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DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF SUBSTITUTED BENZOXAZOLE DERIVATIVES

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ABSTRACT

Benzoxazole and azetidinone are the most important heterocycles exhibiting remarkable anticancer activities. A series of Benzoxazole substituted azetidinone derivatives were synthesized, characterized and evaluated for the anticancer study. The structures were confirmed by ^{1}H NMR, ^{13}C NMR and MASS spectral techniques. The docking studies of synthesized compounds were done to find the binding activity of the derivatives to the VEGFR-2 receptors.

KEYWORDS: Benzoxazole, Azetidinone, Anticancer activity.

INTRODUCTION

Cancer is a disease in which some of the cell grow uncontrollably and invade to other body parts. It starts almost anywhere in the human body, which is made up of millions of cells. Cancer cells do not have any programming so they do not possess any physiological function.[1-2] there are 10 basic principles to understand the molecular basis of cancer proposed by Doughs and Hanahan and Robert Weinberg in 2000. The ten hallmarks include activating invasion and metastasis, inducing angiogenesis, genome instability and mutation, resisting cell death, degenerating cellular energetic, sustaining proliferative signaling, evading growth suppression, avoiding immune destruction, enabling replicative immunity, tumor promoting inflammation. The target selected for this study was based on these 10 hallmarks. Sustaining proliferative signaling, genome instability and mutation, inducing angiogenesis, invasion and metastasis were important hallmarks for different types of solid tumors.[3] Benzoxazole and its derivatives show different biological activities such as anti-bacterial, anti- fungal, anti-histaminic and anti-cancer properties.[4-5] Azetidinones are the simplest beta lactam exhibiting anti-tubercular, anti-HIV, anti-inflammatory activity etc.[6] The Vascular Endothelial Growth Factor, a tyrosine kinase receptor is one of the most suitable targets for cancer. Agents which

could inhibit VEGFR are directly related to blockade of regulatory process of cellular proliferation. [7]

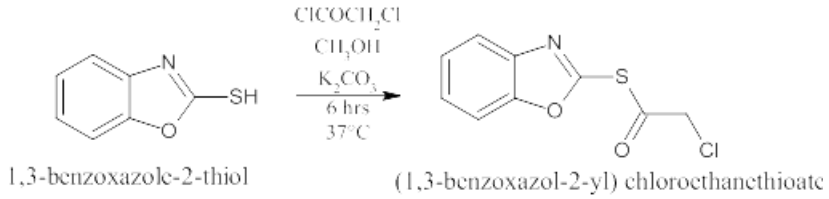
MATERIALS AND METHODS

IR spectra were determined using JASCO FT-IR-40000cm^{-1} using KBr disc. Analytical grade chemicals and solvents are used in this study. Melting points of the compounds were recorded using melting point apparatus. The progress of reaction was checked by TLC plate on suitable solvent system on glass plate coated with silica gel. The solvent system used in this study was petroleum ether: ethyl acetate (4:1).

Procedure for synthesis

Step 1: Synthesis of 2-chloroacetyl Mercapto benzoxazole

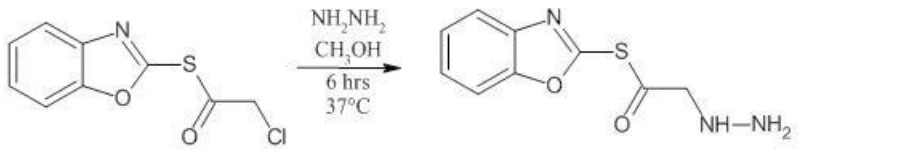
An equimolar solution of 2-mercaptobenzoxazole (0.06 mole) and chloroacetyl chloride (0.06 mole) in methanol (30 mL) in the presence of anhydrous potassium carbonate (2 g) was kept at room temperature for about 25 hours. The solvent was removed *in vacuo* and the residue was recrystallized from chloroform to furnish compound.



