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SYNTHESIS AND *IN-VITRO* ANTIOXIDANT, ANTICANCER ACTIVITY OF SCHIFF'S BASES AND AZETIDINONE OF 6-ETHOXY 2-AMINO BENZOTHIAZOLE DERIVETIVES

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ABSTRACT

A series of Schiff's base derivatives have been synthesized and incorporated with azetidinone as biologically effective agent with good therapeutic values having minimum toxic levels. 6-ethoxy 2-amino benzothiazole was synthesized from 6-ethoxy aniline by refluxing with ammonium thiocyanate in acidic medium. 6-ethoxy 2- amino benzothiazole was then condensed with various aromatic aldehydes by using ethanol as solvent to yield different Schiff's bases. Azetidinones derivatives were synthesized from Schiff's bases in the presences of chloroacetyl chloride, triethylamine and dioxane as solvent. The structures of synthesized compounds were characterized by IR, ^{1}H-NMR and Mass spectral analysis. Purity of the individual compound was confirmed by TLC. The synthesized compounds were evaluated for their *in-vitro* antioxidant activity and compared with ascorbic acid as standard. The compounds having more antioxidant activity were evaluated for their anticancer activity by Micro culture tetrazolium assay (MTT assay).

KEYWORDS: 6-ethoxy 2-amino benzothiazole, Schiff's bases, Azetidinones, Antioxidant activity, Anticancer activity.

INTRODUCTION

The human body possess innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase and glutathione peroxidase. The unbalance between formation and detoxification of free radical species results in the progression of

oxidative stress and leads to the development of chronic and degenerative diseases. Therefore, inhibition of oxidative damage by supplementation with an antioxidant and/or free radical scavengers might reduce the risk of these diseases.[1,2] The chemistry and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. A number of heterocyclic derivatives containing nitrogen and sulphur as hetero atoms serve as unique and versatile scaffolds for experimental drug design.[3] Benzothiazole is the one of the most important heterocyclic compound that has received overwhelming response owing to its diversified molecular design and remarkable optical and electronic properties.[4] Benzothiazole derivatives posses a wide spectrum of biological applications such as antitumor, antimicrobial, schicotosomicidal, anti-inflammatory, anticonvulsants, antidiabetic, antipsychotic and diuretic etc.[5] The feasible positions for substitution group at C-2 position exhibit various pharmacological activities such as anticancer, anthelmintic and cardiovascular.[6] Indole with azetidinone have been reported to antioxidant and anticancer activity.[7] The proposed work is based upon the development of newer analogues of benzothiazoles and azetidinones followed by their biological evaluation such as antioxidant activity and anticancer activity.

MATERIALS AND METHODS

General experimental work

All reactions were carried out under prescribed laboratory conditions, All reactions requiring anhydrous conditions, were conducted inflame dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization technique, wherever necessary. Their melting points were checked with the available literature. Melting points of synthesized compounds were determined by open end capillary tube method. NMR spectra were recorded on BRUKER-SPECTROSPIN 400MHz spectrometer in DMSO, Tetra methyl silane (TMS; $\delta = 0.00$ ppm) served as internal standards for ^{1}H NMR and chemical shifts were measured in parts per million.[8] IR spectra were measured using Shimadzu FT-IR in the range of 4000-450 cm^{-1} KBr pellet technique. Mass spectra were measured on mass spectroscopy and molecular ion peak are recorded in m/z ratio.[9]

