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Novel Benzimidazole/Hydrazone Derivatives as Promising Anticancer Lead Compounds: Design, Synthesis and Molecular Docking Study

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Abstract

A novel series of benzimidazole-hydrazone derivatives was designed and synthesized as a potential anti-cancer agents. The structure of the target compounds was assured using different spectroscopic methods such as IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. These new compounds were examined and evaluated for their antiproliferative activities against 60 different cell lines. Combination of benzimidazole scaffold with hydrazone group exhibited promising anti-cancer activity. Compounds **3a** and **3b** showed significant activity against different cancer cells lines between 50-84 % of growth inhibition. A molecular modeling study for compounds **3a** and **3b** into VEGFR-2 active site was performed with reasonable docking scores.

Key words
Benzimidazole, Hydrazone, Anti-cancer, Molecular docking

1. Introduction

Cancer is one of the most tremendous illness in the world which is considered as the 1st life threatening disease [1]. Chemotherapy is the main cancer treatment strategy while patients administered the available chemotherapeutics are suffering undesirable side effects [2] hence, new research studies are stressed by scientists in order to discover new anticancer promising agents. Because of the structure similarity to the naturally occurring purines, some benzimidazole derivatives have proved biological importance; [3] such as: anti-cancer [4-6] **(Figure 1)**, antimicrobial [7-10], antifungal [11-15], antiviral [18-20], anthelmintic [21-22], antihypertensive [23-27], antitubercular [28-31], antiulcer activity [32], anti-histaminic [33] and anti-oxidant [34-36].

Imine group (-C=N-) included in hydrazones is considered an essential moiety of fictional group plays essential role in biological system such as mechanism of transformation reaction and racemization **(Figure 2)** [37].

The promising anticancer activity of benzimidazole and hydrazones compounds encouraged us to merge between these two scaffolds and design new benzimidazole compounds bearing hydrazone moiety **(Figure 3)**. Consequently, we aimed to synthesize novel benzimidazole-hydrazones derivatives with different substituent as electron withdrawing and electron donating groups **(Figure 3).**

2. Experimental

2.1. Chemistry

2.1.1. Synthesis of methyl 4-(5-methyl-1*H*-benzimidazol-2-yl) benzoate 1

Mixture of 5-methyl-1,2-phenylenediamine (2.0 mmol, 0.25 g) and methyl 4-formyl benzoate (2.0 mmol, 0.33 g) in presence of $Na_2S_2O_5$ (2.4 mmol, 0.5 g) in DMF (30 mL). The reaction mixture was poured into ice-water; the formed precipitate was filtered off and re-crystallized from ethanol to afford **1** [38]

2.1.2. Synthesis of 4-(5-methyl-1H-benzimidazol-2-yl) benzohydrazide 2

Mixture of Compound **1** (2 mmol, 0.5 g) and hydrazine hydrate (2 mmol, 5 mL) in ethanol (15 mL) were heated under reflux for 7 h. The mixture was cooled and poured into iced-water. The obtained crude solid was filtered off, washed with water, dried and recrystallized from absolute ethanol to afford **2** [11].

Dark grey **(**77 % yield**),** m.p. 218-220 °C, IR (KBr, υmax cm^{-1}**):** 3484, 3475 (forked NH_2), 3316 (-NH), 3022 (CH aromatic), 1631 (C═O), 1567 (C═C), 1441 (C═N); ^{1}HNMR (DMSO-d_6, 400 MHz, δ ppm): δ (ppm) 2.43 (s, 3H, C**H**$_3$), 4.73 (s, 2H, N**H**$_2$, D_2O exchangeable), 7.05 (d, 1H, *J*=12 Hz, Ar-H),7.35 (m, 2H, Ar-2H), 7.98 (dd, 2H, *J*=12 Hz, Ar-2H), 8.23 (dd, 2H, *J*=12 Hz, Ar-2H), 9.90 (s, 1H, N**H** amide, D_2O exchangeable), 12.88 (brs, 1H, N**H** benzimidazole, D_2O exchangeable).

2.1.3. General method for synthesis of N'-benzylidene-4-(5-methyl-1*H*-benzimidazol-2-yl) benzohydrazide derivatives [3a-3b]

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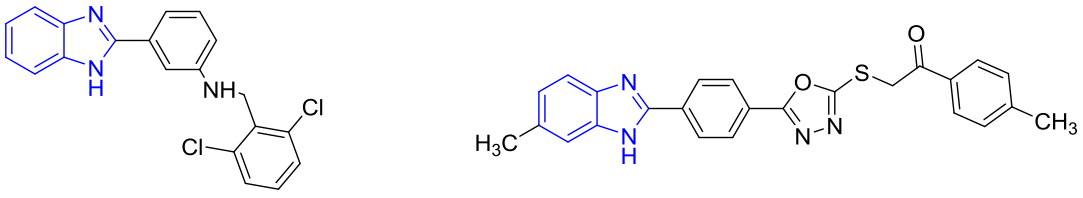
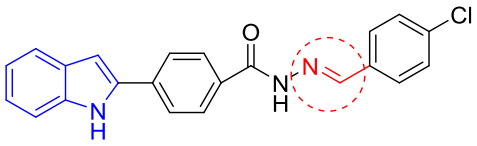


I
MCF-7 cell lines(IC_{50}=6 µM)

II
DNA topoisomerase I(IC_{50}=13 µM)

Benzimidazole derivatives

Figure (1): Chemical structure of biologically active benzimidazole derivatives.



