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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF NOVEL HYBRID MOIETIES-BENZOTHIAZOLES AND AZETIDINONES

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Abstract: A new series of 1-(3-chloro-2-oxo-4-substituted phenyl azetidin-1-yl)-3-(substituted benzo[d] thiazol-2-yl) urea **(7a-j)** were synthesized using appropriate synthetic route. The structure of the synthesized compounds have been established by elemental analysis and spectroscopic data (IR, ^{1}H NMR and Mass). The minimum inhibitory concentrations (MICs, µg mL^{-1}) of the chemical compounds assays were carried out by broth microdilution method. Two Gram-positive (*Bacillus cereus* MTCC 4317 and *Staphylococcus aureus* MTCC 3160) and two Gram-negative (*Escherichia coli* DH5 alpha MTCC 1652 and *Pseudomonas aeruginosa* MTCC 4676) bacteria were used as quality control strains. *Aspergillus niger* MTCC 282, *Alternaria solani* MTCC 2101, *Fusarium culmorum* MTCC 2090 and *Rhizopus stolonifer* MTCC 2591 strains were used for antifungal activity. Ampicillin sodium salt and Fluconazole were used as standard antibacterial and antifungal drugs respectively. The compounds showed good to moderate inhibition against the various strains.

Keywords: Benzothiazole; azetidin-2-one; Schiff base; antibacterial and antifungal activity; MIC

Introduction:

Multidrug resistance against antimicrobial agents is one of the most critical problems of today's medicinal scenario. There is a urgent need to develop new and different antimicrobial agents which can be used as drugs to treat chronic conditions. In the design of new compounds, synthesis of hybrid molecules through the combination of different pharmacophores in a single structure may lead to compounds with better antimicrobial activity.

Benzothiazoles represent a keymotif in heterocyclic chemistry and occupy a significant place in medicinal chemistry due to their capability to exhibit a wide range of biological activities viz. antihelmintic, anti-inflammatory,

antimicrobial, antiviral and antitubercular[1-6]. Similarly, the azetidin-2-one derivatives have been reported to possess a wide range of biological activities such as antihyperlipidemic, antibacterial, antifungal, anti-inflammatory, antiparkinsonian, anticonvulsant, antitubercular, analgesic and anticancer[7-19]. The 2-azetidinone (β-lactam) ring system is the common structural feature of a number of broad spectrum β-lactam antibiotics including penicillins, cephalosporins, nocardicins and monobactams which have been widely used as chemotherapeutic agents to cure microbial infections. Furthermore, they were highlighted as a potent mechanism based inhibitor of several enzymes like human tryptase, chymase, thrombin etc.

Looking to the promising antimicrobial activity of 2-azetidinone and benzothiazole analogues synthesized by us, it was thought of interest to combine the heterocyclic rings together in a single molecular framework in order to increase the additive effect towards the biological activity. The substitution pattern of benzothiazole and azetidin-2-one rings was variably chosen so as to confer varied electronic environment to the molecules.

Results and discussion:

Chemistry

In this present work, a series of novel compounds **(7a-j)** was synthesized. Starting from substituted benzothiazole **(2a-c),** we have synthesized 1-(3-chloro-2-oxo-4-substituted phenyl azetidin-1-yl)-3-(substituted benzo[d] thiazol-2-yl) urea **(7a-j)** according to the procedure outlined in Scheme 1. The required 2-substituted benzylidene-N-(substituted benzo[d]thiazol-2-yl) hydrazine carboxamide **(6a-j)** was synthesized by reacting N-(substituted benzo[d] thiazol-2-yl) hydrazine carboxamide **(5a-c)** with substituted aromatic aldehydes in ethanol. Compounds **(7a-j)** were synthesized in a single step by reacting 2-substituted benzylidene-N-(substituted benzo[d]thiazol-2-yl) hydrazine carboxamide **(6a-j)** with chloroacetylchloride and trimethylamine in the presence of dioxane respectively.The structure of the synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass spectrum).

The IR spectra of compounds **(6a-j)** showed absorption peaks at 3308-3329 and 1661-1689 cm^{-1} due to N-H and C=O stretching vibrations. The stretching of the C=O of β-lactam appeared at 1761-1789 cm^{-1} in the spectra of derivatives together with the C=O stretching at 1660-1690 cm^{-1} confirmed the formation of the compounds **(7a-j)**.

The ^{1}H NMR spectra of compounds **(6a-j)** showed a multiplet at δ 6.48-7.90 ppm for the aromatic ring and singlets at δ 6.25 and 7.95 ppm for –NH and –N=CH respectively.

