17%), 269.08 (2%)

4-(3,4-Dimethoxy-phenyl)-6-methyl-pyrimidine-2-thiol (RBK-14)

Melting point: 189-190^0 C; Yield: 67%; IR: 3287.71 (CH stretch aromatic), 2248.76 (SH), 1633.01 (C=C); NMR: 7.15 (s, 1H, CH pyrimidine), 6.88 (s, 1H, CH benzene), 6.72 (d, 2H, CH, benzene), 3.79 (d, 2H, CH), 3.0 (s, 1H, SH, pyrimidine), 2.35 (s, 3H, CH); Mass: 262.08 (100%), 263.08 (15.5%), 264.07 (4.4%)

4-(3,4-Dimethoxy-phenyl)-6-methyl-pyrimidine-2-ol (RBK-15)

Melting point: 143-144^0 C; Yield: 73%; IR: 3287.71 (CH, stretch aromatic), 3264.11 (OH), 1633.01 (C=C); NMR: 5.0 (s, 1H, OH, pyrimidine), 7.32 (m, 5H, CH, benzene), 6.88 (s, 1H, CH benzene), 6.72 (d, 2H, CH, benzene), 6.68 (s, 1H, CH pyrimidine), 3.79 (d, 2H, CH); Mass: 308.12 (100%), 309.12 (20.4%), 310.12 (2.7%)

4-Methyl-6-styryl-pyrimidine-2-thiol (RBK-16)

Melting point: 155^0 C; Yield: 81%; IR: 3042.79 (CH stretch aromatic), 2850.79 (CH stretch alkyl), 2354.11 (C-SH), 1617.76 (C=C); NMR: 7.21 (s, 1H, CH benzene), 7.10 (s, 1H, CH pyrimidine), 6.99 (d, 2H, CH ethylene), 3.0 (s, 1H, SH, pyrimidine), 2.35 (s, 3H, CH, methyl); Mass: 228.07 (100%), 229.08 (14.6%), 230.07 (4.7%)

2.3. In-vitro anti-cancer activity

Sulforhodamine B (SRB) assay experimental procedure [18]

The cell line MCF-7(for breast cancer) was grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well micro titer plates in 90 µl at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, one plate of each cell line was fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs were solubilized in appropriate solvent. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations. After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mMtriz base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percentage growth of inhibition = Control – sample / control × 100

2.4. In vitro anticataract activity [19]

Lens culture

Fresh goat eyeballs were obtained from slaughter house and immediately transported to the laboratory at 0-4^{0}C. The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl: 140 mM, KCl: 5 mM, $MgCl_2$: 2 mM, $NaHCO_3$: 0.5 mM, $NaH(PO_4)_2$: 0.5 mM, $CaCl_2$: 0.4 mM, and Glucose: 5.5 mM,) at room temperature and PH-7.8 for 72 hours. Penicillin G 32% and streptomycin 25% were added to the culture media to prevent bacterial contamination. At high concentrations, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols causing over hydration and oxidative stress. This lead to cataractogenesis.

Study groups

Lenses were divided into Group 1: aqueous humor + 5.5 mM glucose (negative control); Group 2: aqueous humor + 55 mM glucose (positive control); Group 3: aqueous humor + 55 mM glucose + 20 µg/ml synthesized compounds and Group 4: aqueous humor+ 55 mM glucose + 20 µg/ml standard epalrestat.

Photographic evaluation

After 72 hours of incubation, lenses were placed on a wired mesh with posterior surface touching the mesh and the pattern of the mesh (number of squares clearly visible through the lens) was observed through the lens as a measure of lens opacity.

The degree of opacity was graded as "0"- absence of opacity; "1"- slight degree of opacity; "2"- presence of diffuse opacity; "3"- presence of extensive thick opacity.

3. Results and Discussions

Docking studies were performed on VLife MDS 4.1 using batch docking method. VEGF receptor is selected as a biological target for carrying out the docking study. The crystal structure of VEGF (PDB CODE - 2vpf) was obtained from protein data bank (Figure 1). The docking of the title compounds yielded fitness scores ranging from -3.0792 to -4.7485 (Table1). The docking study revealed that the title compounds have good interaction with 2vpf and compounds RBK-1, RBK-2, RBK-3, RBK-4, and RBK-5 are potential candidates as a anticancer and anticataract agent because of the best negative dock score. Compound RBK-2 bind with 2vpf of VEGF receptor by forming hydrogen bond interaction with amino acid residue PHE294H 2.268 , PHE283H 1.576 as well as Van der Waals interaction with amino acid residue SER297H 3.491, LYS295H 3.197, PHE294H 3.446, HIS334E 3.880, ASN310G 3.436 and pi stacking interaction with amino acid residue HIS334E 4.868. (Figure 3-5)