III
VEGFR (IC_{50}= 8 µM)

Figure (2): Chemical structure of compounds containing imine pharmacophore with anti cancer activity.

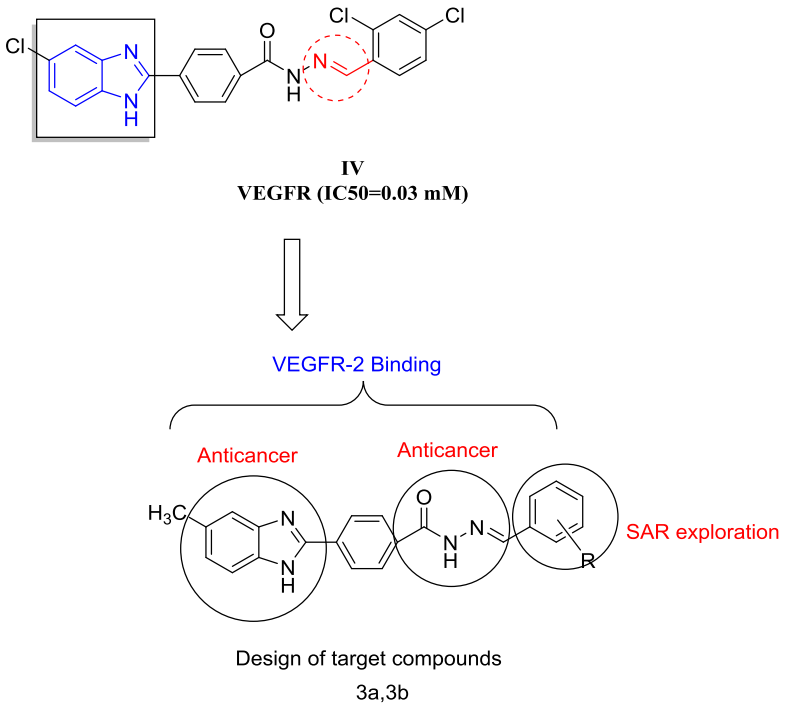


Figure (3): Benzimidazoles having hydrazone pharmacophore with breast cancer and design of target compounds

Equimolar quantities (2 mmol, 0.5 g) of compound **[2]** and appropriate different substituted benzaldehydes in 25 mL of ethanol were refluxed for 6 h in the presence of catalytic amount of glacial acetic acid. The resulting solid was filtered and recrystallized from absolute ethanol [37].

2.1.3.1. Synthesis of N'-(2,4-di-chlorobenzylidene)-4-(5-methyl-1*H*-benzimidazol-2-yl)benzohydrazide [3a].

Light green, 85% yield, m.p. 240-242 ^{o}C, IR (KBr, υ_{max} cm^{-1})**:** 3489-3423 (NH benzimidazole, NH hydrazone), 3046 (CH aromatic), 2922 (CH aliphatic), 1660 (C═O), 1614 (C═C), 1470 (C═N); ^{1}HNMR (DMSO-d$_6$, 400 MHz, δ ppm): 2.44 (s, 3H, C**H$_3$**), 7.08 (d, 1H, *J*=8.00 Hz, Ar-H), 7.42 (s, 1H, Ar-H), 7.53 (dd, 2H, *J*=8.00 Hz, Ar-2H), 7.72 (s, 1H, Ar-H), 8.05 (d, 1H, *J*=8.00 Hz, Ar-H), 8.12 (dd, 2H, *J*=8.00 Hz, Ar-2H), 8.31 (d, 2H, *J*=8.00 Hz, 2Ar-H), 8.85 (s, 1H, C**H**), 12.24 (s, 1H, N**H** hydrazone, D_2O exchangeable); ^{13}C NMR ($CDCl_3$-*d$_3$*, 100 MHz, δ ppm): 21.81 (**C**H3), 124.67, 126.77, 128.51, 128.60, 128.85, 129.85, 130.38, 131.11, 132.51, 133.56, 134.05, 134.40, 135.65, 143.35, 150.21, 163.06 (**C**H=N), 172.08 (**C**=O); Elemental analysis calculated for $C_{22}H_{16}Cl_2N_4O$ (M.Wt. 423.30): C, 65.42; H, 3.81; N, 13.24; Found C, 65.16; H, 3.59; N, 13.51.