The disappearance of the singlet peak of –N=CH and the presence of a singlet peak at δ 7.79 ppm of -N-CH of β lactam ring proved that these compounds participated in the cyclization reaction and formed the desired compounds **(7a-j).** The elemental analysis and molecular ion peak of compounds **(7a-j)** were consistent with the assigned structures.

Biology

Antimicrobial activity

The invitro antimicrobial assessment of synthesized compounds was carried out for determination of MIC against the four strains of bacteria (two gram positive and two gram negative) and antifungal activity against the four strains of fungi by using broth microdilution method. The compounds showed good to moderate inhibition against the various strains.

The compounds **7c, 7d, 7g, 7h** and **7j** showed comparatively good activity against all bacterial strains. Compounds **7a**, **7e** and **7i** exhibited moderate activity against all the bacterial strains **(Table S1).**

Material and Methods:

General

Melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected. All the compounds were subjected to elemental analysis (CHN) and the measured values agreed within ± 0.4% with the calculated ones. The IR spectra were recorded in KBr pellets on SCHIMADZU 8400 S FT IR spectrophotometer. ^{1}H NMR spectra were obtained from JEOL AL 300 FT NMR using TMS as internal standard in $CDCl_3$ / DMSO-d_6. The Mass spectra were recorded on JEOL SX 102/DA600 using Argon/Xenon as FAB gas. The purity of synthesized compounds were checked by TLC using Silica gel "G" as adsorbent and visualization was accomplished by UV light or iodine. The reaction pathway has been summarized in **Scheme S1**.

Synthesis of 4-Bromo/ 4-Fluoro/ 5,7-Dimethylphenylthiourea (2a-c):

4-Bromo/ 4-Fluoro/ 3,5-Dimethyl aniline (**1**, 0.1 mol), hydrochloric acid (9 mL) and water (25 mL) were taken and refluxed for 30 min in a round bottomed flask. The contents were cooled down to room temperature and then ammonium thiocyanate (0.1 mol) was added. The reaction mixture was again refluxed for 4 h. The solid obtained was cooled down, filtered, washed well with water, dried and crystallized from ethanol.

Synthesis of 6-Bromo/ 6-Fluoro/5,7--Dimethyl 2-aminobenzothiazole (3a-c):

In a round-bottomed flask equipped with a mechanical stirrer and a dropping funnel, phenylthiourea (**2**, 0.1 mol) and chloroform (100 mL) were taken. A solution of bromine (0.1 mol) in chloroform (100 mL) was added dropwise with stirring for a period of two hours. Temperature of reaction mixture remains below 5^{o}C during the reaction. Stirring was continued for a period of 4 h. After the addition of bromine solution, the contents of round-bottomed flask were refluxed for about 4 h till the evolution of HBr ceased. The solid obtained was treated with SO_2 water and filtered. The filtrate was neutralized with aqueous ammonia solution. The precipitate was filtered, washed well with water, and crystallized from ethanol.

Synthesis of 1-(substituted benzo[d]thiazol-2-yl)urea (4a-c):

To the solution of sodium cyanate, dissolved in minimum quantity of water, glacial acetic acid (5 mL) was added. An alcoholic solution of 2-amino benzothiazole **(3a-c)** was added and the solution was heated till the contents of the mixture became turbid and the volume reduced to half of the original. The contents were further added to ice cool water. The solid obtained was filtered off, dried and recrystallized from a suitable solvent.

1-(6-Bromo benzo[d]thiazol-2-yl)urea 4a. Yield 90%; mp 125^{o}C; IR (KBr): ν 3308 (NH), 1625 (C=O), 1558 (C=N), 636 (C-Br), 642 (C-S-C benzothiazole) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.12 (s, 1H, NHC=O), 6.67-6.72 (m, 3H, Ar-H), 6.32 (s, 2H, NH_2) ppm; MS, m/z: 272 [M^+]. Anal. Calcd. for $C_8H_6BrN_3OS$: C, 35.29; H, 2.20; N, 15.44 %. Found: C, 35.36; H, 2.25; N, 15.49 %.

1-(6-Fluoro benzo[d]thiazol-2-yl)urea 4b. Yield 86%; mp 105^{o}C; IR (KBr): ν 3318 (NH), 1638 (C=O), 1565 (C=N), 1225 (C-F), 649 (C-S-C benzothiazole) cm^{-1}; ^{1}H NMR ($CDCl_3$):

δ 9.19 (s, 1H, NHC=O), 6.69-6.75 (m, 3H, Ar-H), 6.39 (s, 2H, NH_2) ppm; MS, m/z: 211 [M^+]. Anal. Calcd. for $C_8H_6FN_3OS$: C, 45.50; H, 2.84; N,19.90%. Found: C, 45.54; H, 2.87; N, 19.92%.

