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Original article

Synthesis and Antibacterial Activity of Some Azetidinone Derivatives Containing 2-Amino 6,7 Substituted Benzothiazole

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A novel series of azetidinone AZ1-6 been prepared from the building blocks 2, 3, 4 (trisubstituted benzaldehyde)-*N*-(6,7 substituted-1,3-benzothiazol-2-yl) semicarbazone [2.026a-o]. All of the synthesized compounds have been confirmed by elemental analyses, IR and ^{1}H NMR spectral data. These newly synthesized compounds were screened for their antibacterial activity. Variable and modest activity was observed against the investigated strains of bacteria, however compounds AZ2, AZ3, AZ5 and AZ6 revealed significant antibacterial activity against *Bacillus subtilis* and *Pseudomonas* compared to the reference drug Procaine penicillin and Streptomycin.

Key words: Schiff base, Azetidinone, 2-Amino 6,7 substituted benzothiazole , Antibacterial activity.

2-Amino-6,7-Sübstitüe Benzotiyazol İçeren Bazı Azetidinon Türevlerinin Sentezi ve Antibakteriyel Aktiviteleri

2, 3, 4 (trisübstitüe benzaldehit)-*N*-(6, 7 sübstitüe-1,3-benzotiyazol-2-il) semikarbazon [2.026a-o] dan hareketle yeni bir seri azetidinon (AZ1-6) türevi hazırlanmıştır. Sentezlenen bileşiklerin yapıları elemental analiz, IR ve ^{1}H NMR verileriyle kanıtlanmış ve antibakteriyel aktiviteleri denenmiştir. Bileşiklerden AZ2, AZ3, AZ5 ve AZ6 *Bacillus subtilis* ve *Pseudomona'*a karşı referans bileşikler Prokain penisilin ve Streptomisin ile kıyaslandıklarında kayda değer aktiviteye sahip bulunmuştur.

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INTRODUCTION

The number of life threatening infections caused by multidrug resistant Gram-positive and Gram-negative pathogens has reached an alarming level in hospitals and the community. Infections caused by these organisms create a serious challenge to the scientific community and the need for an effective therapy has led to a search for novel antibacterial agents.

Various famous antibiotics like penicillins, cephalosporins and carbapenems are associated with antitumor (1) anti-inflammatory (2) antituberculer (3) activity due to the presence of 2-azetidinone ring in them. Similarly benzothiazole also have considerable attention because of their various biological and pharmacological activities like antituberculer (4), antimicrobial (5), anti-inflammatory (6).

Small ring heterocycles containing nitrogen, sulfur and oxygen gained great importance since a long time due to their important medicinal properties. It is well known that when one biologically active molecule linked to other, the resultant molecule generally has increase the potency. Therefore it was thought worthwhile to synthesize better kinds of drugs by incorporating azetidinone in benzothiazole moiety. Keeping in view of diverse activity of benzothiazole and azetidinone nucleus, it is planned to synthesis the title compound and screens them for their possible antibacterial activities.

We have already reported some of our work on the synthesis and biological properties of various azetidinones derivatives (7).These compounds were screened for their anticonvulsant and anthelmentic activities and it was found that some of them have moderate to good biological properties. The biological significance of this class of compounds impelled us to continue working on the synthesis of new azetidinone derivatives.

EXPERIMENTAL

Laboratory chemicals were supplied by chemdise chemical Ltd. Melting point of synthesized compounds were determined in open capillary and are uncorrected.IR spectra were recorded in Thermo Scientific; NICOLET iS10 spectrophotometer in KBR disc.1HNMR spectra were recorded on 400 MHZ spectrophotometer in DMSO-d_6 as a solvent and TMS as an internal stander. The purity of the compounds was checked by TLC and R_f value is given in table 1. Elemental analyses of all the compounds were in agreement with the calculated values. Antibacterial activity was performed at department of microbiology (N.R vekaria institute of pharmacy), Junagadh, India. Antibacterial screening using cup-plate diffusion method (8).

The building blocks 2-amino-6,7 substituted benzothiazole [**2.023a-c**] were prepared according to the reported procedures (8).