2.1.3.2. Synthesis of N'-(3, 4, 5 tri-methoxybenzylidene)-4-(5-methyl-1*H*-benzimidazol-2-yl)benzohydrazide [3b].

Canary yellow, 92% yield, m.p. 243-245 ^{o}C, IR (KBr, υmax cm^{-1}): 3448-3412(NH benzimidazole, NH hydrazone), 3042 (CH aromatic), 2923 (CH aliphatic), 1644 (C═O), 1604 (C═C), 1463 (C═N); ^{1}HNMR (DMSO-d$_6$, 400 MHz, δ ppm): 2.45 (s, 3H, C**H$_3$**), 3.77 (s, 3H, OC**H$_3$**), 3.86 (s, 6H, 2OC**H$_3$**), 7.07 (m, 3H, Ar-3H), 7.42 (s, 1H, Ar-H), 7.53 (d, 1H, *J*=8.00 Hz, Ar-H), 8.09 (dd, 2H, *J*=8.00 Hz, Ar-2H), 8.30 (dd, 2H, *J*=8.00 Hz, Ar-2H), 8.43 (s, 1H, CH), 11.79 (s, 1H, N**H** hydrazone, D_2O exchangeable); ^{13}C NMR ($CDCl_3$-*d$_3$*, 100 MHz, δ ppm): 21.81 (**C**H3), 56.45, 60.61 (O**C**H3), 104.86, 124.57, 126.72, 128.78, 130.26, 132.39, 133.46, 134.53, 139.79, 148.56, 150.32, 153.69, 163.04(**C**H=N), 172.03(**C**=O); Elemental analysis calculated for $C_{25}H_{24}N_4O_4$ (M.Wt. 444.49): C, 67.55; H, 5.44; N, 12.60; Found C, 67.31; H, 5.22; N, 12.38.

2.2. Screening of anticancer activity

According to the protocol of Drug Evaluation Branch of the National Cancer Institute (NCI), Bethesda, USA. These studies were carried out using cells derived from NCI 60 cell line panels (six cell lines of Leukemia, nine cell lines of Lung cancer, six cell lines of CNS cancer, seven cell lines of Colon cancer, eight cell lines of Melanoma, six cell lines of Ovarian cancer, eight cell lines of Renal cancer, two cell lines of Prostate cancer and eight cell lines of breast cancer) representing on full nine human systems as Leukemia, Melanoma and cancers of Lung, Colon, Brain, Breast, Ovary, Kidney and Prostate**.**

2.2.1. In vitro Anticancer Screening Procedure:

NCI screening is evaluated of all compounds against the 60 cell lines at a single dose of 1 x 10^{-5} M (molar) or 15 µg/mL. Growth percentages of cells treated with the compound is expressed to anticancer activity for each of the examined compound as compared with untreated control ones. Sulforhodamine B (SRB) solution (100 µL) at 0.4 % (w/v) in 1 % acetic acid added to each well and plates incubated for 10 min at RT. Percentages of growth were measured spectrophotometrically [39].

2.3 Molecular modeling and docking

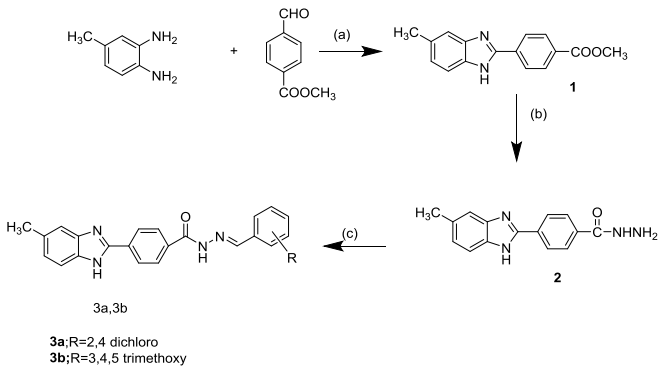
The nature of the interaction of the most active synthesized compounds and VEGFR-2 active site can be clarified, a molecular docking study was performed from protein data bank using crystal structure data for VEGFR-2 (PDB: ID 2OH4) active site. Molecular modeling of compounds **3a, 3b** and co-crystallized ligand, Sorafenib **(Figure 4)** using MOE 2019 modeling software.

Co-crystallized ligand was removed to prepare the enzyme for docking then the enzyme was 3D protonated, in which hydrogen atoms were added to their standard geometry. The conformers generated were docked into the VEGFR-2 receptor with MOE-dock using the triangle matcher placement method and the London dG scoring function. A molecular mechanics force field refinement was carried out on the top 30 poses generated. Sorafenib was redocked as a reference into the active site of VEGFR-2 to validate the docking protocol. The hydrogen bond lengths and amino acid interactions were summarized in **(Table 2).**

3. Result and discussion

3.1. Chemistry

In the present study, some N'-(substitutedbenzylidene)-4-(5-methyl-1*H*-benzimidazol-2-yl)benzohydrazide derivatives (**3a-3b**) were synthesized according to (Scheme 1). Aimed compounds were acquired at three steps. At the beginning, Equimolar mixture of 5-methyl-1,2-phenylenediamine and methyl-4-formyl benzoate in presence of $Na_2S_2O_5$ in DMF to afford **1**. Mixture of compound **1** and hydrazine hydrate in ethanol were heated till reflux for 7 h to obtain compound **2**. Condensation of hydrazide with variously substituted benzaldehydes gave the aimed compounds **3a-3b**.



Conditions and reagents: (a) DMF, $Na_2S_2O_5$, (b) $NH_2.NH_2.H_2O$,ehanol, (c) different substituent aldehyde, few drops of CH_3COOH.