1-(5,7-dimethylbenzo[d]thiazol-2-yl)urea 4c. Yield 90% ; mp 132°C; IR (KBr): ν 3300 (NH), 1630 (C=O), 1565 (C=N), 2915 (Me), 645 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.12 (s, 1H, NHC=O), 6.65-6.89 (m, 2H, Ar-H), 6.35 (s, 2H, NH_2) 2.35 (s, Me) ppm; MS, m/z: 221 [M^+]. Anal. Calcd. for $C_{10}H_{11}N_3OS$: C, 54.29; H, 4.97; N, 19.00 %. Found: C, 54.23; H, 4.90; N, 19.08 %.

Synthesis of N-(substituted benzo[d]thiazol-2-yl)hydrazine carboxamide (5a-c):

A equimolar mixture of compound **4** and hydrazine hydrate was dissolved in methanol at room temperature. To the solution, conc. NaOH was added and refluxed for 6 h. The reaction mixture was poured onto the crushed ice and the solid obtained was filtered off, dried and recrystallized from a suitable solvent.

N-(6-Bromo benzo[d]thiazol-2-yl)hydrazine carboxamide 5a. Yield 85% ; mp 145 °C; IR (KBr): ν 3302 (NH), 1665 (C=O), 1592 (C=N), 658 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.10 (s, 1H, NHC=O), 7.71-7.75 (m, 3H, Ar-H), 6.35 (s, 2H, NH_2), 7.25 (s, 1H, $NHNH_2$) ppm; MS, m/z: 287 [M^+]. Anal. Calcd. for $C_8H_7BrN_4OS$: C, 33.45; H, 2.44; N, 19.51%. Found: C, 33.49; H, 2.46; N, 19.57%.

N-(6-Fluoro benzo[d]thiazol-2-yl)hydrazine carboxamide 5b. Yield 79%; mp 141°C; IR (KBr): ν 3308 (NH), 1668 (C=O), 1595 (C=N), 659 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.16 (s, 1H, NHC=O), 7.72-7.79 (m, 3H, Ar-H), 6.39 (s, 2H, NH_2), 7.31 (s, 1H, $NHNH_2$) ppm; MS, m/z: 226 [M^+]. Anal. Calcd. for $C_8H_7FN_4OS$: C, 42.48; H, 3.10; N, 24.78%. Found: C, 42.45; H, 3.13; N, 24.81%.

N-(5,7-dimethylbenzo[d]thiazol-2-yl) hydrazine carboxamide 5c. Yield 85%; mp 141°C; IR (KBr): ν 3305 (NH), 1650 (C=O), 1575 (C=N), 652 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.15 (s, 1H, NHC=O), 7.50-7.80 (m, 2H, Ar-H), 6.35 (s, 2H, NH_2), 7.30 (s, 1H, $NHNH_2$) ppm; MS, m/z: 236 [M^+]. Anal. Calcd. for $C_{10}H_{12}N_4OS$: C, 50.84; H, 5.08 ; N, 23.72 %. Found: C, 50.80; H, 5.14; N, 23.80 %.

General procedure for the synthesis of 2-Substituted benzylidene -N- (substituted benzo[d]thiazol-2-yl)hydrazine carboxamide (6a-j):

A equimolar mixture of **4** (0.1 mol) and substituted benzaldehyde (0.1 mol) was dissolved in methanol at room temperature. Few drops of glacial acetic acid was added to the reaction mixture and was then refluxed on a water bath for 5-6 h. It was then allowed to cool, poured onto crushed ice and recrystallized from methanol.

***N*-(6-Bromobenzo[d]thiazol-2-yl)-2-(2-chlorobenzylidene) hydrazine carboxamide 6a.** Yield 70%; mp 172°C; IR (KBr): ν 3315 (NH), 1665 (C=O), 1587 (C=N), 652 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.05 (s, 1H, NHC=O), 7.71-7.79 (m, 7H, Ar-H), 6.15 (s, 1H, NH), 7.95 (s, 1H, N=CH) ppm; MS, m/z: 409.5 [M^+]. Anal. Calcd. for $C_{15}H_{10}BrClN_4OS$: C, 543.95; H, 2.44; N, 13.67 %. Found: C, 43.90; H, 2.39; N, 13.61 %.

N-(6-Bromobenzo[d]thiazol-2-yl)-2-(2-methylbenzylidene) hydrazine carboxamide 6b. Yield 65%; mp 145°C; IR (KBr): ν 3308 (NH), 1665 (C=O), 1580 (C=N), 642 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.08 (s, 1H, NHC=O), 7.70-7.78 (m, 7H, Ar-H), 6.12 (s, 1H, NH), 7.89 (s, 1H, N=CH) ppm; MS, m/z: 389 [M^+]. Anal. Calcd. for $C_{16}H_{13}BrN_4OS$:

C, 49.35; H, 3.34; N, 14.40 %. Found: C, 49.39; H, 3.36; N, 14.45 %.