PROCEDURE

Step-1: Synthesis of 6-ethoxy-1,3-benthiazol-2-amine

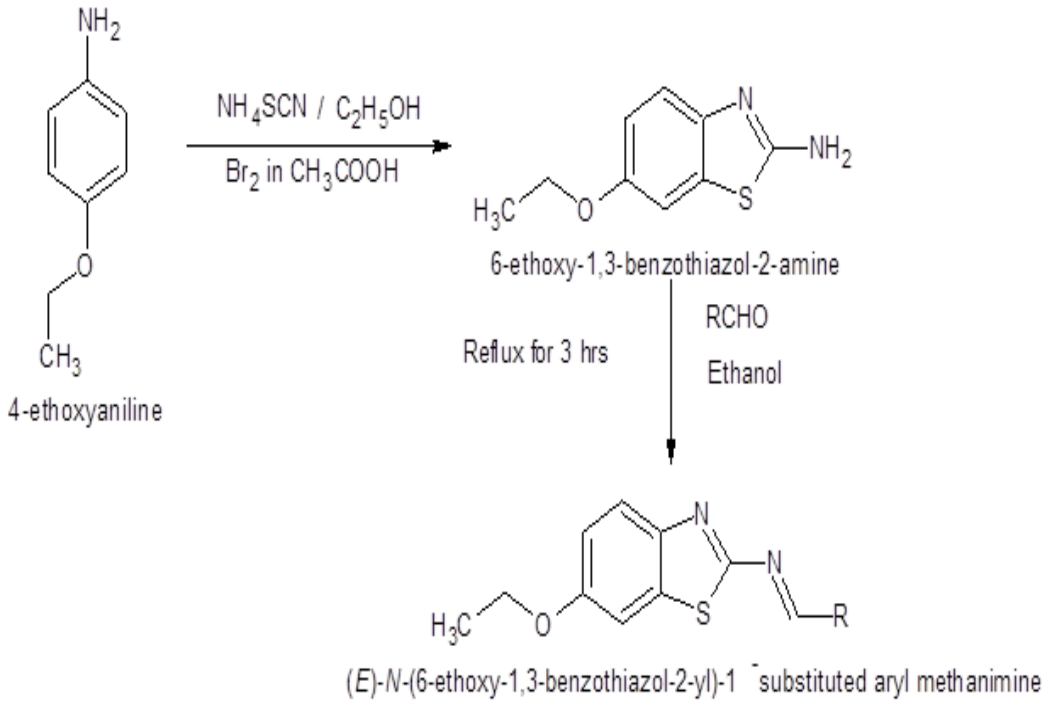
Equimolar quantities of ethoxyaniline (0.02mol), and ammonium thiocyanate (1.5g, 0.02mol) were dissolved in ethanol containing 2ml of Conc. Hydrochloric acid. To this bromine in glacial acetic acid (2.7ml, 0.05mol) was added and the reaction mixture was refluxed for 1hr. Then, it was cooled in ice-water mixture. The precipitate obtained was strained well, filtered, washed with cold water and then dried. The crude product was re-crystallized from rectified spirit.[10]

Step 2: Synthesis of Schiff's bases (KS1-KS5)

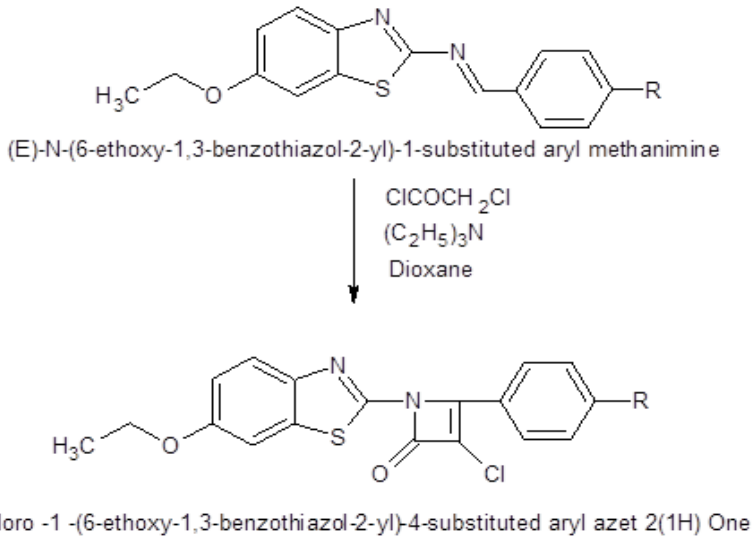
A mixture of 2-amino-substituted benzothiazole (0.02 mol) and *p*-chlorobenzaldehyde, anisaldehyde, N, N- dimethyl amino benzaldehyde, salicylaldehyde, cinnamaldehyde (0.02 mol) was refluxed in absolute ethanol (40 ml) for 3 hrs. The excess solvent was distilled off and then the resulting solid was washed with water, dried and re-crystallized from ethanol.[11]