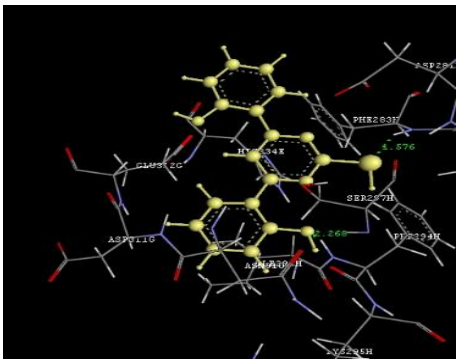


Figure 3. hydrogen bonding of RBK-2 with VEGF.

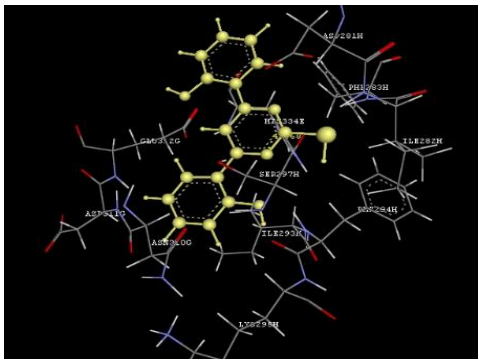


Figure 4. pi-stacking interaction of RBK-2 with VEGF.

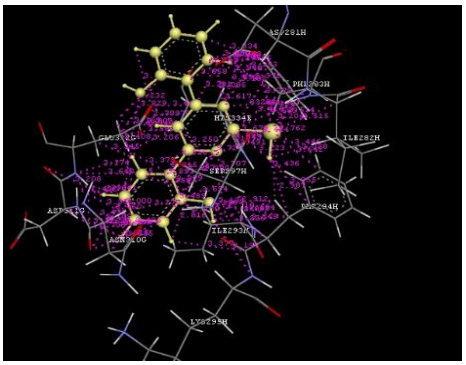
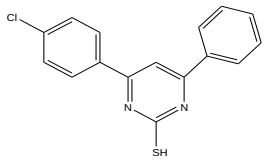
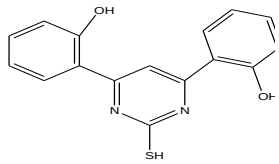
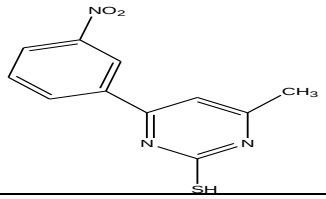
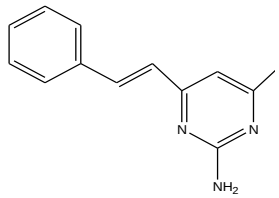
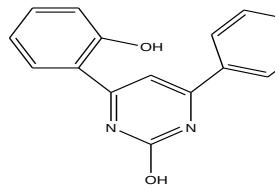
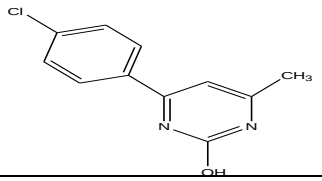
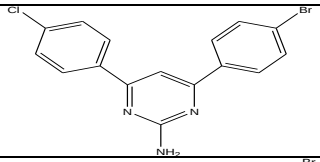
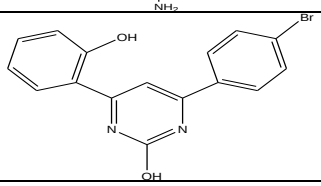
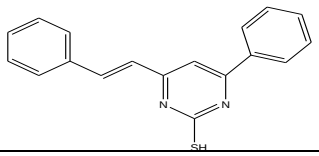
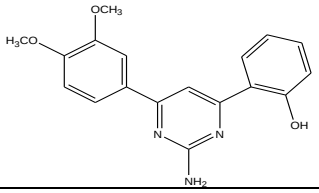
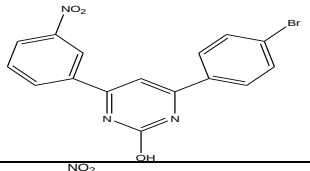
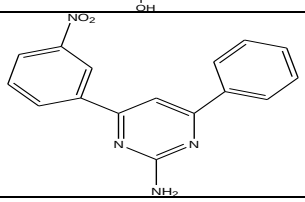
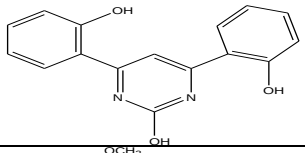
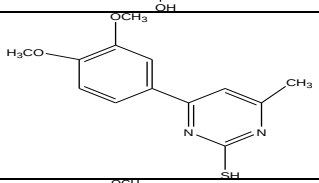
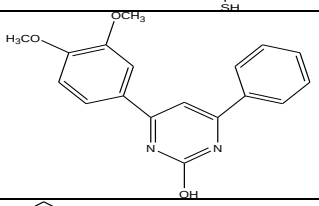
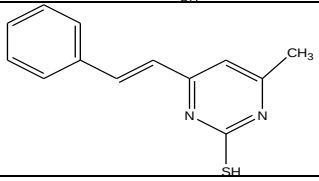


Figure 5. VDW interaction of RBK-2 with VEGF.