General method for synthesis of Ethyl (6,7-substituted-1,3-benzothiazol-2-yl) carbamate

2 amino benzothiazole (0.066 mole) 13.5 g, absolute alcohol 30 ml anhydrous K_2CO_3 (2 g) and ethyl chloroformate (0.0064 mole) 0.7 g, were added under cooled at 0-5^{0}C. The mixture was refluxed for 7- 8 hours at 60-70 ^{0}C. The solution filtered and the residue was washed with ethanol and the solvent was evaporated under reduced pressure to get the product as solid which was recrystalised with ethanol.

General method for synthesis of preparation of N-(6,7-Substituted 1,3-benzothiazol-2-yl) hydrazine carboxamide

Ethyl (6,7-Substituated-1,3-benzothiazol-2-yl) carbamate (0.021 mole) 5.5 g, treated with 4 ml hydrazine hydrate was dissolved in ethanol (30 ml). The reaction mixture was refluxed for 5 hours and cooled to room temperature. The separated carbamoyl hydrazides were filtered and residue was washed with ethanol and recrystalised with alcohol.

General method for synthesis of preparation of 2, 3, 4 (trisubstituted benzaldehyde)-N-(6, 7-substituted-1,3-benzothiazol-2-yl) semicarbazone

5.21 g of *N*-(6-fluro-7-chloro-1,3-benzothiazol-2-yl) hydrazine carboxamide (0.02 mole) was dissolved in absolute ethanol and substituted benzaldehyde (0.02 mole) 2.40 g were added and refluxed for 3 hours and the solvent was removed under reduced pressure to yield Schiff base.

General method for synthesis of Schiff base to azetidinones

To a solution of Schiff base (0.10 mol) in DMF, chloroacetyle chloride (0.10 mol) and triethlyle amine (0.10 mol) were added and reaction mixture was stirred for 24 hr. The reaction mixture was poured into cooled water and the liberated compound was extracted with chloroform. Evaporation of the compound afforded the corresponding azetidinones. Physical data of compounds synthesized are summarized in Table 1**.**

1-(3-chloro-2-oxo-4-(o-tolyl) azetidin-1-yl)-3-(4-chloro-5-fluorobenzo[d]thiazol-2-yl)urea [AZ1]

IR (KBr)*v* cm^{-1}: 1650 (C=O), 3064 (NH), 1602 (C =N), 687 (C-Cl), 1169 (C-F), 720 (C-S-C). ^{1}HNMR (DMSO-d6) δ(ppm): 7.1 (m, 6H , Ar-H) , 2.52 (s, 3H , CH_3) , 5.2 (s, 1H , NH), 4.2 (s , 1H , Azetidinone), 8.6 (s, 1H, CONH) , 5.25 (s, 1H, CH-Cl), **MS:** 439.01(12.53%) M: M+2: M+4 (9:6:1), 228.1 (100%), 201 (12.24%), 209 (8%), 194 (27%). *Anal. Calc.* for $C_{18}H_{13}Cl_2FN_4O_2S$: C, 49.21; H, 2.98; N,12.75.Found: C,49.20; H, 2.96; N, 12.74%.

1-(3-chloro-2-oxo-4-(p-methoxy)azetidin-1-yl)-3-(4-chloro-5-fluorobenzo[d]thiazol-2-yl)urea [AZ2]

IR (KBr)*v* cm^{-1}: 1652(C=O), 3095 (NH), 1612 (C =N), 711 (C-Cl), 1122 (C-F), 717 (C-S-C). ^{1}HNMR (DMSO-d6) δ(ppm): 6.9 (m, 6 H , Ar- H) , 4.3 (s, 3H , -OCH_3) , 6.0 (s, 1H , NH), 4.2 (s, 1H, Azetidinone), 5.5 (s,1H, CH-Cl) , 9.1 (s, 1H, CONH). *Anal. Calc*. for $C_{18}H_{13}Cl_2FN_4O_3S$: C,47.48; H,2.88; N,12.31. Found: C,47.47; H,2.89; N,12.30 %.

1-(3-chloro-2-oxo-4-(P-methoxy)azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea [AZ3]

IR (KBr)*v* cm^{-1}: 1685 (C=O), 3092 (NH), 1600 (C =N), 1153 (C- F), 720 (C-S-C). ^{1}HNMR (DMSO-d6) δ(ppm): 8.14 (m, 7 H , Ar- H) , 3.7 (s, 3H , -OCH_3) , 5.83 (s, 1H , NH), 4.3 (s, 1H, Azetidinone), 5.65 (s, 1H, CH-Cl) , 7.94 (s, 1H ,CONH). *Anal. Calc*. for $C_{18}H_{14}ClFN_4O_3S$: C,51.37; H,3.35; N,13.31. Found: C,51.38; H, 3.36; N,13.30 %.