Scheme (1): Synthesis of compounds 3a and 3b

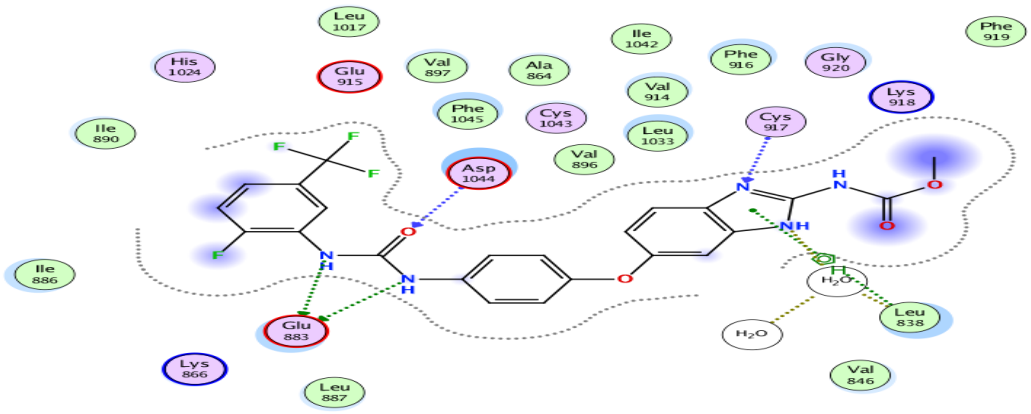


Figure (4): 2D binding of Sorafenib with active site of VEGFR-2.

Table 1: Percentage growth inhibition (GI %) of tumor cell lines at 10 mM concentration for compounds **3a** and **3b**.

Subpanel cancer cell Lines	% Growth Inhibition (GI %)	
	3a	**3b**
Leukemia		
CCRF-CEM	47.86	38.77
RPMI-8226	10.02	52.78
HL-60(TB)	NT	6.85
K-562	NT	56.75
MOLT-4	NT	25.36
Non-Small Cell Lung Cancer		
A549/ATCC	NA	16.22
EKVX	NA	32.47
HOP-62	14.99	25.18
HOP-92	13.66	53.66
NCI-H226	7.24	50.00
NCI-H23	12.53	29.93
NCI-H322M	27.90	7.44
NCI-H460	3.37	35.49
NCI-H522	21.63	26.92
Colon Cancer		
COLO 205	14.58	73.01
HCC-2998	6.09	13.26
HCT-116	56.60	56.09
HCT-15	29.23	5.19
HT29	NA	24.96
KM12	29.43	38.72
SW-620	52.55	14.75
CNS Cancer		
SF-268	9.51	13.45
SF-295	NA	25.12
SF-539	2.34	36.80
SNB-19	5.38	28.74
SNB-75	8.71	29.44
U251	4.38	31.20

Table 1: Percentage growth inhibition (GI %) of tumor cell lines at 10 mM concentration for compounds **3a** and **3b** (cont.).

Subpanel cancer cell Lines	% Growth Inhibition (GI %)	
	3a	**3b**
Melanoma		
LOX IMVI	14.02	12.90
MALME-3M	4.35	80.33
M14	26.52	54.28
MDA-MB-435	6.02	61.08
SK-MEL-2	NA	27.93
SK-MEL-28	NA	52.16
SK-MEL-5	0.82	70.24
UACC-257	NA	44.40
UACC-62	4.19	55.78
Ovarian Cancer		
IGROV1	9.02	24.06
OVCAR-3	19.90	3.54
OVCAR-4	79.76	14.13
OVCAR-5	2.26	14.89
OVCAR-8	28.37	22.94
NCI/ADR-RES	7.81	1.35
SK-OV-3	NA	36.87
Renal Cancer		
786-0	33.22	49.01
A498	15.30	34.06
ACHN	0.04	27.49
CAKI-1	12.44	38.81
RXF 393	NA	10.90
SN12C	5.82	13.17
TK-10	65.51	60.20
UO-31	19.61	29.19
Prostate Cancer		
PC-3	30.57	5.82
DU-145	13.78	22.40
Breast Cancer		
MCF7	30.51	84.33
MDA-MB-231/ATCC	25.34	15.29
HS 578T	10.35	45.91
BT-549	2.11	18.05
T-47D	30.2	54.24
MDA-MB-468	NA	4.05

NT: not tested NA: GI% ≤ zero

Table 2: Molecular docking data for compounds **3a, 3b** and Sorafenib in VEGFR-2 active site (PDB ID: 2OH4).

Compound No.	VEGFR-2				
	Affinity kcal/mol	**Distance (in A°) from main residue**	**Amino acids residue**	**Functional group**	**Type of interactions**
Sorafenib	-19.10	3.02	Glu 883	N12	H-donor
		2.93	Glu 883	N15	H-donor
		2.80	HOH 167	N26	H-donor
		3.03	Cys 917	N28	H-acceptor
		2.84	Asp 1044	O14	H-acceptor
		4.29	Leu 838	5-ring	pi-H
3a	-14.67	3.23	Glu 883	Cl	H-donor
3b	-20.75	2.97	Cys 917	N10	H-donor
		4.42	Leu 838	5-ring	pi-H

Structure elucidations of target compounds were carried out with IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analyses. IR spectra showed, stretching absorptions that were appeared at 3489-3423 cm^{-1} proved N-H bond of the hydrazide group and benzimidazole. Furthermore, carbonyl (C=O) function was appeared at 1660–1644 cm^{-1}. The stretching absorption at about 1614–1604 cm^{-1} were observed for C═C and 1470-1463 C═N. ^{1}H-NMR spectra of the compounds, schiff base protons (─CO**NH**N═C**H**─) were determined at about 8.43–8.85 and 11.79–12.24 ppm, respectively. The NH proton of the benzimidazole ring was not appeared due to its acidity and rotatable proton [40]. The aromatic protons assigned at range from 6-8 ppm. In the ^{13}C-NMR spectra, characteristic hydrazone (─**CO**NHN═CH─) signal at 163.08–174.04 due to carbonyl (C═O) group. Compounds **3a** and **3b** provided acceptable elemental analyses results.