1,3-benzoxazole-2-thiol (1,3-benzoxazol-2-yl) chloroethanethioate

Step 2: Synthesis of (2-Hydrazinoacetyl)-mercaptobenzoxazole

To a solution of 1 (0.02 mole) and hydrazine hydrate (0.02 mole) in methanol (30 mL) was kept at room temperature for about 20 hours. The solvent was removed *in vacuo* and the resulting solid was dried recrystallized from chloroform to produce analytical pure material.

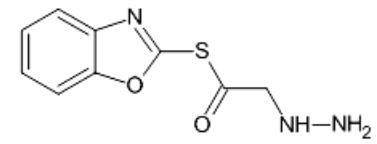


(1,3-benzoxazol-2-yl) chloroethanethioate (1,3-benzoxazol-2-yl) hydrazinylethanethioat

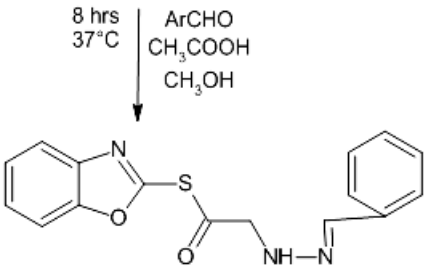
Step 3: Synthesis of - (Aryliden-hydrazinoacetyl)-mercaptobenzoxazole

A mixture of compound **2** (0.008 mole) and benzaldehyde (0.008 mole) and 2-3 drops of glacial acetic acid in ethanol (25 mL) was kept at room temperature for about 24 hours. The solvent was removed *in vacuo* and the resulting solid was dried and recrystallized. Likewise,

other compoundswere prepared in a similar way using different carbonyl compounds.[8]



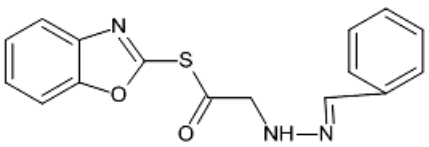
(1,3-benzoxazol-2-yl) hydrazinylethanethioate



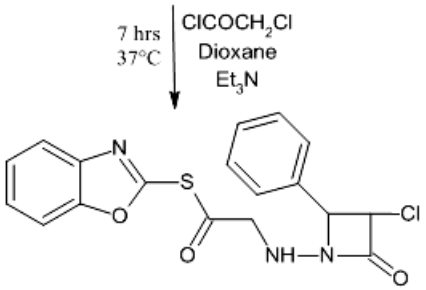
(1,3-benzoxazol-2-yl) [(2*E*)-2-benzylidenehydrazinyl]ethanethioat

Step 4: Synthesis of benzoxazole substituted azetidinone derivatives

A mixture of Schiffs base (0.01 mole) and triethylamine (3-4 drops) was dissolved in 1, 4-Dioxan(50 ml), cooled and stirred. To this well-stirred cooled solution, Chloro acetyl chloride (0.015 mol, ml) was added drop wise. Then stirred for an additional 3 hours at room temperature and reflux it for 7 hrs. The reaction mixture was filtered to remove triethylamine hydrogen chloride and the resultant solution was concentrated, cooled and poured into ice-cold water with stirring. The solid thus obtained was recrystallized from ethanol to yield benzoxazole substituted azetidinone derivatives.



(1,3-benzoxazol-2-yl) [(2*E*)-2-benzylidenehydrazinyl]ethanethioate



(1,3-benzoxazol-2-yl) [(3-chloro-2-oxo-4-phenylazetidin-1-yl)amino]ethanethioat

Table 1: Physical data of synthesized compounds.