Step 3: Synthesis of azetidinones from Schiff's bases (KS6-KS10)

Triethylamine (0.01 mol) in 1, 4-dioxane, chloroacetyl chloride (0.01mol) was added drop wise to a solution of compound Schiff's base (0.005 mol) at room temperature. The reaction mixture was stirred for 30 min. The mixture was then refluxed for 3 hrs on a water bath. The solid obtained after removal of 1, 4-dioxane was re-crystallized from ethanol.[12]



Scheme-1: Step 1&2



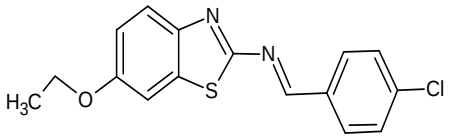
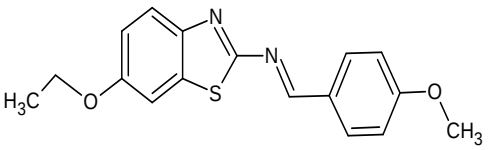
Scheme 2: Step 3.

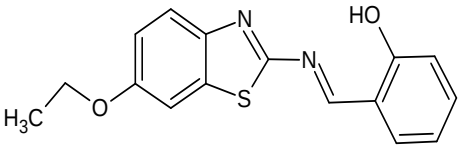
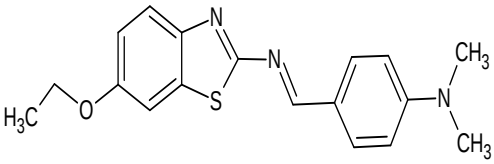
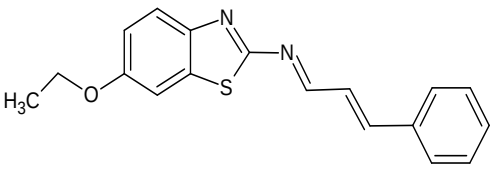
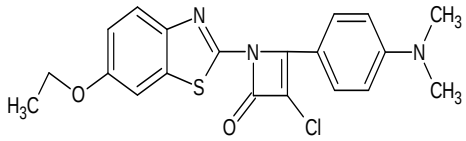
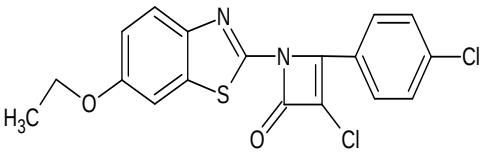
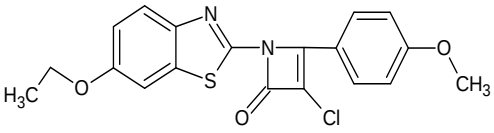
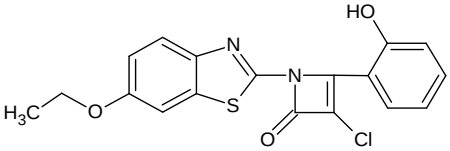
R= - Cl, - OCH_3, -OH, - $N(CH_3)_2$, - H

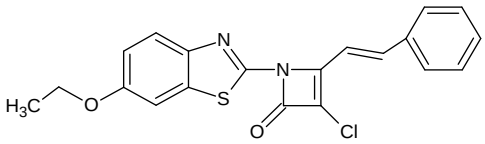
Table 1: Physical data of synthesized compound.

