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SYNTHESIS AND IN-VITRO BIOLOGICAL EVALUATION OF SCHIFF'S BASE AND AZETIDINONE OF 6-ETHOXY 2-AMINO BENZOTHIAZOLE DERIVETIVES

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ABSTRACT

A series of Schiff's base of several 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* anti microbial activity against several *staphylococcus aureus* and klebsiella pneumoniae. All the compounds have shown significant anti-inflammatory and antibacterial activity which was compared with Diclofenac and Amikacin respectively as standard.

KEYWORDS: 6-ethoxy 2-amino benzothiazole, Schiff's bases, Azetidinones, Anti-inflammatory activity, Antibacterial activity.

INTRODUCTION

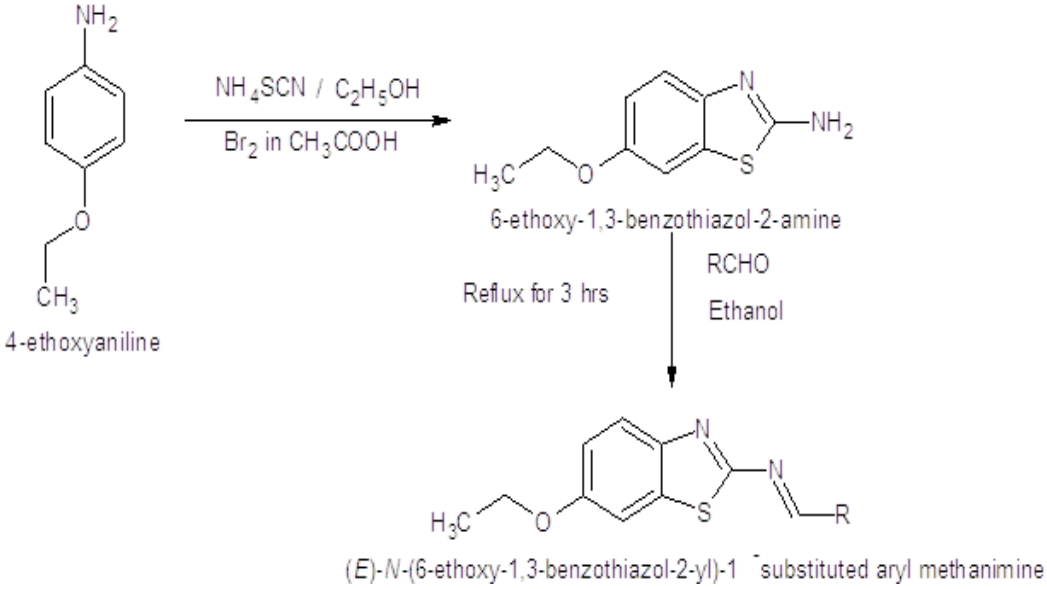
The chemistry and biological study of heterocyclic compounds has been an interesting field for a long time in medicinal chemistry. A number of heterocyclic derivatives containing nitrogen and sulphur as hetero atoms serve as unique and versatile scaffolds for experimental drug design.[1] Proposed work is based upon the development of newer analogues of benzothiazoles and azetidinones followed by their biological evaluation. 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.[2] Benzothiazole derivatives posses a wide spectrum of biological applications such as antitumor, antimicrobial, schicotosomicidal, anti-inflammatory, anticonvulsants, antidiabetic, antipsychotic and diuretic etc.[3] 2-substituted benzothiazole has immersed in its usage as a core structure in the diverse therapeutic applications. 2-Azetidines have been extensively investigated by the organic chemists due to their close association with various types of biological activities. Azetidines -2-one also have great importance because of the β- lactum derivatives possess antibacterial property. Recently, some other types of biological activity beside the antibacterial activity have been reported in compounds containing 2-Azetidinone ring.[4] Hence Azetidinone or β-lactum ring in several families of bicyclic antibiotics had stimulated our continuous interest because of their high antibacterial activity. Azetidinones can be prepared from Schiff's bases which are the condensation products of carbonyl and amino compounds.[5]

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.[6] 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.[7]