Sl. No.	Compound code	Molecular Formula	Solubility	Melting point (^{0}C)	R*f*	Yield (%)
1.	CK-1	$C_{18}H_{14}ClN_3O_3S$	Methanol	189-192	0.54	70
2.	CK-2	$C_{18}H_{13}Cl_2N_3O_3S$	Methanol, Ethanol	185-189	0.44	74
3.	CK-3	$C_{18}H_{14}ClN_3O_4S$	Methanol	180-186	0.45	72
4.	CK-4	$C_{19}H_{16}ClN_3O_3S$	Methanol	182-187	0.57	68
5.	CK-5	$C_{18}H_{13}ClN_4O_5S$	Methanol, Ethanol	184-190	0.53	70

Table 2: Synthesis of benzoxazole substituted azetidinone derivatives.

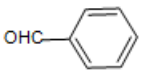
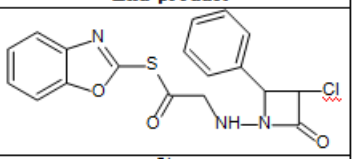
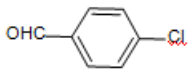
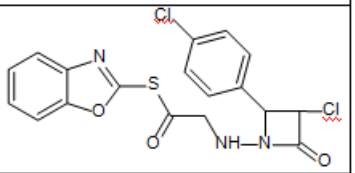
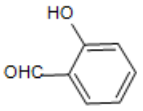
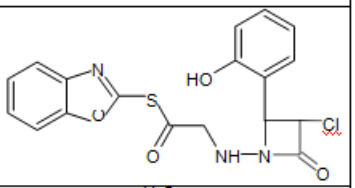
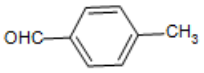
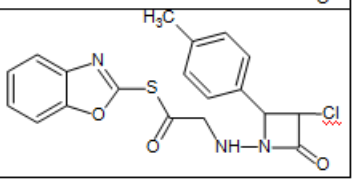
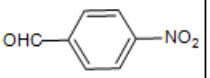
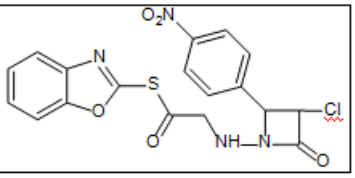
Sl. no.	Compound	Ar	End product
1	CK-1		
2	CK-2		
3	CK-3		
4	CK-4		
5	CK-5		

Table 3: IR Spectral Data.

Compound	IR (KBr cm^{-1}): Absorption Band Appear
CK-1	3346.53, 1550.49, 1594.36, 1619.91, 660 (for benzoxazole nucleus) 2923- 1344.14 (NH stretch), 1727.72(C=O), 750.174 (Cl)
CK-2	3422.53, 1548.56, 1551.45, 1573.63, 1240, 1057.76, 754.995 (benzoxazole nucleus), 3422.06 (NH), 1660.41(C=O), 820.563 (Cl)
CK-3	3203.18, 1564.995, 1529.95, 1347.03, 1253.5,

	754.995 (benzoxazole), 1700.01(C=O), 3563.81(OH Stretch)
CK-4	3387.35, 1584.24, 1619.91, 1647.88, 1051.01, 668.214(Benzoxazole nucleus), 2956.34-1347.03(NH stretch), 1689.34(C=O)
CK-5	3446.6, 1590.66, 1584.68, 1577, 1250.32, 1026.55, 680.5(benzoxazole), 3089-1320.14(NH stretch), 1690.19(C=O), 1637.75 (NO2 stretch)

Table 4: NMR Spectral Data.

