RESEARCH ARTICLE

Synthesis, Characterization and Antimicrobial Activity of Novel 2-Azetidinones Compounds

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ABSTRACT

A series of 2-azetidinone derivatives were synthesized by the formation of triazole, followed by synthesis of Schiff's bases by condensation of various substituted aldehydes. The Schiff's bases further reacted to chloroacetyl chloride in the presence of triethyl amine, and undergoes cyclocondensation to give substituted 3-chloro-1-(3-mercapto-5-((5-methoxy-*1H*-indol-1-yl) methyl)-4*H*-1,2,4-triazol-4-yl)-4-phenylazetidin-2-one which on further reaction with ethyl chloroacetate, gives substituted ethyl 2-((4-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-((5-methoxy-*1H*-indol-1-yl)methyl)-4,5-dihydro-*1H*-1,2,4-triazol-5-yl) thio)acetate. Synthesized compounds were by characterized by fourier-transform infrared (FTIR), nuclear magnetic resonance (NMR) (^{1}H, ^{13}C), and mass spectroscopy. All synthesized compounds were evaluated for its *in-vitro* antimicrobial activity against Gram-positive and gram-negative bacterial as well as fungal strains. The antimicrobial activity results revealed that A-VI, A-IX, A-XVIII, displayed equipotent activity when compared with the standard drug. The compounds with p-chloro and p-nitro group exhibited the most potent antimicrobial agent.

Keywords: 2-Azetidinone, Synthesis, Characterization, Antimicrobial, Phenylazetidin-2-one, Triazole.

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INTRODUCTION

The nucleus of azetidinones (also known as β-lactam) plays a key role in the biology of a large family of antibiotics. Azetidinones are characterized by the presence of a four-monomer ring and are distinguished by their side chains, unsaturation, heteroatoms, and five- or six-member rings.[1-4] They occupy a central position among the most important moieties in medicine because of their diverse and potent antibiotic properties. Schiff base[5-11] has received the most attention as a potential antimicrobial agent in the last 20 years. It has good antimicrobial and antifungal properties and can be prepared by an acid-catalyzed aldehyde-to-ketone reaction with amines. 2-Azetidinones[12-16] have a broad range of promising biological activities. These compounds have been found to be useful in a wide range of diseases and disorders such as microbial infections, coronary artery disease (atherosclerosis), inflammatory disorders, autoimmune diseases, asthma, thrombocytopenia, rheumatoid arthritis, pneumonitis, retinitis, esophagus, colitis, osteoporosis, diabetes and cancer. Future research on this scaffold may provide some additional lead compounds for the development of active drugs for a variety of diseases. Antimicrobial drug design tends to be based on clubbing 2- or 3-heterocyclic molecules with different sites or mechanisms of action. This has led to the formation of compounds containing substituted Indole ring systems with substituted triazole ring systems and azetidinone ring systems to serve as the new scaffold for developing new antimicrobial agents. In the context of the above discussion, the main focus was on synthesizing indole-triazole-azetidinone substitution to form new antimicrobial agents.

MATERIALS AND METHODS

Chemicals

Every chemical used in the synthesis was bought from Merck and Sigma-Aldrich. The remaining reagents and solvents were all of analytical quality and were obtained from different commercial suppliers.

Instrumentation

Melting points are uncorrected and were measured using the Perfit melting point instrument. The Win IR FTS 135 equipment was used to record the infrared spectra of KBr discs. Using TMS (Sigma-Aldrich) as an internal standard, the Bruker spectrospin DPX-300 NMR captured the proton nuclear magnetic resonance (^{1}H-NMR) (400 MHz) spectra. The instrument used to obtain the mass spectra was the JEOL-JMS-DX 303. The Perkin-Elmer

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240C analyzer was used to perform elemental studies (C, H, and N). Every chemical was within ± 0.4% of the values predicted by theory. Thin-layer chromatography (TLC) plates coated with silica gel G were used for TLC (Merck).