Sr.no	Code	Molecular formula	Physical nature	% yield	m.p	R_f value
1	**KS1**	C16H13ClN2OS	Yellow solid	72.58	230	0.60
2	**KS2**	C17H16N2O2S	Yellow solid	83.49	238	0.53
3	**KS3**	C16H14N2O2S	Pale yellow solid	90.21	241	0.57
4	**KS4**	C18H19N3OS	Orange solid	90.00	233	0.58
5	**KS5**	C18H16N2OS	Red solid	89.66	248	0.50
6	**KS6**	C20H18ClN3O2S	Orange solid	76.05	195	0.50
7	**KS7**	C18H12Cl2N2O2S	Brown solid	73.07	201	0.48
8	**KS8**	C19H15ClN2O3S	Darkbrown solid	75.12	215	0.47
9	**KS9**	C18H13ClN2O3S	Black solid	80.64	198	0.51
10	**KS10**	C20H15ClN2O2S	Palebrown solid	78.04	213	0.54

Table 2: Spectral data of synthesized compounds.

Sr.no	Code	Structure and IUPAC name	IR	^{1}HNMR	Mass
1	KS1	 (*E*)-1-(4-chlorophenyl)-*N*-(6-methoxy-1,3-benzothiazol-2-yl)methanimine	1261.45(C-O-C) 1512.19(C=N) 804.32(C-N) 626.87(C-S) 694.37(Ar-Cl) 1477.47(C=C)	7.8(q,6H,Ar-H) 8.0(d,4H,Ar-H) 3.94(m,5H,O-CH_2CH_3) 9.99(s,1H,N=CH)	316.80
2	KS2	 (*E*)-*N*-(6-methoxy-1,3-benzothiazol-2-yl)-1-(4-methoxyphenyl)methanimine	1251.8(C-0-C) 1512.19(C-=N) 902.69(C-N) 651.94(C-S) 1274.95(Ar-OCH_3) 1465.19(C=C)	7.95(m,6H,Ar-H) 7.26(d,4H,Ar-H) 3.99(m,5H, OCH_2CH_3) 3.92(s,5H, OCH_3) 9.89(s,1H,N=CH)	312.38

3	KS3	 2-{(*E*)-[(6-methoxy-1,3-benzothiazol-2-yl)imino]methyl}phenol	1259.52(C-O-C) 1440.83(C=N) 1120.64(C-N) 659.66(C-S) 3633.89(Ar-OH) 1490.97(C=C) 3032.10(C-H)	6.98(m,6H,Ar-H) 7.42(q,4H,Ar-H) 3.98(m,4H,Ar-H) 9.99(s,5H,OCH_2CH_3) 8.80(s,1H,Ar-OH)	298.36
4	KS4	 4-{(*E*)-[(6-methoxy-1,3-benzothiazol-2-yl)imino]methyl}-*N*,*N*-dimethylaniline	1232.51(C-0-C) 1587.99(C=N) 1174.65(C-N) 680.87(C-S) 1371.01(N-CH_3) 1535.34(C=C) 3028.24(C-H)	6.73(t,6H,Ar-H) 6.92(d,4H,Ar-H) 3.17(m,5H,O-CH_2CH_3) 9.37(s,1H,N=CH) 3.09(s,6H,N-$(CH_3)_2$)	325.43
5	KS5	 (1*E*,2*E*)-*N*-(6-methoxy-1,3-benzothiazol-2-yl)-3-phenylprop-2-en-1-imine	1249.87(C=C) 1510.26(C=N) 1087.85(C-N) 686.66(C-S) 1612.49(Ali-C=C) 1463.97(C=C) 3032.10(C-H)	7.43(m,6H,Ar-H) 7.20(d,4H,Ar-H) 4.00(m,5H,O-CH_2CH_3) 9.66(s,1H,N=CH) 1.19(t,2H,CH=CH)	308.39
6	KS6	 CH_4 3-chloro-4-[4-(dimethylamino)phenyl]-1-(6-methoxy-1,3-benzothiazol-2-yl)azet-2(1*H*)-one	1249.87(C-O-C) 920.05(C-N) 682.8(C-S) 1365.00(N-CH_3) 725.23(C-Cl) 1720.5(N-C=O)	6.96(m,6H, Ar-H) 7.81(q,4H,Ar-H) 3.18(m,5H,O-CH_2CH_3) 3.09(s,6H,N-$(CH_3)_2$)	399.89
7	KS7	 3-chloro-4-(4-chlorophenyl)-1-(6-methoxy-1,3-benzothiazol-2-yl)azet-2(1*H*)-one	1205.51(C-O-C) 1261.45(C=N) 1745.58(C-N) 582.5(C-S) 636.51(C-Cl) 1512.19(C=C) 3030.17(C-H)	7.54(m,6H,Ar-H) 6.96(m,4H,Ar-H) 3.95(t,5H,O-CH_2CH_3)	391.27
8	KS8	 3-chloro-1-(6-methoxy-1,3-benzothiazol-2-yl)-4-(4-methoxyphenyl)azet-2(1*H*)-one	1259.52(C-O-C) 1130.29(C-N) 758.02(C-Cl) 632.65(C-S) 1755.22(N-C=O) 1598.99(C=C)	6.99(m,6H,Ar-H) 7.82(d,4H,Ar-H) 4.01(m,5H,O-CH_2CH_3) 3.88(s,3H,O-CH_3)	386.85
9	KS9	 3-chloro-4-(2-hydroxyphenyl)-1-(6-methoxy-1,3-benzothiazol-2-yl)azet-2(1*H*)-one	1720.5(C-O-C) 1259.52(C-N) 759.95(C-Cl) 626.87(C-S) 1753.29(N-C=O) 3570.24(Ar-OH)	7.26(m,6H,Ar-H) 9.22(d,4H,Ar-H) 3.94(m,5H,O-CH_2CH_3) 11.02(s,1H,Ar-H)	372.82