1-(3-chloro-2-oxo-4-(o-tolyl) azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea [AZ4]

IR (KBr)*v* cm^{-1}: 1671 (C=O), 3094(NH), 1604 (C=N), 1157(C-F), 728 (C-S-C). ^{1}HNMR (DMSO-d6) δ(ppm): 7.85 (m, 7H, Ar- H) , 2.16 (s, 1H , CH_3) , 5.98 (s, 1H, NH), 3.57 (s, 1H, Azetidinone), 5.64 (s, 1H, CH-Cl), 8.77(s, 1H, CONH). *Anal. Calc*. for $C_{18}H_{14}ClFN_4O_2S$: C,53.40; H,3.49; N,13.84. Found: C,53.41; H,3.50; N,13.85 %.

1-(3-chloro-2-oxo-4-(p-methoxy)azetidin-1-yl)-3-(4-chlorobenzo[d]thiazol-2-yl)urea [AZ5]

IR (KBr)*v* cm^{-1}: 1680 (C=O), 3095(NH), 1615 (C=N), 685 (C-Cl), 725 (C-S-C). ^{1}HNMR (DMSO-d6) δ(ppm): 7.68 (m, 7 H , Ar- H) , 4.3 (s, 3H ,OCH_3), 5.98 (s, 1H, NH), 4.23 (s, 1H, Azetidinone), 3.92 (s, 1H, CH-Cl) , 8.42 (s, 1H,CONH). *Anal. Calc*. for $C_{18}H_{14}Cl_2N_4O_3S$: C, 49.44; H, 3.23; N,12.81. Found: C, 49.45; H, 3.24; N, 12.82%.

1-(3-chloro-2-oxo-4-(o-tolyl) azetidin-1-yl)-3-(4-chlorobenzo[d]thiazol-2-yl)urea [AZ6]

IR (KBr)*v* cm^{-1}: 1675(C=O), 3095(NH), 1605 (C=N), 680 (C-Cl), 720 (C-S-C). ^{1}HNMR (DMSO-d6) δ(ppm): 7.68 (m, 7H, Ar-H) , 2.95 (s, 3H , OCH_3) , 6.0 (s, 1H, NH), 4.23 (s, 1H, Azetidinone), 3.95 (s, 1H, CH-Cl) , 8.45 (s, 1H, CONH). *Anal. Calc*. for $C_{18}H_{14}Cl_2N_4O_2S$: C,51.32; H,3.35; N,13.30. Found:C, 51.33; H, 3.36; N, 13.31%.

Antibacterial screening

All microbial cultures were collected from Micropharm diagnostic center, Gandhinagar, Gujarat, India. and tested against known drugs Streptomycin and Procaine penicillin. Nutrient agar medium was used as nutrient medium to grow and dilute the drug suspension for the test. DMF was used as diluents to get desired concentration of drugs to test upon standard bacterial strains.

The fresh culture of bacteria are obtained by inoculating bacteria into peptone water liquid media and incubated at 37 ± 2˚ C for 18-24 hours. This culture commixed with nutrientr media (20%) and poured into petri dishes by following aseptic techniques. After solidification of the media bores are made at equal distance by utilizing sterile steel cork borer (8 mm diameter). Into these cups different concentrations of standard drugs and synthesized compounds are introduced where dimethyl formamide was utilized as a control. After exordium of standard drugs and synthesized compounds, the plates were placed in a refrigerator at 80-100^{0}C for felicitous diffusion of drugs into the media. After two hours of gelid incubation, the petriplates are transferred to incubator and maintained at 370±20C for 18-24 hours. After the incubation period, the petriplates were observed for zone of inhibition by utilizing vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs.

RESULT AND DISCUSSION

Chemistry

2-amino 6,7 substituted benzothiazole **2.023a-c** and ethyl chloroformate in presence of anhydrous $K_2CO_3$and ethanol solvent were heated to form *N*-(6,7-Substituted 1,3-benzothiazol-2-yl) hydrazine carboxamide **2.024** which on further heating in ethanol and subsequent reaction with hydrazine hydrazine hydrate to form 2, 3, 4 (trisubstituted benzaldehyde)-*N*-(6,7-substituted-1,3-

benzothiazol-2-yl)semicarbazone **2.025.** After condensing, **2.025** with aromatic aldehyde in DMF solvent, Schiff bases **2.026a-o** were obtained. Finally, azetidinones **AZ1-6** were prepared by refluxing the Schiff base **5** and chloroacetylchloride in triethyl amine.