Synthesis

Synthesis of ethyl-N-indolyl acetate

A 100 mL dry conical flask containing 10 mL of acetone was used to dissolve 1.1 g of indole (0,01 mol). This was mixed with 1.2 mL (0.01 mol) of ethyl chloroacetate. After adding 2.3 g of potassium carbonate, the liquid was covered with cotton and given a 24-hour stir with a magnetic stirrer. After pouring the mixture into around 100 mL of ice water, it was refrigerated for the whole night. After that, ether was used to extract the ester that had been produced above, and an ester was obtained by discarding the ether.

Synthesis of N-indolyl acetyl hydrazine

About 0.96 mL of hydrazine hydrate (0.01 mol) was added to 2 g of Indoly ester 1 (0.01 mol) and refluxed for a duration of 12 to 16 hours. After adding the aforementioned combination to a beaker filled with ice-cold water, it was refrigerated for the whole night. The result had a yield of 89% and a melting point of 49°C. It was filtered at the pump, periodically cleaned with ice-cold water, and then recrystallized using pure alcohol.

Synthesis of preparation of 2-(2-(5-methoxy-1H-indol-1-yl) acetyl)hydrazine-1-carbodithioic acid

In 50 mL of 100% ethanol was used to dissolve 0.03 mol of potassium hydroxide. After the mixture had cooled in an ice bath, hydrazide 2 (0.02 mol) was stirred in. Small amounts of carbon disulfide (0.025 mol) were added to this while stirring continuously. For 16 hours at RT, the reaction mixture was constantly stirred. Filtration was used to gather the precipitated potassium dithiocarbazinate, which was then cleaned with anhydrous ether and vacuum-dried. Without more purification, the potassium salt that was thus produced was utilized in the next procedure. Yield: 89%, IR (KBr): 3142 cm^{-1} (Aromatic C-H Stretching), 2954 cm^{-1} (Aliphatic C-H Stretching), 1531 cm^{-1} (Aromatic C=C Stretching), 717 cm^{-1} (C-S bending); δ 1.17 (3H, t, *J* = 7.1 Hz), 3.59-3.69 (2H, 3.64 (s), 3.64 (s)), 4.07–4.19 (2H, 4.13 (q, *J* = 7.1 Hz), 4.13 (q, *J* = 7.1 Hz)), 4.64–4.76 (2H, 4.70 (d, *J* = 6.1 Hz), 4.70 (d, *J* = 6.1 Hz)), 5.25 (1H, d, *J* = 8.1 Hz), 5.44-5.65 (2H, 5.50 (d, *J* = 8.1 Hz), 5.59 (t, *J* = 6.1 Hz)), 6.53-6.73 (3H, 6.60 (ddd, *J* = 8.8, 2.0, 0.5 Hz), 6.66 (ddd, *J* = 8.3, 2.0, 0.5 Hz)), 7.23 (2H, ddd, *J* = 8.3, 1.1, 0.5 Hz), 7.69-7.81 (2H, 7.75 (dd, *J* = 8.5, 1.8 Hz), 7.75 (dq, *J* = 8.5, 0.5 Hz)), 7.88 (1H, dt, *J* = 8.8, 0.5 Hz), 8.63 (1H, ddt, *J* = 2.0, 1.8, 0.5 Hz)