3.2. Screening of anticancer activity

The screening results were reported **(Table 1)** as the percent growth of inhibition compared to untreated control cells. Compound **3b** which possessing tri-methoxy substituent as electron donating group is more active than compound **3a** which possessing di-chloro substituent as electron withdrawing group, which reflects a positive influence of substituent on the antiproliferative activity. Compound **3b** showed promising activities against a majority of the tested cancer cell lines such as leukemia (GI%=65.75), non-small cell lung cancer (GI%=53), colon cancer (GI%=56.09), melanoma (GI%=80.33), renal cancer (GI%=60.20) and breast cancer (GI%=84.33). While compound **3a** showed moderate inhibitory effect against leukemia (GI%=47.86), colon cancer (GI%=56.60), ovarian cancer (GI%=79.76) and renal cancer (GI%=65.51).

3.3 Molecular modeling

Molecular Operating Environment MOE version 2019 is used to perform molecular modeling study. Structures of **3a** and **3b** where built in MOE database. The X-ray crystal structure of Sorafenib bound to the VEGFR-2 enzyme binding site (PDB: ID 2OH4) active site was obtained from protein data bank at research collaboration for Structural Bioinformatics (RSCB) protein database [PDB][3].

Hydrazone derivatives **3a** and **3b** were fully fitted within VEGFR-2 active site with high affinity (-14.67 and -20.75 Kcal/mol, respectively) in comparison with Sorafenib (-19.10 kcal/mol). Compound **3a** showed binding through the Cl atom which is an electron donor, in addition to an extra binding with secondary amino group to Glu 883. In compound **3b**, the binding was observed throughout the imidazole ring via hydrophobic interaction and electron donor interaction, respectively which bound with amino groups to Cys 917 and Leu 838 (**Figure 5-9**). Thus, the molecular docking results ensure that compounds **3a** and **3b** bind to VEGFR-2 active site with the same manner of Sorafenib. Nevertheless, 3b is more active than 3b according to binding with active site

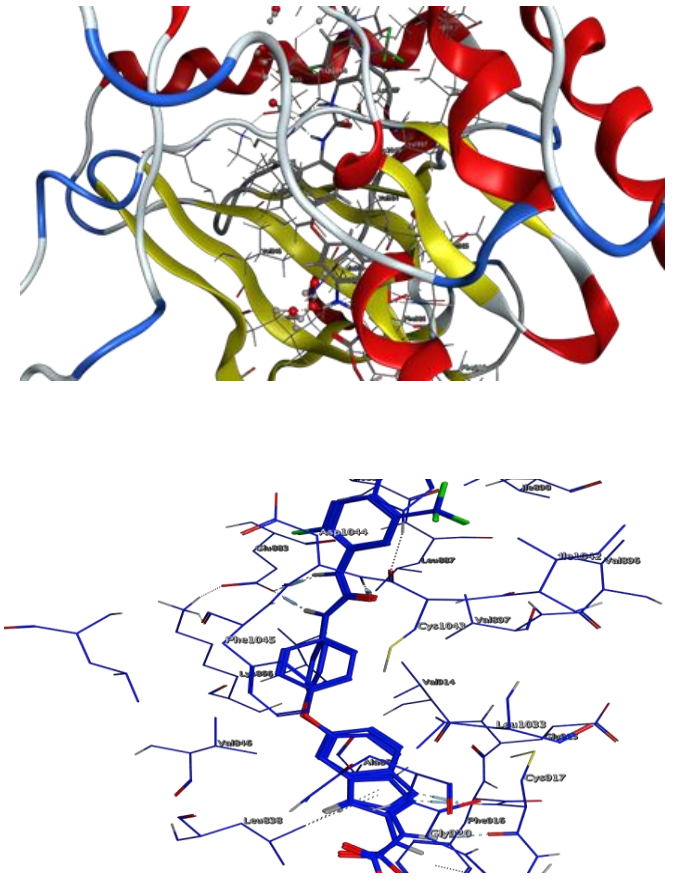


Figure (5): 3D binding of Sorafenib with active site of VEGFR-2**.**

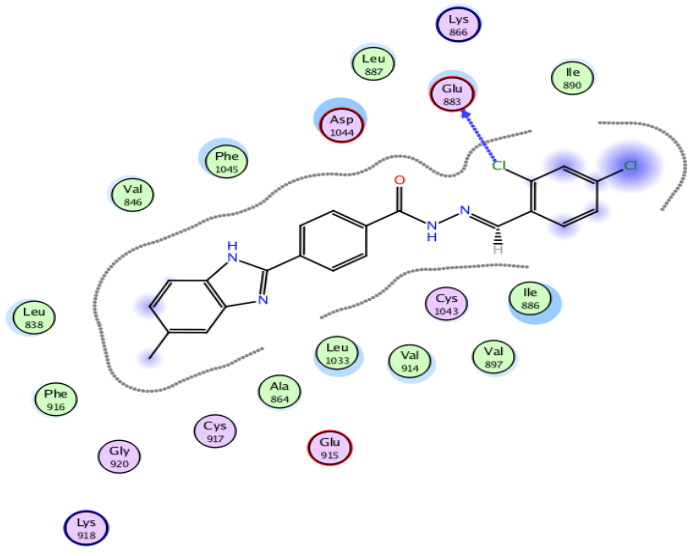


Figure (6): 2D binding of compound **3a** with active site of VEGFR-2.