N-(6-bromobenzo[d]thiazol-2-yl)-2-(2-methoxybenzylidene) hydrazine carboxamide 6c. Yield 68%; mp 157°C; IR (KBr): ν 3320(NH), 1661 (C=O), 1585 (C=N), 655 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.07 (s, 1H, NHC=O), 7.72-7.85 (m, 7H, Ar-H), 6.15 (s, 1H, NH), 7.92 (s, 1H, N=CH) ppm; MS, m/z: 405 [M^+]. Anal. Calcd. for $C_{16}H_{13}BrN_4O_2S$: C, 47.40, H, 3.21, N,13.82 %. Found: C, 47.36, H, 3.18, N, 13.80 %.

N-(6-Bromobenzo[d]thiazol-2-yl)-2-(4-nitrobenzylidene) hydrazine carboxamide 6d. Yield 58%; mp 162°C; IR (KBr): ν 3325 (NH), 1675 (C=O), 1595 (C=N), 650 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.20 (s, 1H, NHC=O), 7.79-7.85 (m, 7H, Ar-H), 6.25 (s, 1H, NH), 7.99 (s, 1H, N=CH) ppm; MS, m/z: 420 [M^+]. Anal. Calcd. for $C_{15}H_{10}BrN_5O_3S$: C, 42.85; H, 2.38; N, 16.67 %. Found: C, 42.82; H, 2.35; N, 16.65 %.

N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(2-chlorobenzylidene) hydrazine carboxamide 6e. Yield 79%; mp180°C; IR (KBr): ν 3319 (NH), 1678 (C=O), 1588 (C=N), 654 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.15 (s, 1H, NHC=O), 7.72-7.83 (m, 7H, Ar-H), 6.16 (s, 1H, NH), 7.96 (s, 1H, N=CH) ppm; MS, m/z: 348.5 [M^+]. Anal. Calcd. for $C_{15}H_{10}FClN_4OS$: C, 51.65; H, 2.87; N, 16.07 %. Found: C, 51.71; H, 2.95; N, 16.09 %.

N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(2-methylbenzylidene)hydrazine carboxamide 6f. Yield 65%; mp 149°C**;** IR (KBr): ν 3308 (NH), 1665 (C=O), 1578 (C=N), 642 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.05 (s, 1H, NHC=O), 7.71-7.79 (m, 7H, Ar-H), 6.11 (s, 1H, NH), 7.92 (s, 1H, N=CH) ppm; MS, m/z: 328 [M^+]. Anal. Calcd. for $C_{16}H_{13}FN_4OS$: C, 58.53; H, 3.96; N, 17.07 %. Found: C, 58.49; H, 3.95; N, 17.05 %.

N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(2-methoxybenzylidene)hydrazinecarboxamide 6g. Yield 72%; mp 136°C; IR (KBr): ν 3318 (NH), 1662 (C=O), 1587 (C=N), 652 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.08 (s, 1H, NHC=O), 7.79-7.86 (m, 7H, Ar-H), 6.15 (s, 1H, NH), 7.81 (s, 1H, N=CH) ppm; MS, m/z: 344 [M^+]. Anal. Calcd. for $C_{16}H_{13}FN_4O_2S$: C, 55.81; H, 3.78; N, 16.28 %. Found: C, 55.85; H, 3.81; N, 16.35 %.

N-(6-Fluorobenzo[d]thiazol-2-yl)-2-(4-Nitrobenzylidene)hydrazinecarboxamide 6h. Yield 70%; mp 136°C; IR (KBr): ν 3325 (NH), 1687 (C=O), 1590 (C=N), 648 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.25 (s, 1H, NHC=O), 7.79-7.89 (m, 7H, Ar-H), 6.21 (s, 1H, NH), 8.01 (s, 1H, N=CH) ppm; MS, m/z: 359 [M^+]. Anal. Calcd for $C_{15}H_{10}FN_5O_3S$: C, 50.14; H, 2.78; N, 19.50 %. Found: C, 50.19; H, 2.85; N, 19.57 %.

N-(5,7-Dimethylbenzo[d]thiazol-2-yl)-2-(2-chlorobenzylidene)hydrazinecarboxamide 6i. Yield 73%; mp 146°C; IR (KBr): ν 3329 (NH), 1682 (C=O), 1593 (C=N), 652 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.23 (s, 1H, NHC=O), 7.66-7.81 (m, 6H, Ar-H), 6.25 (s, 1H, NH), 8.10 (s, 1H, N=CH) ppm; MS, m/z: 358.5 [M^+]. Anal. Calcd. for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.18; N, 15.62 %. Found: C, 56.99; H, 4.27; N, 15.73 %.