The 2,4,6-substituted pyrimidine (Table 1) derivatives were prepared from chalcone derivative by condensing various ketones with aromatic aldehyde in presence of ethanol and sodium or potassium hydroxide (Figure 2). The purity of the synthesized compounds was monitored by TLC. The solvent used for development of TLC was hexane:ethyl acetate (8:2) and visualization was done using iodine vapor. Their structures were confirmed by IR, ^{1}H NMR and mass spectra.

Table 1. Physicochemical characteristic of title compounds.

Compound Code	Molecular Structure	Molecular Formula	Molecular Weight	Melting Point (^{0}C)	Yield (%)	R_f Value	Docking score
RBK-1		$C_{16}H_{11}N_2SCl$	298	114	84.33	0.33	-3.3375
RBK-2		$C_{16}H_{12}N_2O_2S$	296	136	81.11	0.29	-4.9900
RBK-3		$C_{11}H_9N_3O_2S$	247	148	68.59	0.336	-4.0755
RBK-4		$C_{13}H_{13}N_3$	211	138	54.27	0.47	-4.5369
RBK-5		$C_{16}H_{12}N_2O_2$	264	128	72.48	0.39	-3.7375
RBK-6		$C_{16}H_9N_2OCl$	220	154	71.09	0.45	-4.5641
RBK-7		$C_{16}H_{11}N_3C$	360	162	4	0.	-

		lBr			5.35	65	3.7640
RBK-8		$_2$Br	$C_{16}H_{11}N_2O$	343	146	5 7.45	3. 23	- 3.8011
RBK-9			$C_{18}H_{14}N_2S$	290	136	8 4.10	3. 00	- 3.6790
RBK-10			$C_{18}H_{17}N_3O_3$	264	132	6 8.44	2. 64	- 3.7855
RBK-11			$C_{16}H_{10}N_3O_3Br$	372	149	7 7.36	2. 74	- 3.0792
RBK-12			$C_{16}H_{12}N_4O_2$	292	158	6 3.47	2. 73	- 3.1281
RBK-13			$C_{16}H_{12}N_2O_3$	280	161	5 4.28	3. 39	- 4.7485
RBK-14			$C_{13}H_{14}N_2O_2S$	262	189	4 7.82	4. 15	- 3.4453
RBK-15			$C_{18}H_{16}N_2O_3$	308	143	8 1.96	3. 66	- 3.4499
RBK-16			$C_{13}H_{12}N_2S$	228	155	3 1.76	3. 18	- 4.3708

The *in-vitro* anticancer activity was performed by **Sulforhodamine B (SRB)** assay and results shown in Table 2.

Table 2. *In-vitro* anti-cancer activity.

Compound Code	GI_{50} (µMol)
RBK-1	> 80
RBK-2	47.1
RBK-3	>80
RBK-4	>80
RBK-5	>80
Adriamycin	< 10

In-vitro anticancer screening reveals that the compound **RBK-2** shows mild anticancer activity. The *in-vitro* anticataract activity was performed on the lens of the goat eye. Compounds **RBK-2** and **RBK-3,** shows significant anticataract activity like standard epalrestat which is shown in Table 3 and Figure 6-11.

Table 3. Degree of opacity of goat lens after treatment.

Compound	Degree of opacity
Negative control	0
Positive control	3
RBK-2	1
RBK-3	1
RBK-18	1
Epalrestat	0

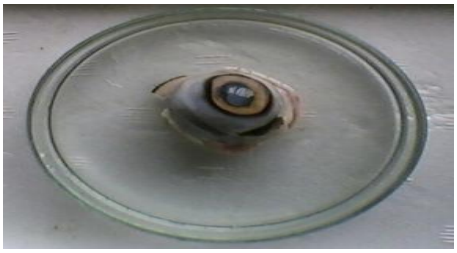


Figure 6. goat eye before placed in aqueous humor.

Figure 7. goat lens in aqueous humor + 5.5 mM glucose (negative control).