Compound	^{1}H NMR and ^{13}C NMR
CK-1	^{1}H NMR: δ6.5-8 (Aromatic proton) δ4.7, 3.4 (aliphatic proton), δ4.4 (CH Cl).
CK-2	^{1}H NMR: δ6.9-7.5 (Aromatic proton) δ4.6, 4.2 (aliphatic proton), δ4.5(CH-Cl) ^{13}C NMR: δ117.21-135.73 (Aromatic carbon, aliphatic CH2), 172.34,166.03(C=O), 156.88(C-S), 153.71(C-O)
CK-3	^{1}H NMR: δ6.7-7.5 (Aromatic proton) δ4.8 (aliphatic proton), δ4.2 (CH-Cl).
CK-4	^{1}H NMR: δ6.8-8.4 (Aromatic protonδ4.7 (CH2) δ4.2 (CH-Cl) δ3.8 (CH2).
CK-5	^{1}H NMR: δ6.5-8 (Aromatic proton) δ4.7, 3.4(aliphatic proton), δ4.4 (CH-Cl) δ6.9-8. 7(Aromatic proton), δ4.9 (CH2), δ4.2 (CH-Cl), δ3.1 (CH2). ^{13}C NMR: δ117.21-135.73 (Aromatic carbon, aliphatic CH2), δ 172.24, 156.10(C=O), δ 153.69 (C-S), δ 140.67 (C-O).

Table 5: Mass spectral data.

Compound	Mass
CK-2	M+2 peak-425.500, Base peak-348.550
CK-5	MASS M+2 peak-435.100, base peak- 282.900.

MTT Assay

The MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals (Insoluble) by living cells, which represents mitochondrial activity. Only the compound CK-5 is opted in MTT assay with various concentrations like 50µg, 100 µg, 200 µg, 400 µg, 800 µg. SKMEL cell is taken for the study. Percentage viability of each treatment was calculated using the formula:

$$Percentage\ viability = OD\ Of \frac{TEST}{CONTROLL} * 100$$

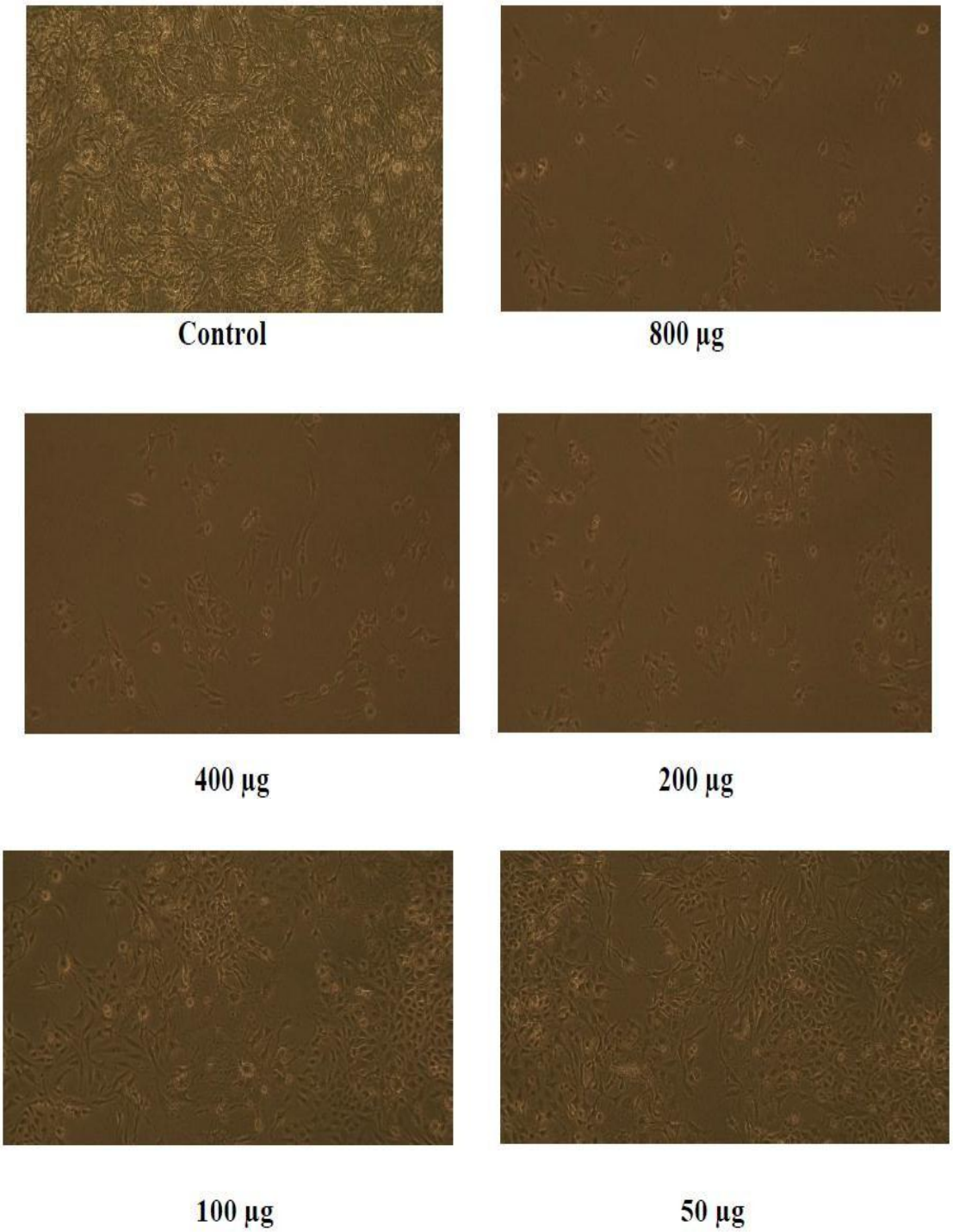


Fig. 1: Images of MTT assay of CK-5 on SKMEL cell line.

Percentage inhibition of each treatment was calculated using the formula

$$\textbf{Percentage inhibition} = 100 - \textbf{percentage viability}$$

Molecular docking

The molecular docking studies of synthesized compounds with VEGFR-2 are done. The schematic 3D representation of compounds with receptor VEGFR-2 (4DBN) was visualized

and shown in Figure 1. The docking score of compounds and the standard (sorafenib) with 4DBN is given in table 6. Various hydrogen bond interactions were shown with Ser 535 for derivative CK-1, Val 599for derivative CK-2, Phe 594 for derivative CK-3, Asp 593 for derivative CK-4, Phe 582 for derivative CK-5. From the docking studies, derivative CK-5, CK-1 and CK-2 Showed high docking score which indicate that these compounds possess high affinity andhigh polar interaction towards protein 4DBN.[9]

Table 6: Docking score of Derivatives and Standard (Sorafenib) with protein 4DBN (ligand Binding domain of vascular endothelial growth factor.

S. No.	Compound code	Docking score (Kcal/mol)
1.	CK-1	-8.6
2.	CK-2	-8.4
3.	CK-3	-8.1
4.	CK-4	-8.0
5.	CK-5	-9.3
6.	Standard (Sorafenib)	-10.1