N-(5,7-Dimethylbenzo[d]thiazol-2-yl)-2-(4Nitrobenzylidene) hydrazinecarboxamide6j. Yield 65%; mp 110°C; IR (KBr): ν (3335 (NH), 1689 (C=O), 1599 (C=N), 645 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.20 (s, 1H, NHC=O), 7.77-7.91 (m, 6H, Ar-H), 6.29 (s,1H, NH), 8.15 (s, 1H, N=CH) ppm); MS, m/z: 369 [M^+]. Anal. Calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.28; H, 4.06; N, 18.97 %. Found: C, 55.20, H, 4.04, N, 18.95 %. **General**

procedure for the synthesis of 1-(3-chloro-2-oxo-4-substituted phenylazetidin-1-yl)-3-(substitutedbenzo[d]thiazol-2-yl)urea (7a-j)

A mixture of **6** (0.01 mol) in 20 mL of dioxane was added to a well stirred mixture of chloroacetyl chloride (0.015 mol) and triethylamine (Et_3N) (0.015 mol) dissolved in dioxane (10 mL) at 0-5°C. Then the reaction mixture was stirred for 8-10 h and kept for two days at room temperature and further treated with cold water. The solid thus separated was filtered, washed several times with water and recrystallized from methanol.

1-(3-Chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-3-(6-bromobenzo[d] thiazol-2-yl)urea 7a. Yield 77%; mp 240°C; IR (KBr): ν 3249 (NH), 3125 (C-H aromatic), 1755 (C=O β lactam ring), 1675 (C=O), 1570 (C=C aromatic), 1449 (C-N benzothiazole), 648 (C-S-C benzothiazole) cm^{-1}; ^1H NMR($CDCl_3$): δ 10.49 (s, 1H, CONH), 7.79 (s, 1H, N-CH β lactam ring), 6.62-6.73 (m, 7H, Ar-H) ppm; MS, m/z: 486 [M^+]. Anal. Calcd. for $C_{17}H_{11}BrCl_2N_4O_2S$: C, 41.97; H, 2.26; N, 11.52 %. Found: C, 41.95; H, 2.25; N, 11.50 %.

1-(3-Chloro-2-oxo-2-p-tolyl azetidin-1-yl)-3-(6-bromobenzo[d]thiazol-2-yl)urea 7b. Yield 73%; mp 215°C; IR (KBr): ν 3253 (NH), 3120 (C-H aromatic), 1770 (C=O β lactam ring), 1678 (C=O), 1578 (C=C aromatic), 1459 (C-N benzothiazole), 652 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.45 (s, 1H, CONH), 7.78 (s, 1H, N-CH β lactam ring), 6.72-6.85 (m, 7H, Ar-H),2.35 (s, 3H, CH_3) ppm; MS, m/z: 465.5[M^+]. Anal. Calcd. for $C_{18}H_{14}ClBrN_4O_2S$: C, 46.40; H, 3.01; N, 12.03 %. Found: C, 46.45; H, 3.04; N, 12.08 %.

1-(3-Chloro-2-(2-methoxyphenyl)-4-oxoazetidin-1-yl)-3-(6-bromobenzo[d] thiazol-2-yl)urea 7c. Yield 68%; m.p. 230°C**;** IR (KBr): ν 3262 (NH), 3110 (C-H aromatic), 1779 (C=O β lactam ring), 1682 (C=O), 1530 (C=C aromatic), 1450 (C-N benzothiazole), 659 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.52 (s, 1H, CONH), 7.69 (s, 1H, N-CH β lactam ring), 6.52-6.69 (m, 7H, Ar-H), 3.88 (s, 1H, OCH_3) ppm); MS, m/z: 481.5[M^+]. Anal. Calcd. for $C_{18}H_{14}ClBrN_4O_3S$: C, 44.86; H, 2.90; N, 11.63 %. Found: C, 44.95; H, 2.98; N, 11.70 %.

1-(3-Chloro-2-(4-nitrophenyl)-4-oxo-azetidin-1-yl)-3-(6-bromobenzo[d]thiazol-2-yl)urea 7d. Yield 75%; mp 210°C; IR (KBr): ν 3268 (NH), 3115 (C-H aromatic), 1789 (C=O β lactam ring), 1672 (C=O), 1532 (C=C aromatic), 1460 (C-N benzothiazole), 665 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.45 (s, 1H, CONH), 6.62-6.78 (m, 7H, Ar-H), 7.78 (s, 1H, N-CH β lactam ring) ppm; MS, m/z: 496.5[M^+]. Anal. Calcd. for $C_{17}H_{11}ClBrN_5O_4S$: C, 41.08; H, 2.21; N, 14.10 %. Found: C, 41.05; H, 2.17; N, 14.04 %.