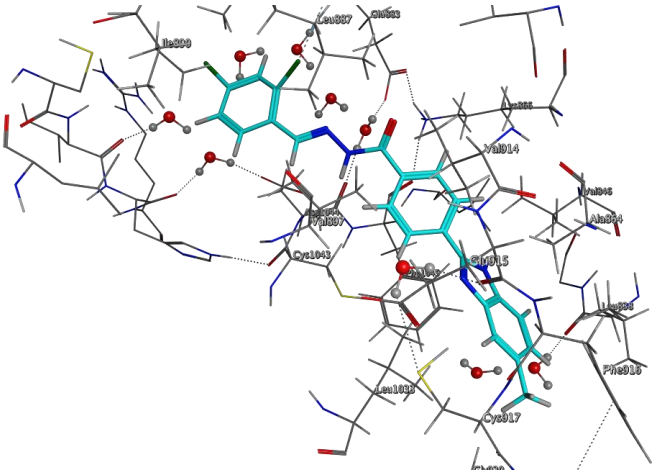


Figure (7): 3D binding of compound **3a** with active site of VEGFR-2.

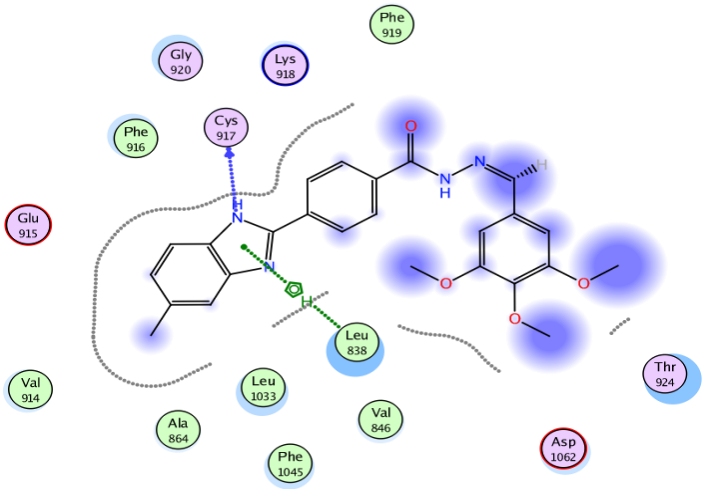


Figure (8): 2D binding of compound **3b** with active site of VEGFR-2.

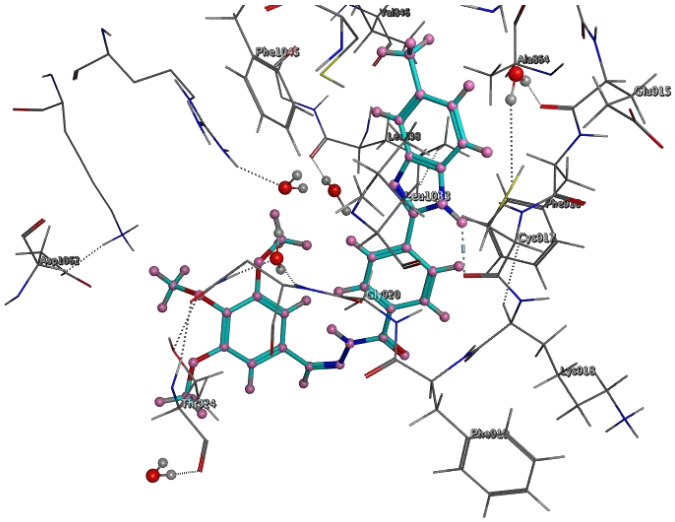


Figure (9): 3D binding of compound **3b** with active site of VEGFR-2.

Conclusion

In this work, benzimidazole bearing hydrazone moiety based compounds were designed and synthesized to afford compounds **3a** and **3b**. Molecular docking ensured that compound **3b** which has an electron donating group (tri-methoxy substituent) can bind with the active site of VEGFR-2 with reasonable docking scores than compound **3a** which as electron withdrawing group (di-chloro substituent). Type of substituent on the hydrazone moiety indicated deep impact on the binding mode, where electronic effect contributes to the interaction with the conserved amino acids as well as the reference Sorafenib. We aimed for further investigation of action mechanism.

References

[1] P. Singla, V. Luxami, R. Singh, V. Tandon, K. Paul, Novel pyrazolo [3, 4-d] pyrimidine with 4-(1*H*-benzimidazol-2-yl)-phenylamine as broad spectrum anticancer agents: synthesis, cell based assay, topoisomerase inhibition, DNA intercalation and bovine serum albumin studies. *Eur. J. Med. Chem.*, 126 (2017), 24-35.

[2] J. Akhtar, A. A. Khan, Z. Ali, R. Haider, M. S. Yar, Structure-activity relationship (SAR) study and design strategies of nitrogen-containing heterocyclic moieties for their anticancer activities. *Eur. J. Med. Chem.*, 125 (2017), 143-189.

[3] A. S. Mostafa, R. M. Gomaa, M. A. Elmorsy, Design and synthesis of 2-phenyl benzimidazole derivatives as VEGFR-2 inhibitors with anti-breast cancer activity. *Chem. Biol. Drug Des.*, 93(2019), 454-463.

