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Synthesis and Antimicrobial Activity of Some Azetidinone Derivatives Containing Substituted Benzothiazole

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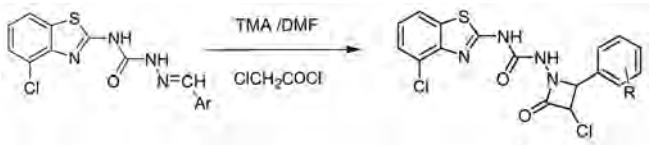
ABSTRACT Ethyl (7-chloro-1,3-benzothiazol-2-yl) carbamate **2.024** was prepared and treated with hydrazine hydrate to yield of N-(7-chlorobenzothiazol-2-yl) hydrazine carboxamide derivatives **2.025.** Reaction of the hydrazine carboxamide derivative **2.025** with substituted benzaldehyde afforded the corresponding Schiff base **2.026.** 2-Azetidinones derivatives **1A- 5A** were obtained in high yield by reaction of **2.026** with chloroacetyl chloride, triethylamine**.** Compounds **1A-5A** were evaluated for their antimicrobial activity against Gram-positive and Gram-negative bacteria, compounds 1A and 5A showed the significant activity against Bacillus *subtilis* and *Pseudomonas* aeruginosa *when compared with the standard.* All synthetic derivatives were also tested against two fungal stains; however, they were weekly active against *Candida albicans* and *Aspergillus flavus*. The synthesized compounds were characterized by different spectroscopy techniques.



KEY WORDS 2-Amino-7-chlorobenzothaizole, Schiff **base, Antibacterial activity, Antifungal activity.**

INTRODUCTION

The development of simple synthetic routes to widely used organic compounds using readily available reagents is one of the main objectives of organic synthesis. Nitrogen heterocycles are of special interest because they constitute an important class of natural and nonnatural products, many of which exhibited useful biological activities. Investigation of the 2- amino substituted benzothaizole heterocycles has shown that they possess varied biological properties such as antitubercular,[1] antimicrobial,[2] anti-inflammatory,[3] anthelmintic,[4] cardiovascular,[5] and anticancer[6] activity. In addition, 2-azetidinones derivatives have exhibited a wide range of biological activities.[7] Furthermore, they can be used as intermediates in the synthesis of highly functionalized compounds. As part of our research projects aim of this work was the synthesis of some new benzothiazole-based azetidinone derivatives utilizing 2-amino-7-chlorobenzothaizole **2.023** as a key starting synthon.

The aim of our study was to further evaluate the antimicrobial activity of 2-azetidinones by making some structure modifications. It is evident that the $-OCH_3$ group at C-4 position of phenyl ring is playing a very crucial role in the antibacterial activity. A total of five 2-azetidinones (1A-5A) were synthesized and evaluated for their antimicrobial activities. To the best of our knowledge, all the synthesized derivatives in this study are new.

EXPERIMENTAL

Laboratory chemicals were supplied by Chemdise Chemical Ltd. (Rajkot, India). Melting point of synthesized compounds was determined in open capillary and is

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uncorrected. IR spectra were recorded in Thermo Scientific; NICOLET iS10 spectrophotometer in KBR disc. Nuclear magnetic resonance (1HNMR) spectra were recorded on 400 MHZ spectrophotometer in dimethyl sulfoxide-d6 as a solvent and tetramethylsilane as an internal stander. The purity of the compounds was checked by thin layer chromatography. Elemental analyses of all the compounds were in agreement with the calculated values.

BIOASSAY

Antibacterial assay

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 a nutrient medium to grow and dilute the drug suspension for the test. Dimethylformamide (DMF) was used as diluents to get desired concentration of drugs to test for standard bacterial strains. The fresh culture of bacteria is obtained by inoculating bacteria into peptone water liquid media and incubated at 37 ± 2°C for 18–24 h. This culture commixed with nutrient media (20%) and poured into Petri dishes by following aseptic techniques. After solidification of the media, bores are made at an 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 dimethylformamide was utilized as a control. After exordium of standard drugs and synthesized compounds, the plates were placed in a refrigerator at 80–100°C for felicitous diffusion of drugs into the media. After 2 h of gelid incubation, the Petri plates are transferred to an incubator and maintained at 370 ± 20°C for 18–24 h. After the incubation period, the Petri plates were observed for the zone of inhibition by utilizing vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drugs.