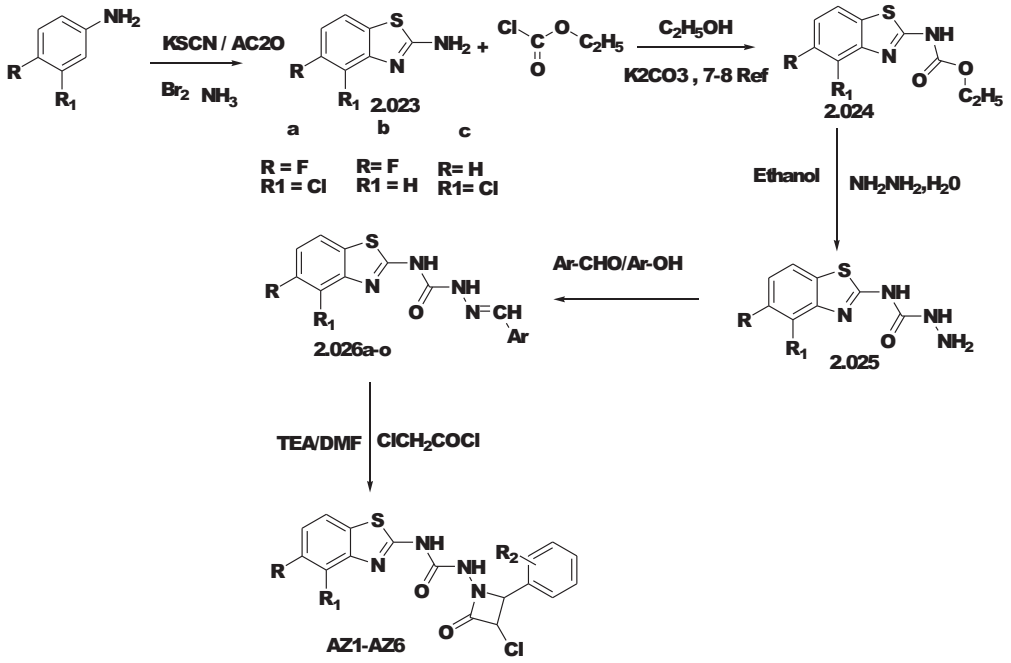
Structure of compounds were confirmed by elemental analyses and IR spectra (cm^{-1}) absorption band band of **2.023a** at 3095 cm^{-1} for (NH), 1603 $cm^{-1}$for (C=N), 1155 $cm^{-1}$for (C-F), 711 cm^{-1} for (C-Cl). Similarly on the basis of IR spectrum, the formation of compounds 2.023b and 2.023c were also established.

^{1}H-NMR Spectra of **2.023** at 400 MHz in DMSO-d_6 displayed characteristic signal for presence of proton in the molecule. It exhibited a broad multiplet at δ 7.97 which was attributed to the presence of aromatic proton. A singlet at δ 4.0 shows the presence of two proton of NH_2.

For ethyl (6,7-substituted-1,3-benzothiazol-2-yl) carbamate (**2.024**) characteristic absorption band was at 3085 cm^{-1} for (NH), 1608 $cm^{-1}$for (C=N), 1157 $cm^{-1}$for (C-F), 710 $cm^{-1}$for (C-Cl).

Ethyl (6,7-substituted -1,3-benzothiazol-2-yl) carbamate (**2.024**) exhibited multiplet at δ 7.20 for aromatic hydrogen. At δ 8.0, 1.30 and 4.12 singlet peak were observe due to presence of one, three, two proton of NH, CH_3, CH_2 .