1-(3-Chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-3-(6-fluorobenzo[d]thiazol-2-yl)urea 7e. Yield 79%; mp265°C; IR (KBr): ν 3250 (NH), 3120 (C-H aromatic), 1761 (C=O β lactam ring), 1675 (C=O), 1565 (C=C aromatic), 1445 (C-N benzothiazole), 655 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.25 (s, 1H, CONH), 7.75 (s, 1H, N-CH β lactam ring), 6.50-6.75 (m, 7H, Ar-H); MS, m/z: 425 [M^+]. Anal. Calcd. for $C_{17}H_{11}FCl_2N_4O_2S$: C, 48.00; H, 2.58; N,13.17 %. Found: C, 48.05; H, 2.61; N, 13.19 %.

1-(3-Chloro-2-oxo-2-o-tolyl azetidin-1-yl)-3-(6-fluorobenzo[d] thiazol-2-yl)urea7f. Yield: 71%; mp 231°C; IR (KBr): ν 3261 (NH), 3140 (C-H aromatic), 1768 (C=O β lactam ring), 1672 (C=O), 1562 (C=C aromatic), 1460 (C-N benzothiazole), 659 (C-S-C benzothiazole) cm^{-1}; ^1H NMR($CDCl_3$): δ 10.41 (s, 1H, CONH), 7.78 (s, 1H, N-CH β lactam ring), 6.59-6.72 (m, 7H, Ar-H), 2.35 (s, 3H, CH_3) ppm; MS, m/z:

404.5 [M^+]. Anal. Calcd. for $C_{18}H_{14}FClN_4O_2S$: C, 53.40; H, 3.46; N, 13.84 %. Found: C, 53.37; H, 3.45; N, 13.81 %.

1-(3-Chloro-2-(2-methoxyphenyl)-4-oxo-azetidin-1-yl)-3-(6-fluorobenzo[d] thiazol-2-yl)urea 7g. Yield 72%; mp 238 °C; IR (KBr): ν 3261 (NH), 3125 (C-H aromatic), 1782 (C=O β lactam ring), 1671 (C=O), 1552 (C=C aromatic), 1462 (C-N benzothiazole), 629 (C-S-C benzothiazole) cm^{-1}; ^1H NMR($CDCl_3$): δ 10.42 (s, 1H, CONH), 7.77 (s, 1H, N-CH β lactam ring), 6.52-6.62 (m, 6H, Ar-H), 3.82 (s, 3H, OCH_3) ppm; MS, m/z: 420.5 [M^+]. Anal. Calcd. for $C_{18}H_{14}FClN_4O_3S$: C, 51.36; H, 3.33; N, 13.31 %. Found: C, 51.39; H, 3.39; N, 13.37 %.

1-(3-Chloro-2-(4-nitrophenyl)-4-oxo-azetidin-1-yl)-3-(6-fluorobenzo[d] thiazol-2-yl)urea 7h. Yield 69%; m.p. 202°C; IR (KBr): ν 3265 (NH), 3135 (C-H aromatic), 1785 (C=O β lactam ring), 1678 (C=O), 1548 (C=C aromatic), 1459 (C-N benzothiazole), 665 (C-S-C benzothiazole) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.48 (s, 1H, CONH), 7.72 (s, 1H, N-CH β lactam ring), 6.48-6.62 (m, 7H, Ar-H) ppm; MS, m/z: 435.5 [M^+]. Anal. Calcd. for $C_{17}H_{11}FClN_5O_4S$: C, 46.84; H, 2.52; N, 16.07 %. Found: C, 46.85; H, 2.55; N, 16.11 %.

1-(3-Chloro-2-(2-chlorophenyl)-4-oxo-azetidin-1-yl)-3-(5,7-dimethylbenzo[d] thiazol-2-yl)urea 7i. Yield: 76%; m.p. 212°C; IR (KBr): ν 3245 (NH), 3130 (C-H aromatic), 1748 (C=O β lactam ring), 1668 (C=O), 1439 (C=C aromatic), 1450 (C-N benzothiazole), 635 (C-S-C benzothiazole) cm^{-1}; ^1H NMR($CDCl_3$): δ 10.48 (s, 1H, CONH), 7.69 (s, 1H, N-CH β lactam ring), 6.58-6.65 (m, 6H, Ar-H) ppm; MS, m/z: 435 [M^+]. Anal. Calcd. for $C_{19}H_{16}Cl_2N_4O_2S$: C, 52.41; H, 3.67; N,12.87 %. Found: C, 52.45; H, 3.69; N, 12.92 %.