10	KS10	 3-chloro-1-(6-methoxy-1,3-benzothiazol-2-yl)-4-[(*E*)-2-phenylethenyl]azet-2(1*H*)-one	1249.87(C-0-C) 648.08(C-Cl) 1174.65(C-N) 686.66(C-S) 1957.75(N-C=O) 1662.64(Ali-C=C)	6.73(m,4H,Ar-H) 7.27(d,4H,Ar-H) 3.98(m,5H,O-CH_2CH_3) 1.79(s,2H,CH=CH)	382.86

Biological Evaluation

***in-vitro* Antioxidant activity[13]**

All the synthesized compounds were screened for their *in-vitro* antioxidant activity by phospho molybdenum method at concentration 100 µg/ml, 200 µg/ml and 300 µg/ml. Dimethyl sulfoxide (DMSO) was used as solvent control. The absorbance was measured at 695 nm. Ascorbic acid was used as standard for comparison. All the synthesized compounds showed varying degree of anti-oxidant activity. The results are shown in Table 3.

***in-vitro* Anti-cancer activity[14,15]**

Among the synthesized compounds, the compounds showing more anti-oxidant activity such as **KS4** and **KS6** were screened for their *in-vitro* anti-cancer activity by Micro culture tetrazolium assay at different concentration such as 0.25 µM, 2.5 µM, 25 µM, 50 µM and 100 µM. The absorbance was measured at 570 nm. The percentage of cell viability and cell inhibition were calculated with respect to control. The cytotoxicity was obtained by comparing the absorbance between the samples and the control. The synthesized compounds showed varying degree of anti-cancer activity. The results are shown in Table 4.