Procedure



Scheme-1

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

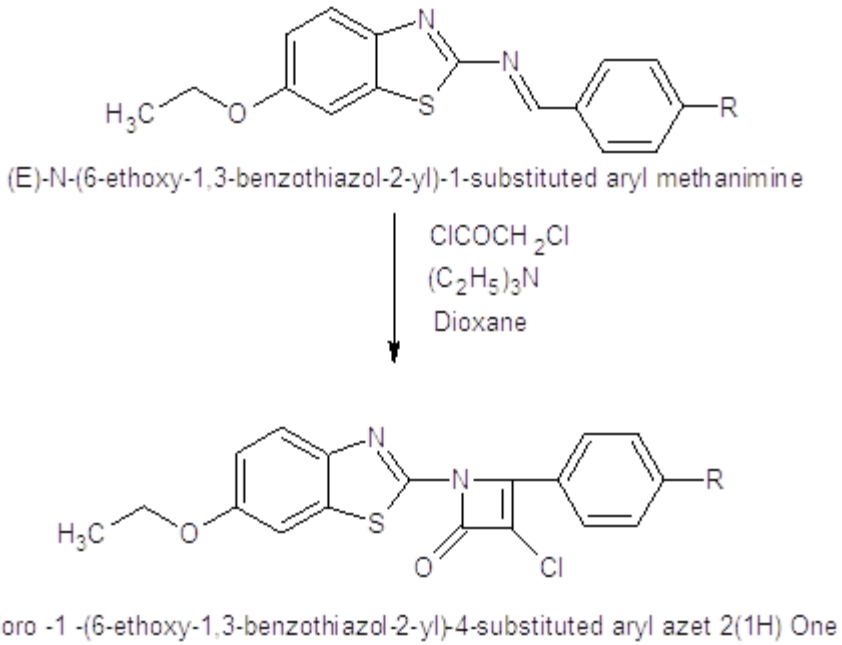
Equimolar quantities of ethoxyaniline (0.02mol), and ammonium thiocyanate (1.5g, 0.02mol) were dissolved in ethanol containing 2ml of Conc. Hydrochloric acid. To this bromine in glacial acetic acid (2.7ml, 0.05mol) was added and the reaction mixture was refluxed for 1hr. Then, it was cooled in ice-water mixture. The precipitate obtained was strained well, filtered, washed with cold water and then dried. The crude product was re-crystallized from rectified spirit.[8]

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.[9]

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.[10]



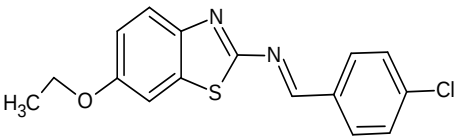
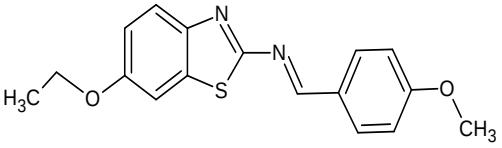
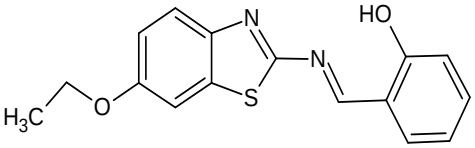
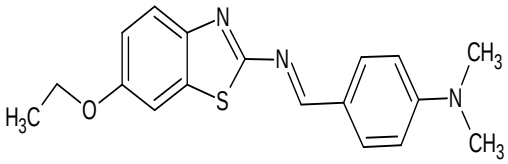
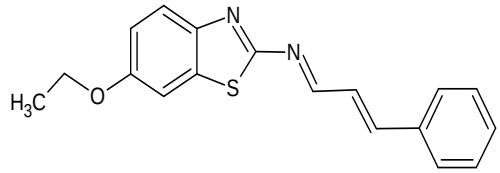
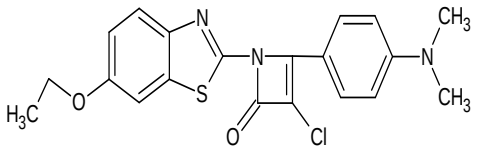
Scheme 2

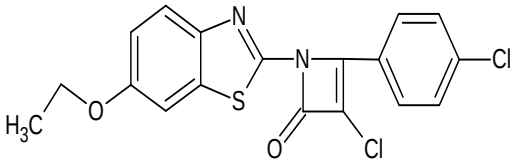
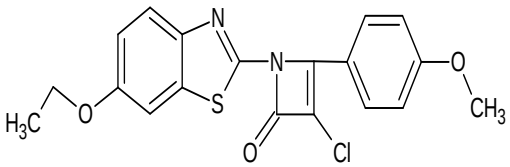
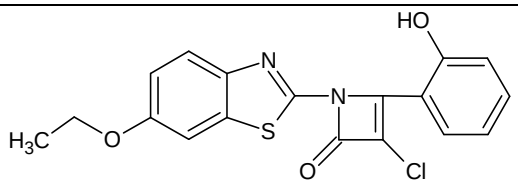
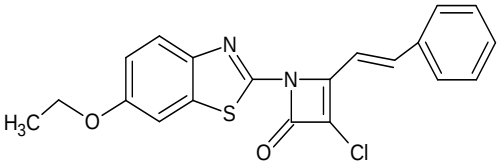
R= - Cl, - OCH_3, -OH, - N $(CH_3)_2$, - H

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 Antimicrobial activity

All the synthesized compounds were screened for their *in-vitro* antibacterial activity by cup plate method in Mullar hinton nutrient agar culture medium against *staphylococcus aureus* and klebsiella pneumoniae at concentration 50 µg/ml and 100 µg/ml. DMSO was used as solvent control. The zone of inhibition of synthesized compound was measured and compared with standard Amikacin. All the synthesized compounds showed varying degree of antibacterial activity.[11] The results are shown in Table 3.