Antifungal assay

The 48 hold fungal culture inoculated into nutrient broth by following aseptic techniques and incubated for 48 h at 37° ± 2°C in an incubator. This culture mixed with Potato dextrose agar media (20%) and poured into Petri plates. After solidification five bores are made at an equal distance using sterile steel cork borer (8 mm in diameter). Into these cups, different concentrations of standard drug and synthesized compounds along with control (dimethylformamide) introduced. After the introduction of standard drug and compounds, these plates are placed in a refrigerator at 8°–10°C for 2 h for proper diffusion of the drugs. After 2 h of cold incubation, the Petri plates are transferred to an incubator and maintained at 37° ± 2°C for 24–36 h. After the incubation period, the plates were observed for the zone of inhibition by using vernier scale. Results evaluated by comparing the zone of inhibition shown by the synthesized compounds with a standard drug. The results are the mean value of the zone of inhibition measured in millimeter of two sets. The standard drug and synthesized compounds were dissolved in minimum quantity of DMF and adjusted, to make up the volume with DMF to get 50 µg/ml and 100 µg/ml concentrations. The griseofulvin used as a standard drug.

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

General method for synthesis of ethyl (7-chloro-1,3-benzothiazol-2-yl) carbamates (2.024)

2-Aminobenzothiazole (13.5 g), absolute alcohol 30 ml anhydrous K_2CO_3 (2 g) and ethyl chloroformate (0.7 g), were mixed under cooled at 0–5°C. The mixture was refluxed for 7–8 h at 60–70°C. The solution was filtered, and the residue was washed with ethanol, and the solvent was evaporated under reduced pressure to give the product as solid which was recrystallized with ethanol.

General method for synthesis of preparation of N-(7-chloro-1,3-benzothiazol-2-yl) hydrazine carboxamides (2.025)

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

General method for synthesis of preparation of 2, 3, 4 (trisubstituted benzaldehyde)-N-(7-chloro-1,3-benzothiazol-2-yl) semicarbazones (2.026)

Compound **2.025** (2.21g) was dissolved in absolute ethanol and substituted benzaldehyde (2.40 g) was added. The mixture was refluxed for 3 h, and the solvent was removed under reduced pressure to yield Schiff base.

General method for synthesis of Schiff base to azetidinones (1A-5A)

To a solution of Schiff base (0.10 mol) in DMF, chloroacetyl chloride (0.10 mol), and triethyl amine (0.10 mol) were added, and reaction mixture was stirred for 24 h. The reaction mixture was poured into cooled water, and the liberated compound was extracted with chloroform. Evaporation of the compound afforded the corresponding azetidinone.

Spectroscopic data

1-(3-Chloro-2-oxo-4-(p-methoxy)azetidin-1-yl)-3-(7-chlorobenzo[d]thiazol-2yl)urea (1A)

Pale yellow powder, mp: 170°C, yield 90%. IR (KBr) (vmax/cm^{-1}): 1680 (C=O), 3095 (NH), 1615 (C =N), 685 (C-Cl), 725 (C-S-C).^{1}HNMR: δ 7.68 (m, 7 H, Ar- H), 4.3 (s, 3H, -OCH_3), 5.98 (s, IH, NH), 4.23 (S, IH, azetidinone), 3.92 (s, IH, CH-Cl), 8.42 (s, IH, CONH). Analytical calculated for $C_{18}H_{14}Cl_2N_4O_3S$: C, 49.44; H,3.23; N,12.81. Found: C, 49.43; H, 3.21; N, 12.80%.

1-(3-Chloro-2-oxo-4-(o-tolyl) azetidin -1-yl)-3-(7-chlorobenzo [d]thiazol-2-yl)urea (2A)

Pale yellow powder, mp: 119°C, yield 85%. IR (KBr) (vmax/cm^{-1}): 1675 (C=O), 3095(NH), 1605 (C =N), 680 (C-Cl), 720 (C-S-C), ^{1}HNMR: δ 7.68 (m, 7 H, Ar- H), 2.95 (s, 3H, -OCH$_3$), 6.0 (s, IH, NH), 4.23 (S, IH, azetidinone), 3.95 (s, IH, CH-Cl), 8.45 (s, IH, CONH). Analytical calculated for $C_{18}H_{14}Cl_2N_4O_2S$: C, 51.32; H, 3.35; N, 13.30. Found: C, 51.23; H, 3.36; N, 13.33%.

1-(3-Chloro-2-oxo-4-(m-tolyl) azetidin -1-yl)-3-(7-chlorobenzo [d]thiazol-2-yl)urea (3A)

Pale yellow powder, mp: 119°C, yield 85%. IR (KBr) 1600 (C=O), 3092 (NH), 1610 (C =N), 1150 (C-F); ^{1}H NMR 7.84 (m, 6 H, Ar- H), 2.18 (S, IH, CH$_3$), 11.34 (S, IH, NH), 3.56 (S, IH, azetidinone), 5.67 (S, IH, CH-Cl), 8.72 (S, IH, CONH). Analytical calculated for $C_{18}H_{14}ClFN_4O_2S$: C, 53.40; H, 3.49; N, 13.84. Found: C, 53.39; H, 3.38; N, 13.83%.