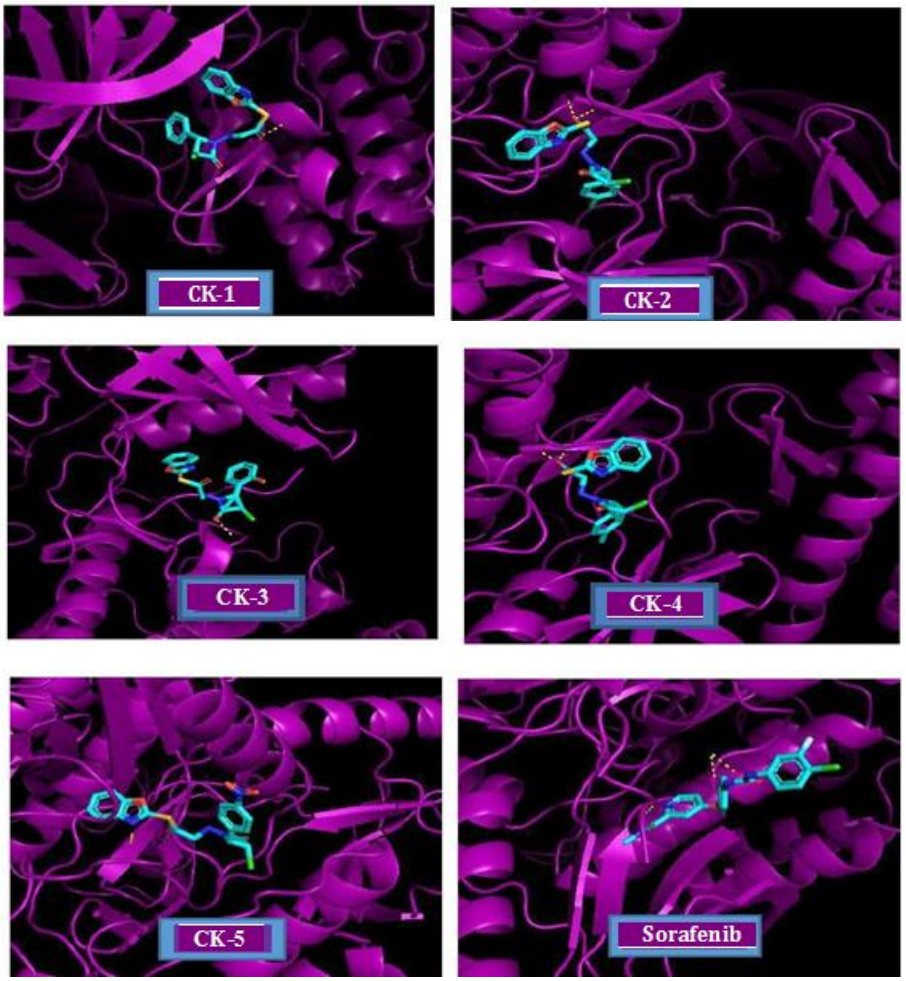


Fig. 2: Docked images of derivatives and standard (sorafenib) with protein 4 DBN (Ligand-binding domain of vascular endothelial growth factor 2.

RESULTS AND DISCUSSION

All the compounds were synthesized and purified. The characterization was done by spectral techniques includes IR spectroscopy, proton NMR, ^{13}C NMR and MASS spectral techniques. The anticancer study of Benzoxazole substituted azetidinone derivatives was carried out by MTT Assay.

MTT Assay

The percentage inhibition of the cell is calculated. It is tabulated in table 7.

Table 7: Anti-neoplastic activity of CK-5 on SKMEL cell lines.

Concentration (µg/ml)	% Viability	% Inhibition
50	80.77	19.33
100	89.72	11.28
200	65.04	34.96
400	37.02	62.98
800	25.81	74.19

DISCUSSION

The benzoxazole substituted azetidinone derivatives (CK1 - CK5) were synthesized and characterized. The purity of the synthesized compounds was checked by melting point using digital melting point apparatus. The chemical structure of the derivatives was confirmed by spectral techniques. From docking studies, the study concludes that the designed benzoxazole substituted azetidinone derivatives are found to have good interaction in binding pocket of target 4DBN; derivatives possess good anticancer activity with high binding affinity. The anticancer activity of the synthesized compounds was studied through *invitro* assays like MTT assay. From the biological evaluation the results concluded that the compound CK-5 and CK-2 exhibit good anticancer activity than others.

CONCLUSION

The present study is focused on the design, synthesis and biological evaluation of benzoxazole substituted azetidinone derivatives from 2-mercaptobenzoxazole.

The findings are

- ✓ Five derivatives were prepared through conventional method.
- ✓ IR spectra of five compounds predict the expected frequencies.
- ✓ ^{1}H NMR and ^{13}C NMR spectra of five derivatives provide signals.

- ✓ MASS spectra of two derivatives provide peaks.
- ✓ Anticancer study was carried out and the result is given.
- ✓ Molecular docking studies are conducted and the results are shown.

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