Table 3: Results of antibactirial activity of synthesized compounds by Cup Plate method.

Sample	Diameter of zone of inhibition (in mm)			
	Klebsiella pneumoniae		*Staphylococcus aureus*	
	50 µ g/ml	100 µ g/ml	50 µ g/ml	100 µ g/ml
KS1	7	11	11	13
KS2	R	10	R	11
KS3	10	12	8	12
KS4	11	12	11	13
KS5	11	13	R	12
KS6	12	13	14	11
KS7	8	12	9	13
KS8	6	10	7	11
KS9	7	11	7	12
KS10	12	13	12	13
Control	R	R	R	R
Standard	17	17	17	17

R- Resistant Control - DMSO Standard- Amikacin

In-vitro Anti-inflammatory activity

All the synthesized compounds were screened for their *in-vitro* anti-inflammatory activity by Membrane stabilization assay at different concentration such as 100 µ g/ml, 200 µg/ml and 300 µg/ml. The absorbance was measured at 560 nm.[12] Diclofenac sodium was used as standard for comparison. The percentage of membrane stabilization was calculated as per the formula given below and compared with standard. All the synthesized compounds showed varying degree of anti-inflammatory activity. The results are shown in Table 4.

% Membrane stabilization = $100\text{-}A_{test}/A_{control} \times 100$

Table 4: Results of anti-inflammatory activity by Membrane stabilization assay.

Compounds name	Absorbance			% of activity		
	100µg/ml	200µg/ml	300µg/ml	100µg/ml	200µg/ml	300µg/ml
KS1	0.489±0.003	0.431±0.001	0.395±0.001	74.11	77.18	79.08
KS2	0.554±0.001	0.541±0.002	0.501±0.003	70.67	71.36	73.47
KS3	0.611±0.005	0.599±0.002	0.532±0.002	67.65	68.29	71.83
KS4	0.445±0.003	0.415±0.001	0.341±0.002	76.44	78.03	81.91
KS5	0.698±0.005	0.629±0.002	0.601±0.002	63.04	66.70	68.18
KS6	0.495±0.003	0.441±0.003	0.415±0.001	73.19	76.65	78.03
KS7	0.461±0.003	0.410±0.004	0.425±0.001	75.59	76.29	77.50
KS8	0.751±0.002	0.710±0.001	0.698±0.002	62.40	62.04	63.04
KS9	0.811±0.001	0.799±0.002	0.751±0.001	57.70	57.70	60.24
KS10	0.851±0.002	0.825±0.001	0.798±0.002	56.32	56.32	57.75
STD	0.415±0.003	0.346±0.002	0.295±0.002	81.63	81.68	84.38

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 refluxing with chloroacetyl chloride and triethylamine in 1,4 dioxane. The synthesized compounds were identified by TLC and the spectral data of FT-IR, ^{1}H NMR and Mass spectroscopy. IR 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 spectrums confirm the molecule ion peaks for all synthesized compounds. Based on the spectral data the structures of all synthesized compounds were conformed. The Antibacterial activity of synthesized compounds was done by cub plate method against Klebsiella pneumoniae and Staphylococcus auerus at concentration 50 µg/ml, 100µg/ml and zone of inhibition were compared with standard Amikacin. All the synthesized compounds showed Antibacterial activity. The antibacterial activity of synthesized compounds may be due to the presence of 6-ethoxy 1,3 Benzothiazole and Azetidinones nucleus. Since the first antibiotic penicillin was ß-lactum ring fused with 1,3 thiazolidine showed broad spectrum antibacterial activity. The Anti-inflammatory activity of synthesized compounds was screened by membrane stabilization assay at concentration 100µg/ml, 200µg/ml, 300µg/ml and the percentage of membrane stabilization compared with standard Diclofenac sodium.[12] The synthesized compounds showed Anti-inflammatory activity. The anti-inflammatory activity may be due to the presence of 6-ethoxy 1,3 Benzothiazole and Schiff's base.[1]

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 synthesized compounds showed antibacterial activity against ***Klebsiella pneumonia*** and ***Staphylococcus aureus*** when compared to standard Amikacin. The compounds **KS1, KS3, KS4, KS5, KS6** and **KS10** showed potent antibacterial activity against the tested organisms. The compound **KS5** showed resistant at lower concentration (50µg/ml) but showed more activity at higher concentration (100µg/ml). *In-vitro* anti-inflammatory activity of all synthesized compounds were evaluated and compared with standard Diclofenac. All the compounds showed significant anti inflammatory activity. Among ten synthesized compounds **KS1, KS2, KS4, KS6** and **KS7** shows good anti-inflammatory activity.

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