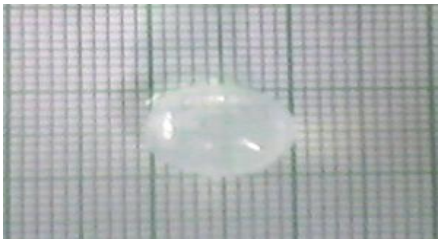


Figure 8. goat lens in aqueous humor + 55 mM glucose (positive control).

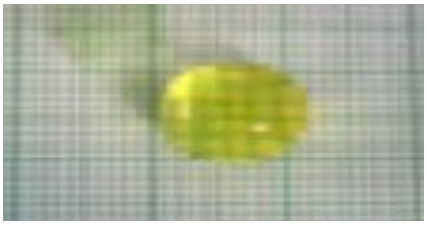


Figure 9. goat lens in aqueous humor + 55 mM glucose +20 µg/ml synthesized compounds (RBK-2).

Figure 10. goat lens in aqueous humor + 55 mM glucose +20 µg/ml synthesized compounds (RBK-3).

Figure 11. goat lens in aqueous humor + 55 mM glucose + 20 µg/ml epalrestat.

4. Conclusion

The molecular docking studies can be used to prioritized 2,4,6-substituted pyrimidine derivatives as a VEGF inhibitors. Compound 4,6-bis-(2-hydroxy-phenyl)-pyrimidine-2-thiol (RBK-2) shows potent anticancer activity as compared to standard drug adriamycin (ADR) where as compounds 4-(4-chloro-phenyl)-6-phenyl-pyrimidine-2-thiol (RBK-1); 4,6-bis-(2-hydroxy-phenyl)- pyrimidine-2-thiol (RBK-2) and 4-methyl-6-(3-nitro-phenyl)- pyrimidine-2-thio (RBK-3) shows potent anticataract activity as like standard drug epalrestat.The present study reveals that a derivatives of pyrimidine shows activity against breast cancer and diabetic retinopathy through inhibition of VEGF.

References

[1] J. G. Hardman, L. E. Limbird, Goodman and Gilman's The Pharmacological Basis of Therapeutics,10th edn., McGraw Hill, New York, 2001.

[2] American Cancer Society. Global Cancer Facts & Figures, 3rd Edition, 2015, 1.

[3] K. Papatheodorou, M. Banach, M. Edmonds, N. Papanas, D. Papazoglou, *J. Diabetes Res.*, 36 (2015) 1-5.

[4] A. Mukherjee, D. Rathore, S. Shree, J. Shaik, *Int. J. Eng. Res. Appl*., 5 (2015) 21-24.

[5] J. Folkman, *Nat. Med*., 1 (1995) 27-31.

[6] G. Bergers, L. E. Benjamin, *Nat. Rev. Cancer*, 3 (2003) 401-410.

[7] N. Ferrara, H. P. Gerber, J. L. Couter, *Nat. Med*., 9 (2003) 669-676.

[8] K. Noonan, D. O'Brien, J. Snoeyink, tnI. J. *Robotics Res*., 24 (2005) 971-982

[9] S. V. Kulkarni, B. Ramesh, *J. Global Pharm. Tech*., 2 (2010) 110-112.

[10] S. A. Khan, O. Alam, N. Siddiqui, W. Ahsan, *Med. Chem. Res*., 19 (2010) 1245–1258.

[11] R. L. Sawant, V. I. Sarode, *Iran. J. Pharm. Res*., 10 (2011) 733-739.

[12] F. M. Awadallah, *Sci. Pharm*., 76 (2008) 415–438.

[13] A. Agrawal, K. Shrivastav, S. K. Puri, M. S. Chauhan, *Bioorg. Med. Chem*., 13 (2005) 6226-6232.

[14] R. L. Sawant, M. S. Bhatia, *Bull. Chem. Soc. Ethiop*., 22 (2008) 1-12.

[15] M. R. Ahmad, V. G. Sastry, Y. R. Prasad, M. H. Khan, N. Bano, S. Anwar, *Eur. J. Chem*., 3 (2012) 94-98.

[16] R. L. Sawant, V. I. Sarode, G. D. Jadhav, J. B. Wadekar, *Med. Chem. Res*., 21 (2012) 1825–1832.

[17] V. D. Joshi, M. D. Kshirsagar, S. Singhal, *Der Pharm. Sinica*, 3 (2012) 343-348.

[18] V.Vichai, K. Kirtikara, *Nat. Protoc*., 1 (2006) 1112 – 1116.

[19] S. Merugu, B. Veeresh, D. Rekulapally, T. Swetha, *Orient Pharm. Exp. Med*., 3 (2009) 245-251.