1-(3-Chloro-2-(4-nitrophenyl)-4-oxo-azetidin-1-yl)-3-(5,7-dimethylbenzo[d] thiazol-2-yl)urea 7j. Yield 66%; mp 225°C; IR (KBr): ν 3258 (NH), 3132 (C-H aromatic), 1779 (C=O β lactam ring), 1665 (C=O), 1449 (C-N benzothiazole), 662 (C-S-C benzothiazole) cm^{-1}; ^1H NMR($CDCl_3$): δ 10.40 (s, 1H, CONH), 7.72 (s, 1H, N-CH β lactam ring), 6.57-6.69 (m, 6H, Ar-H) ppm; MS: m/z 445.5 [M^+].Anal. Calcd. for $C_{19}H_{16}ClN_5O_4S$: C, 51.17; H, 3.59; N, 15.71 %. Found: C, 51.15; H, 3.51; N, 15.65 5 %.

Antimicrobial assay

The minimum inhibitory concentrations (MICs, μg mL^{-1}) of the chemical compounds assays were carried out by broth microdilution method as per NCCLS-1992 manual. Two Gram-positive (*Bacillus cereus* MTCC 4317 and *Staphylococcus aureus* MTCC 3160) and two Gram-negative (*Escherichia coli* DH5 alpha MTCC 1652 and *Pseudomonas aeruginosa* MTCC 4676) bacteria were used as quality control strains. For determining antifungal activities of the compounds, the following reference strains were tested : *Aspergillus niger* MTCC 282, *Alternaria solani* MTCC 2101, *Fusarium culmorum* MTCC 2090 and *Rhizopus stolonifer* MTCC 2591. Ampicillin sodium salt and Fluconazole were used as standard antibacterial and antifungal drugs, respectively. The MICs of tested compounds in μg/mL against certain bacteria and fungi are shown in Table 1.

Solutions of test compounds and standard drugs were prepared in DMSO. Each synthesized drug was diluted obtaining 1000 μg/mL concentration as a stock solution. In primary screening, 500, 250 and 125 μg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were

similarly diluted to obtain 100, 50, 25, 20, 15 µg/mL concentrations. The highest dilution showing at least 99% inhibition was taken as MIC. This means that the lowest concentration of each chemical compound in the tube with no growth (i.e. no turbidity) of inoculated bacteria/ fungi was recorded as minimum inhibitory concentration of that compound. Antibacterial activities of the bacterial strains were carried out in Luria broth (Himedia) medium and all fungi were cultivated in Sabouraud Dextrose Agar (Himedia), at pH 6.9, with an inoculum of 10^8 cfu/mL by the spectrophotometric method and an aliquot of 10 µL was added to each tube of the serial dilution and incubated on a rotary shaker at 37°C for 24 h at 150 rpm. At the end of incubation period, MIC values were recorded.

Compounds **7d, 7h** and **7j** showed good activity against all the fungal strains. Compounds **7a, 7e** and **7i** showed moderate activity against all the fungal strains **(Table S1).** For antimicrobial activity, it was observed that introduction of electron withdrawing groups in the compounds showed considerable increase in antibacterial potency of compounds. The compounds having electron withdrawing groups showed maximum potency than compounds containing electron releasing groups.

Conclusion:

In our study, particularly derivatives having electron withdrawing groups such as nitro and halogen are identified as exhibiting potential antibacterial activity against bacterial strains and antifungal strains. The result also showed that gram negative showed better activity than gram positive organisms. Thus, heterocycles accommodating sub units i.e. benzothiazole and azetidin-2-one are expected to prove the therapeutic relevance and their utility in medicinal chemistry. Our ongoing research focuses on the same molecular hybrids with incorporation of more effective substituents in search of new effective antimicrobial agents.