1-(3-Chloro-2-oxo-4-phenylazetidin -1-yl)-3-(7-chlorobenzo [d]thiazol-2-yl)urea (4A)

Pale yellow powder, mp: 148°C, yield 68%. IR (KBr) 1652 (C=O), 3095 (NH), 1602 (C =N), 711 (C-Cl), 718(C-S-C); ^{1}HNMR 7.68 (m, 8 H, Ar- H), 6.10 (s, IH, NH), 4.52 (s, IH, azetidinone), 4.09 (s, IH, CH-Cl), 8.53 (s, IH, CONH). Analytical calculated for $C_{17}H_{12}Cl_2N_4O_2S$: C, 50.13; H, 2.97; N, 13.76. Found: C, 50.18; H, 2.95; N, 13.71%.

1-(3-Chloro-2-oxo-4-(o-methoxy) azetidin-1-yl)-3-(7-chlorobenzo[d]thiazol-2-yl)urea (5A)

Pale yellow powder, mp: 135°C, yield 77%. IR (KBr) 1650 (C=O), 3090 (NH), 1608 (C =N), 717 (C-Cl), 730(C-S-C); ^{1}HNMR 7.68 (m, 7 H, Ar- H), 5.89 (s, IH, NH), 4.51 (s, IH, azetidinone), 5.40 (s, IH, CH-Cl), 8.50 (s, IH, CONH). MS m=z (%): 437 (14%) M: M+2: M+4 (9:6:1), 229 (100%), 220 (9.15%), 210 (11.25%), 194 (52.24%), 117 (10.25%). Analytical calculated for $C_{18}H_{14}Cl_2N_4O_3S$: C, 49.44; H, 3.23; N, 12.81. Found: C, 51.36; H, 3.34; N, 13.30%.

RESULTS AND DISCUSSION

Chemistry

As part of our continuing interest in the construction of novel heterocycles, we now report the results of our studies involving the reactions of 2,3,4 (trisubstituted benzaldehyde) – N - (7-chloro-1,3-benzothiazol-2-yl) semicarbazone **2.026** and chloroacetyl chloride, triethlyl amine which constitutes a synthesis of azetidinones derivatives (**Scheme 1**).

The structures of compounds **1A-5A** were deduced from their elemental analyses and their IR, ^{1}H NMR and mass spectra. For example, the ^{1}H NMR spectrum of ethyl (7 -Chloro - 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 observed due to the presence of one, three, two proton of NH, CH$_3$, CH$_2$. For N- (7- chloro 1,3 - benzothiazo l - 2 -yl) hydrazine carboxamide (**2.025**) exhibited multiplet at δ 7.95 for the presence of aromatic proton. Compound number (**2.025**) also showed singlet at δ 6.0 and 2.0 for two and one protons of NH$_2$, NH, which confirmed the proposed structure. Similarly, compound **2.026** exhibited a broad multiplet at δ 7.98 due to the presence of aromatic proton. Singlet at δ 3.80 shows the presence of two protons of CH$_2$ and singlet at δ 8.50 showed the presence of CONH.

The IR spectrum of ethyl (7-chloro-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), and 710 cm^{-1} for (C-Cl). 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), and 715 cm^{-1} for (C-Cl).

Antimicrobial activity

Literature reveals that one of the major factors plays a very key role in antibacterial activity is the lipophilicity means that only lipid soluble materials can easily passed through the cell membrane of microorganism.[9] This key factor, as well as the crucial role of chloro group in azetidinones, was our major focus throughout the synthetic work. Therefore, it is worth mentioning that we have avoided the hydroxyl group in our synthesized compounds by keeping in mind the major factor of lipophilicity. Hence, all the synthesized compounds are much lipophilic with no hydroxyl groups, having halogen substituent.