[4] U. A. Çevik, B. N. Sağlık, B. Korkut, Y. Özkay, S. Ilgın, Antiproliferative, Cytotoxic, and Apoptotic Effects of New Benzimidazole Derivatives Bearing Hydrazone Moiety. *J. Heterocycl. Chem.*, 55(2018), 138-148.

[5] N. Shrivastava, M. J. Naim, M. J. Alam, F. Nawaz, S. Ahmed, O. Alam, Benzimidazole scaffold as anticancer agent: synthetic approaches and structure–activity relationship. *Arch. Pharm. Chem. Life Sci.*, 350(2017), 1-80.

[6] H. M. Refaat, Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. *Eur. J. Med. Chem.*, 45(2010), 2949-2956.

[7] E. Menteşe, H. Bektaş, S. Ülker, O. Bekircan, B. Kahveci, Microwave-assisted synthesis of new benzimidazole derivatives with lipase inhibition activity. *J. Enzym. Inhib. Med. Chem.*, 29(2014), 64-68.

[8] K. G. Vaidehi, R. V. Satya, R. R. Bangaramma, R. H. Kumar, Y. R. Sudha, T. R. Kumar, Synthesis, Characterization and antibacterial activity of 2-substituted benzimidazole derivatives. *Int. J. Res. Pharm. Chem.*, 2(2002), 322-326.

[9] F. Alasmary, A. Snelling, M. Zain, A. Alafeefy, A. Awaad, N. Karodia, Synthesis and evaluation of selected benzimidazole derivatives as potential antimicrobial agents. *Molecules*, 20(2015), 15206-15223.

[10] N. C. Desai, D. Pandya, D. Vaja, Synthesis and antimicrobial activity of some heterocyclic compounds bearing benzimidazole and pyrazoline motifs. *Med. Chem. Res.*, 27(2018), 52-60.

[11] A. Karaburun, B. K. Çavuşoğlu, U. A. Çevik, D. Osmaniye, B. Sağlık, S. Levent, Y. Özkay, A. S. Koparal, Z. Kaplancıklı, Synthesis and Antifungal Potential of Some Novel Benzimidazole-1, 3, 4-Oxadiazole Compounds. *Molecules*, 24(2019), 191-205.

[12] A. Mobinikhaledi, N. Foroughifar, M. Kalhor, M. Mirabolfathy, Synthesis and antifungal activity of novel 2-benzimidazolylimino-5-arylidene-4-thiazolidinones. *J. Heterocycl. Chem.*, 47(2010), 77-80.

[13] Y. Ke, X. Zhi, X. Yu, G. Ding, C. Yang, H. Xu, Combinatorial synthesis of benzimidazole-azo-phenol derivatives as antifungal agents. *Comb. Chem. high t. scr.*, 17(2014), 89-95.

[14] W. Si, T. Zhang, Y. Li, D. She, W. Pan, Z. Gao, X. Mei, Synthesis and biological activity of novel benzimidazole derivatives as potential antifungal agents. *J. pestic. Sci.*, 41(2015), 15-19.

[15] P. Keller, C. Müller, I. Engelhardt, E. Hiller, K. Lemuth, H. Eickhoff, S. Rupp, An antifungal benzimidazole derivative inhibits ergosterol biosynthesis and reveals novel sterols. *Antimicrob. Agents Chemother.*, 59(2015), 6296-6307.

[16] K. C. Achar, K. M. Hosamani, H. R. Seetharamareddy, In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. *Eur. J. Med. Chem.*, 45(2010), 2048-2054.