% Cell viability = $A_{test}/A_{control} \times 100$

% Cell inhibition = $100\text{-}A_{test}/A_{control} \times 100$

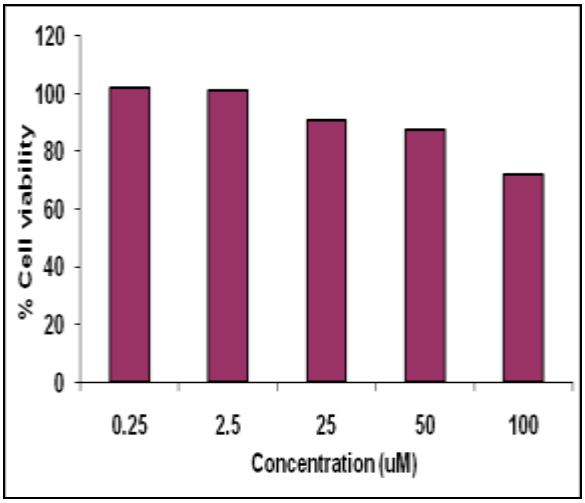
Table 3: Results of Anti-oxidant activity of synthesized compounds by phospho molybdenum method.

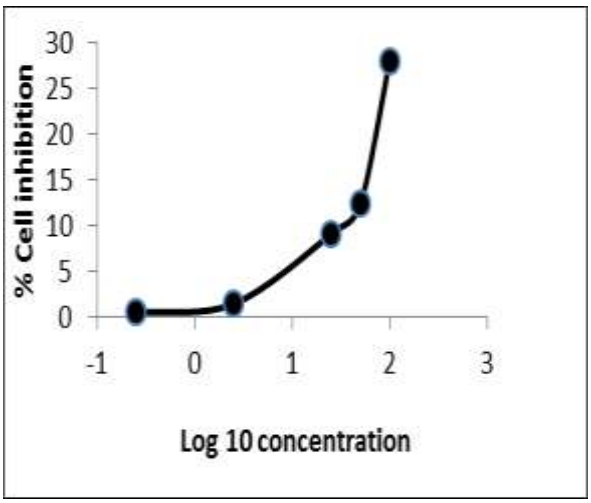
Compounds	Absorbance of different concentration		
	100µg/ml	200µg/ml	300µg/ml
KS1	1.43±0.009	1.76±0.009	1.97±0.007
KS2	1.56±0.007	1.70±0.004	1.73±0.007
KS3	1.45±0.007	1.83±0.007	1.85±0.007
KS4	1.70±0.007	1.96±0.007	2.32±0.009
KS5	1.60±0.004	1.61 ±0.004	1.83±0.009
KS6	1.78±0.007	1.93± 0.004	2.32±0.002
KS7	1.54±0.007	1.88±0.007	1.93±0.009
KS8	1.50±0.007	1.73±0.007	1.83±0.007
KS9	1.59±0.007	1.63±0.007	1.72±0.007
KS10	1.67±0.009	1.80 ±0.007	1.90±0.007
STD	1.81±0.009	2.10±0.009	2.52±0.007

STD-Standard (Ascorbic acid).

Table 4: Results of anti-cancer activity by Micro culture tetrazolium assay of compounds KS4 and KS6.

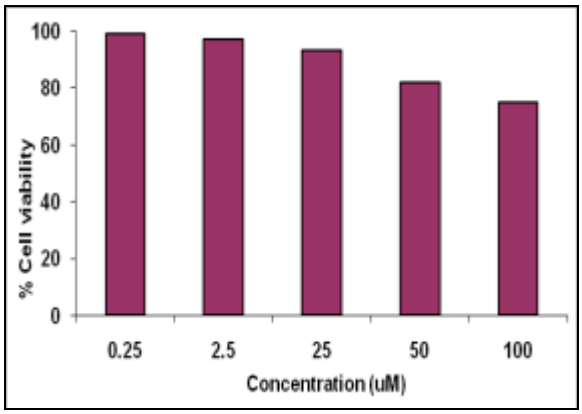
Compounds name	Concentration µM	% Cell viability	% Cell inhibition	IC 50
KS4	0.25	99.521	0.478	>100
	2.5	98.564	1.436	
	25	90.836	9.09	
	50	87.649	12.440	
	100	72.031	27.99	
KS6	0.25	99.203	0.717	
	2.5	97.290	2.631	
	25	93.306	6.698	
	50	82.151	17.942	>100
	100	74.820	25.119	