Similarly compound no **2.025** exhibited characteristic band at 3080 cm^{-1} for (NH), 1602 $cm^{-1}$for (C=N), 1158 $cm^{-1}$for (C-F), 715 cm^{-1} for (C-Cl). For *N*- (6,7- Substituted 1,3-



Scheme 1: Synthetic route for the preparation compounds AZ1-AZ6

Table 1. Physical data of compounds (AZ1-AZ6)

Compound	R	R1	R2	Molecular formula	Molecular weight	Yield %	m.p (^{0}c)
AZ1	F	Cl	2-CH_3	$C_{18}H_{13}Cl_2FN_4O_2S$	439.29	80	220
AZ2	F	Cl	4-OCH_3	$C_{18}H_{13}Cl_2FN_4O_3S$	455.29	69	125
AZ3	F	H	4-OCH_3	$C_{18}H_{14}ClFN_4O_3S$	420.84	80	175
AZ4	F	H	2-CH_3	$C_{18}H_{14}ClFN_4O_2S$	404.84	88	195
AZ5	H	Cl	4-OCH_3	$C_{18}H_{14}Cl_2N_4O_3S$	437.29	90	170
AZ6	H	Cl	2-CH_3	$C_{18}H_{14}Cl_2N_4O_2S$	421.30	85	119

benzothiazol-2-yl) hydrazine carboxamide (**2.025**) exhibited multiplet at δ 7.95 for presence of aromatic proton. Compound number (**2.025**) also show singlet at δ 6.0 and 2.0 for two and one proton of NH_2, NH.

Another characteristic band of **2.026a** also observe at 1554 cm^{-1} for (N=CH), 3175 $cm^{-1}$for (NH), 1600 $cm^{-1}$for (C=N), 1145 $cm^{-1}$for (C-F), 680 $cm^{-1}$for (C-Cl).

Similarly compound **2.026** exhibited a broad multiplet at δ 7.98 due to presence of aromatic proton. Singlet at δ 3.80 shows the presence of two protons of CH_2 and singlet at δ 8.50 shows the presence of CONH. For compound **AZ1** MS: 439.01(12.53%) M: M+2: M+4 (9:6:1), 228.1(100%),201 (12.24%),209(8%), 194(27%).

Antibacterial Activity

Antibacterial activity of the synthesized compounds **AZ1-6** in form of minimum inhibitory concentrations (MICs) was evaluated against various pathogenic bacterial strains *Pseudomonas(***MTCC-1688***)* and *Bacillus subtilis* (***MTCC-736***).Antibacterial activity was carried out by cup-plate diffusion method (8). Minimum inhibitory concentrations (MICs) of the tested compounds are shown in Table 2.

On comparing the antibacterial activity of all synthesized compounds, no zone of inhibition was observed in DMF and among the two minimum inhibitory concentration taken (50-100mg/ml), highest activity were found to be at 100mg/ml on both the pathogenic strain. Particularly compounds **AZ2, AZ3, AZ5 and AZ6** exhibited maximum activity. While compounds **AZ1, AZ4,** exhibited moderate to low activity relative to the reference drug.

CONCLUSION

Nobel azetidinone derivatives were synthesized; starting from building blocks **2.023a-c** and were studied for their antibacterial activity. Overall observation from the results of the antibacterial activity of the synthesized compounds revealed that compounds containing OCH_3 group at C-4 position of phenyl ring and CH_3 group at C-2 position of phenyl ring exhibited maximum and low activity.

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Table 2. Biological profile of compounds **AZ1-AZ6**

S. No	Compounds	Mean zone of inhibition (in mm)			
		Bacillus subtilis		*Pseudomonas*	
		50mg	**100mg**	**50mg**	**100mg**
01	Procaine penicillin	20(100%)	24(100%)	-	-
02	Streptomycin	-	-	20(100%)	25(100%)
03	**AZ1**	13.5 (65%)	17.8 (74.1%)	11.4(55.5%)	19.2(76.8%)
04	**AZ2**	16.2 (81%)	20.8 (86.6%)	18.1(90.5%)	23.1(92.4%)
05	**AZ3**	17.2 (86%)	22.7 (94.5%)	17.5(87.5%)	24.3(97.2%)
06	**AZ4**	11.3 (56.5)	14.5 (72.5%)	10.5(52.5%)	16.2(64.8%)
07	**AZ5**	14.5 (72.5%)	18.3 (91.5%)	14.2(71%)	19.5(78%)
08	**AZ6**	18.1 (90.5%)	21.8 (90.8%)	17.9(89.5%)	24.1(96.4%)

$$\% \text{ Activity index} = \frac{\text{Test compound}}{\text{Stander compound}} \times 100$$

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