[17] M. Gaba, P. Gaba, D. Uppal, N. Dhingra, M. S. Bahia, O. Silakari, C. Mohan, Benzimidazole derivatives: search for GI-friendly anti-inflammatory analgesic agents. *Acta Pharm. Sin. B.*, 5(2015), 337-342.
[18] M. Tonelli, F. Novelli, B. Tasso, I. Vazzana, A. Sparatore, V. Boido, B. Busonera, Antiviral activity of benzimidazole derivatives. III. Novel anti-CVB-5, anti-RSV and anti-Sb-1 agents. *Bioorg. Med. Chem.*, 22(2014), 4893-4909.
[19] Y. M. Shaker, M. A. Omar, K. Mahmoud, S. M. Elhallouty, W. M. El-Senousy, M. M. Ali, H. I. El Diwani, Synthesis, in vitro and in vivo antitumor and antiviral activity of novel 1-substituted benzimidazole derivatives. *J. Enzym. Inhib. Med. Chem.*, 30(2015), 826-845.
[20] A. Dittmer, I. Woskobojnik, R. Adfeldt, J. C. Drach, L. B. Townsend, S. Voigt, E. Bogner, Tetrahalogenated benzimidazole D-ribonucleosides are active against rat cytomegalovirus. *Antivir. Res.*, 137(2017), 102-107.
[21] M. Longo, S. Zanoncelli, P. A. Colombo, M. O. Harhay, I. Scandale, C. Mackenzie, G. Mazué, Effects of the benzimidazole anthelmintic drug flubendazole on rat embryos in vitro. *Reprod. Toxicol.*, 36(2013), 78-87.
[22] B. Munguía, P. Mendina, R. Espinosa, A. Lanz, J. Saldaña, M. J. Andina, L. Domínguez, Synthesis and anthelmintic evaluation of novel valerolactam-benzimidazole hybrids. *Lett. Drug Des. Discov.*, 10(2013),1007-1014.
[23] M. T. Khan, M. T. Razi, S. U. Jan, M. Mukhtiar, R. Gul, A. Hussain, I. Rabbani, Synthesis, characterization and antihypertensive activity of 2-phenyl substituted benzimidazoles. *Pak. J. pharm. Sci.*, 31(2018), 1067-1074.
[24] M. C. Sharma, D. V. Kohli, S. Sharma, A. D. Sharma, Synthesis and antihypertensive activity of some new benzimidazole derivatives of 4′-(6-methoxy-2-substituted-benzimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid in the presences of BF3· OEt2. *Der Pharm. Sinica*, 1(2010), 104-115.
[25] Z. Wu, X. L. Bao, W. B. Zhu, Y. H. Wang, N. T. P. Anh, X. F. Wu, Z. L. Chen, Design, Synthesis, and Biological Evaluation of 6-Benzoxazole Benzimidazole Derivatives with Antihypertension Activities. *ACS med. Chem. Lett.*, 10(2018), 40-43.
[26] D. Mishra, V. Sudha, S. C. Chaturvedi, Docking Studies on Some Benzimidazole and Triazole Analogues as Antihypertensive Agents. *J. Bioinform.*, 5(2018)., 37-39.
[27] A. J. Marcus, I. Iezhitsa, R. Agarwal, P. Vassiliev, A. Spasov, O. Zhukovskaya, N. M. Ismail, Intraocular pressure lowering effect and structure-activity relationship of imidazo [1, 2-a] benzimidazole and pyrimido [1, 2-a] benzimidazole compounds in ocular normotensive rats: insight on possible link with hypotensive activity. *Eur. J. Pharm. Sci.*, 114(2018), 245-254.
[28] S. Yadav, B. Narasimhan, S. M. Lim, K. Ramasamy, M. Vasudevan, S. A. Shah, A. Mathur, Synthesis and evaluation of antimicrobial, antitubercular and anticancer activities of benzimidazole derivatives. *Egyptian J. Basic Appl. Sci.*, *5*(2018), 100-109.
[29] R. S. Keri, C. K. Rajappa, S. A. Patil, B. M. Nagaraja, Benzimidazole-core as an antimycobacterial agent. *Pharmacol. Rep.*, 68(2016), 1254-1265.
[30] D. M. L. Araujo, M. M. Maste, S. Alegaon, A. Saxena, Synthesis, Antitubercular Evaluation And Docking Studies Of Novel Benzimidazole Analogues. *Int. J. Pharm. Sci. Res.*, 9, (2018), 3696-3704.
[31] G. Surineni, Y. Gao, M. Hussain, Z. Liu, Z. Lu, C. Chhotaray, T. Zhang, Design, synthesis, and in vitro biological evaluation of novel benzimidazole tethered allylidenehydrazinylmethylthiazole derivatives as potent inhibitors of Mycobacterium tuberculosis. *MedChemComm*, 10(2019), 49-60.
[32] B. Soni, A Short Review on Potential Activities of Benzimidazole Derivatives. *PharmaTutor*, *2*(2014), 110-118.
[33] K. Anand, S. Wakode, Development of drugs based on Benzimidazole Heterocycle: Recent advancement and insights. *IJCS*, 5(2017), 350-362.
[34] A. S. Alpan, G. Sarıkaya, G. Çoban, S. Parlar, G. Armagan, V. Alptüzün, Mannich-Benzimidazole Derivatives as Antioxidant and Anticholinesterase Inhibitors: Synthesis, Biological Evaluations, and Molecular Docking Study. *Arch. Pharm. Chem. Life Sci.*, 350(2017), 1-15.
[35] N. Karmaker, D. N. Lira, B. K. Das, U. Kumar, A. S. S. Rouf, Synthesis and Antioxidant Activity of Some Novel Benzimidazole Derivatives. *J. Pharm. Sci.*, 16(2017), 245-249.
[36] S. Rajasekaran, G. Rao, A. Chatterjee, Synthesis, Anti-Inflammatory and Anti-oxidant activity of some substituted Benzimidazole Derivatives. *Int. J. Drug Dev. Res.*, 4(2012), 303-309.
[37] Y. Özkay, Y. Tunalı, H. Karaca, İ. Işıkdağ, Antimicrobial activity and a SAR study of some novel benzimidazole derivatives bearing hydrazone moiety. *Eur. J. Med. Chem.*, 45(2010), 3293-3298.
[38] N. Ö. Can, U. A. Çevik, B. N. Sağlık, S. Levent, B. Korkut, Y. Özkay, Z. A. Kaplanciklj, A. S. Koparal, Synthesis, Molecular Docking Studies and Antifungal Activity Evaluation of New Benzimidazole-Triazoles as Potential Lanosterol 14α Demethylase Inhibitors. *J. Chem.*, 2017 (2017), 1-15.
[39] M. R. Boyd, K. D. Paull, Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res.*, 34 (1995), 91-109.
[40] P. A. Gale, J. R. Hiscock, N. Lalaoui, M. E. Light, N. J. Wells, M. Wenzel, Benzimidazole-based anion receptors: tautomeric switching and selectivity. *Org. Biomol. Chem.*, 10(2012), 5909-5915.