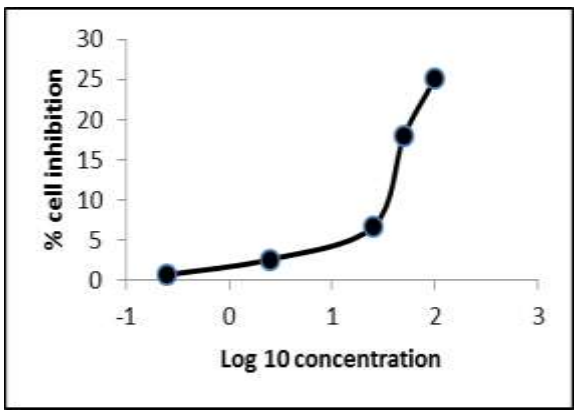




1a **1b**

Diagram 1a, 1b showing the anticancer activity of synthesized compound KS4 by Micro culture tetrazolium assay.





2a **2b**

Diagram 2a, 2b showing the anticancer activity of synthesized compound KS6 by Micro culture tetrazolium assay.

RESULTS AND DISCUSSION

The basic nucleus 6-ethoxy 1,3 benzothiazol-2-amine was synthesized from 4-ethoxy aniline by refluxing with ammonium thiocyanate using bromine in glacial acetic acid. Schiff's bases were synthesized from 6-ethoxy 1,3 benzothiazol-2-amine by condensation with different aldehydes. Then azetin-2-one derivative was synthesized by refluxing chloroacetyl chloride with triethylamine in 1,4 dioxane. The synthesized compounds were identified by TLC. The spectral data of FT-IR, ^{1}H NMR and Mass spectroscopy. IR spectrum data showed relevant data peaks for C=C, C=N, C=O groups. ^{1}H NMR also showed relevant proton peaks for all synthesized compounds. The Mass spectrum data confirm the molecule ion peaks for all synthesized compounds. Based on the spectral data, the structures of all synthesized compounds were conformed. The Anti-oxidant activity of synthesized compounds was done by phospho molybdenum method at concentration 100 µg/ml, 200 µg/ml and 300 µg/ml. The absorbance was compared with standard Ascorbic acid. All the synthesized compounds showed Anti-oxidant activity. Among the synthesized compounds, benzothiazole with Schiff's base have more antioxidant activity than benzothiazole with azetidinone nucleus. The synthesized compounds **KS4** and **KS6** have more antioxidant activity and they were selected for the evaluation of anticancer activity. The anti cancer activity of selected compounds were screened by Micro culture tetrazolium assay at a concentration of 0.25 µM, 2.5 µM, 25 µM, 50 µM and 100 µM. The percentage of cell viability and cell inhibition were calculated with respect to control. The percentage of cell viability and cell inhibition of the synthesized compounds **KS4** and **KS6** at 100 µM was 72.03%, 27.99 %and 74.82%, 25.119% respectively showing anticancer activity. The compounds **KS4** and **KS6** exhibited less anticancer activity because of they have more cell viability and low cell inhibition. The percentage of cell inhibition of synthesized compound **KS4 (**Schiff's base**)** was 27% whereas the compound **KS6 (**3-chloro azetidinone**)** showed percentage of cell inhibition was 25%. The increase in the percentage of cell inhibition may be due to the presence of Schiff's base.[6,17]

CONCLUSION

The present work was very interesting because of the presence of 6-ethoxy 1,3 benzothiazole attached to azetidine-2-one that was ß-lactum. All the synthesized compounds showed antioxidant activity and the synthesized compounds **KS4** and **KS6** showed less anticancer activity. The author has already reported the anti-inflammatory and anti-bacterial activity of Benzothiazole with Schiff's bases and Azetidinones.[16] In the present study, it was observed

Benzothiazole with Schiff's base have good antioxidant activity and anticancer activity.[6,17] Benzothiazole with Azetidinones have good antioxidant activity but less anticancer activity, this may be due to that was β-lactum ring.[7]

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