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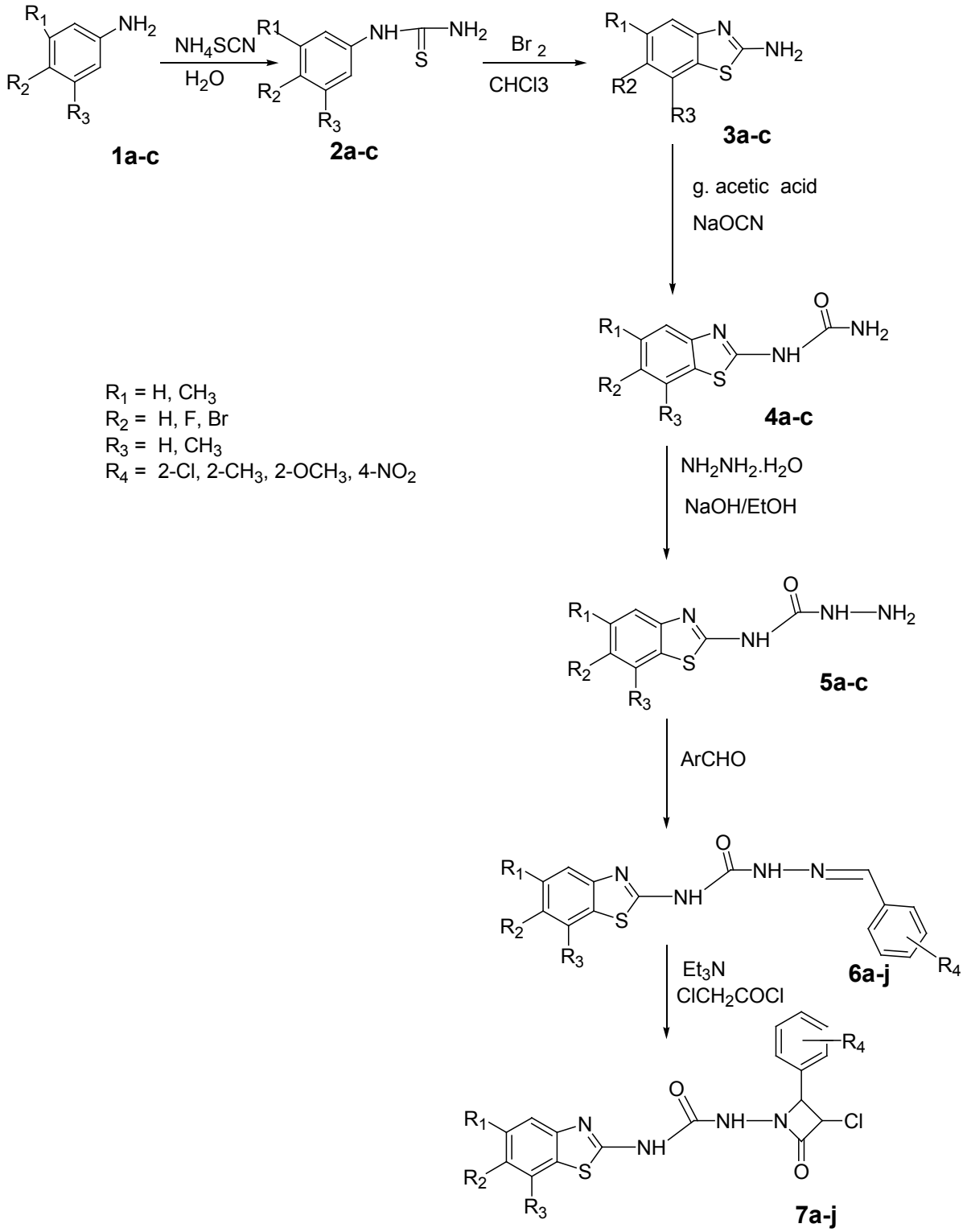

Scheme 1: Synthesis of compounds **7a-j**

Table 1 Antimicrobial activity of synthesized compounds **7a-j**

Compd. No.	[a]MICs of bacterial strains in µg/mL				MICs of fungal strains in µg/mL			
	Bacillus cereus	*Staphylococcus aureus*	*E. coli* DH5 alpha	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Alternaria solani*	*Fusarium culmorum*	*Rhizopus stolonifer*
	MTCC 4317	MTCC 3160	MTCC 1652	MTCC 4676	MTCC 282	MTCC 2101	MTCC 2090	MTCC 2591
7a	34.7	46.2	35.4	43.6	31.6	36.2	33.4	41.2
7b	32.9	53.6	52.3	49.4	30.5	34.8	30.3	39.4
7c	38.4	60.9	69.1	55.8	25.7	29.3	27.2	38.7
7d	40.8	66.1	65.4	61.9	38.9	42.1	39.7	51.8
7e	22.8	43.2	42.8	37.1	20.4	26.2	21.1	30.3
7f	35.7	50.8	48.2	44.3	34.3	37.6	34.8	45.9
7g	32.9	40.3	39.6	41.5	30.1	32.5	30.2	37.6
7h	37.9	62.7	62.6	59.2	38.2	43.4	38.6	48.7
7i	25.7	40.7	41.2	38.8	30.5	31.8	28.7	37.7
7j	28.9	45.6	43.3	40.1	34.3	37.6	35.6	46.5
Ampicillin sodium salt	38	64	64	60	-	-	-	-
Fluconazole	-	-	-	-	36.3	40.3	36.3	48.2

[a]Minimum Inhibitory Concentration