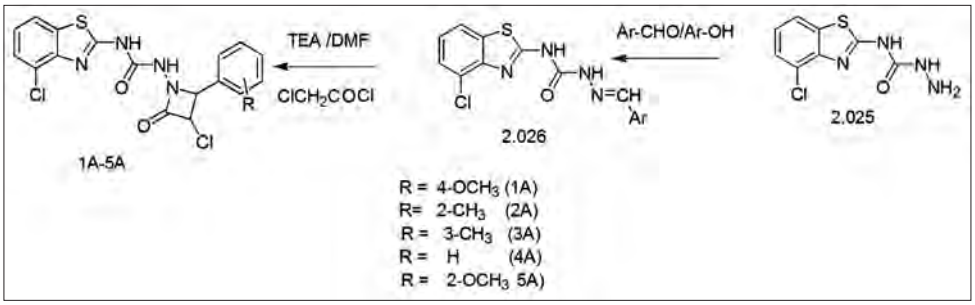
Five newly synthesized compounds **1A-5A** were evaluated to check their antibacterial activity against both Gram-positive and Gram-negative bacterial strains by following standard cup-plat diffusion method.[10] *Bacillus subtilis* (MTCC-736) and *Pseudomonas* (MTCC-1688) were used as Gram-positive and Gram-negative bacterial strains. The results in zone diameter of growth inhibition (mm) showed that all the new synthetic compounds have demonstrated weak to significantly good inhibitory potential against Gram-positive as well as Gram-positive bacteria when compare to the standard procaine penicillin and streptomycin Table 1. Compounds **1A** and **5A** displayed notably good activity against *B. subtilis* and *Pseudomonas* comparable with the standard.

Griseoflavin used as a standard for the antifungal bioassay. These key structural features are also the part of our synthesized derivatives and were taking into account while hoping that our compounds will also show the antifungal activity.

In the inhibitory antifungal activity of all compounds, **1A-5A** were evaluated against two species, i.e., *Candida albicans, Aspergillus flavus* using cup-plate diffusion method.[10] The linear growth of all fungus was obtained by measuring the diameter of the fungal colony after 1 week. **Table 2** summarizes the results as zone diameter of growth inhibition (mm). All the compounds showed weak to moderate activity against *C. albicans, A. flavus,* respectively.

CONCLUSION

New azetidinone derivatives were synthesized; starting from building blocks **2.023** and were studied for their



Scheme 1: Synthetic route for preparation of compounds 1A-5A

Table 1: Antibacterial activity of compounds 1A-5A against Gram-positive and Gram-negative bacteria (inhibition zones in mm using the cup-plate diffusion method)

Name of the compounds	Mean zone of inhibition (in mm)			
	B. subtilis (%)		*Pseudomonas* (%)	
	50 mg	100 mg	50 mg	100 mg
Procaine penicillin	20 (100)	24 (100%)	-	-
Streptomycin	-	-	20 (100)	25 (100)
1A	18.3 (91.5)	21.7 (90.4)	17.5 (87.5)	23.7 (94.8)
2A	13.5 (67.5)	17.4 (72.5)	14.2 (71)	19.5 (78)
3A	11.5 (57.5)	16.6 (69.1)	11.8 (59.0)	18.9 (75.6)
4A	11.3 (56.5)	14.5 (72.5)	10.5 (52.5)	16.2 (64.8)
5A	16.2 (81)	20.8 (86.6)	18.1 (90.5)	23.1 (92.4)

B. subtilis: Bacillus subtilis. Keys: - = No zone of inhibition, Key: 11.3-16.2 mm=weakly active, 13.5-17.4 mm=moderately active, >18.1=good activity

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

Table 2: Antifungal activity (inhibition zones in mm using the cup-plate diffusion method)

Name of the compounds	Mean zone of inhibition (in mm)			
	C. albicans (%)		*A. flavus* (%)	
	50 mg	100 mg	50 mg	100 mg
Griseofulvin	18 (100)	21 (100)	18 (100)	21 (100)
1A	16.3 (90.5)	19.8 (94.2)	16.9 (93.8)	20.2 (96.1)
2A	12.6 (70.0)	16.7 (79.52)	11.7 (65.0)	17.4 (82.5)
3A	9.8 (54.4)	15.8. (75.2)	8.7 (48.30)	14.5 (69.0)
4A	11.3 (62.7)	14.7 (70.0)	10.8 (60.0)	16.4 (78.0)
5A	16.1 (89.4)	19.5 (92.8.0)	16.4 (91.1)	20.0 (95.2)

C. albicans: Candida albicans, A. flavus: Aspergillus flavus. Keys: - = No zone of inhibition, Key: 8.7–16.2 mm=weakly active, 12.6-17.4 mm=moderately active, >19.5=good activity

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

antibacterial and antifungal activity. Overall, observation from the results of the antibacterial and antifungal activity of the synthesized compounds revealed that compounds containing -OCH_3 group at C-4 position of the phenyl ring and CH_3 group at C-2 position of phenyl ring exhibited a maximum and low activity.

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