Synthesis of 5-((5-methoxy-1H-indol-1-yl) methyl)-1,2,4-triazole-3-thiol

For 10 to 15 hours, while shaking periodically, a suspension of potassium dithiocarbazinate 3 (0.02 mol) and hydrazine hydrate 99% (0.04 mol) in 50 mL of ethanol was refluxed. The reaction mixture's colour evolved into a light green as the hydrogen sulfide gas evolved. Over the course of the reaction, a homogeneous mixture was produced. Following RT cooling, the reaction mixture was diluted with 20 mL of cold water. White precipitate, the necessary triazole, was formed upon acidification with dilute hydrochloric acid. It was filtered, cleaned in cold water, dried, and refined *via* DMSO recrystallization. TLC examination revealed that the chemical was homogenous using a solvent solution of toluene: ethylacetate: formic acid (5:4:1, v/v/v). Yield: 61%, Melting point: 210°C, IR (KBr): 3213 cm^{-1} (Aromatic C-H Stretching), 3000 cm^{-1} (Aliphatic C-H Stretching), 1627 cm^{-1} (C=N Stretching), 1510 cm^{-1} (Aromatic C=C Stretching), 744 cm^{-1} (C-S bending); δ 1.17 (3H, t, *J* = 7.1 Hz), 2.29 (3H, s), 3.59–3.69 (2H, 3.64 (s), 3.64 (s)), 4.07–4.19 (2H, 4.13 (q, *J* = 7.1 Hz), 4.13 (q, *J* = 7.1 Hz)), 4.64–4.76 (2H, 4.70 (d, *J* = 6.1 Hz), 4.70 (d, *J* = 6.1 Hz)), 5.30 (1H, d, *J* = 8.1 Hz), 5.53–5.65 (2H, 5.59 (d, *J* = 8.1 Hz), 5.59 (t, *J* = 6.1 Hz)), 6.60 (1H, ddd, *J* = 8.8, 2.0, 0.5 Hz), 7.02 (1H, ddd, *J* = 7.9, 1.6, 1.2 Hz), 7.11–7.38 (3H, 7.17 (ddd, *J* = 8.0, 1.6, 1.2 Hz), 7.26 (ddd, *J* = 8.0, 7.9, 0.5 Hz), 7.33 (td, *J* = 1.6, 0.5 Hz)), 7.69–7.81 (2H, 7.75 (dd, *J* = 8.5, 1.8 Hz), 7.75 (dq, *J* = 8.5, 0.5 Hz)), 7.88 (1H, dt, *J* = 8.8, 0.5 Hz), 8.63 (1H, ddt, *J* = 2.0, 1.8, 0.5 Hz)

Synthesis of 4-(benzylideneamino)-5-((5-methoxy-1H-indol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol

A round-bottom flask was filled with an equivalent volume of compound 4 (0.01 mol) and aldehyde (0.01 mol) in absolute ethanol (30 mL), and four drops of glacial acetic acid were added. After that, it refluxed for ten hours. The suspension was added to ice-cold water after cooling the reaction mixture to room temperature. The resulting product was filtered, repeatedly cleaned with cold water, and refined by recrystallization from ethanol to produce compound crystals with a cream hue. TLC analysis using n-Hexane:Petroleum ether (6:4 v/v) as solvent system. Yield: 69%,Melting point: 152°C, IR (KBr): 3190 cm^{-1} (Aromatic C-H Stretching), 3022 cm^{-1} (Aliphatic C-H Stretching) 1597 cm^{-1} (C=N Stretching) 1550 cm^{-1} (Aromatic C=C Stretching), 756 cm^{-1} (C-S bending); δ 1.17 (3H, t, *J* = 7.1 Hz), 2.29 (3H, s), 3.59-3.69 (2H, 3.64 (s), 3.64 (s)), 4.07–4.19 (2H, 4.13 (q, *J* = 7.1 Hz), 4.13 (q, *J* = 7.1 Hz)), 4.64-4.76 (2H, 4.70 (d, *J* = 6.1 Hz), 4.70 (d, *J* = 6.1 Hz)), 5.30 (1H, d, *J* = 8.1 Hz), 5.53–5.65 (2H, 5.59 (d, *J* = 8.1 Hz), 5.59 (t, *J* = 6.1 Hz)), 6.60 (1H, ddd, *J* = 8.8, 2.0, 0.5 Hz), 7.02 (1H, ddd, *J* = 7.9, 1.6, 1.2 Hz), 7.11–7.38 (3H, 7.17 (ddd, *J* = 8.0, 1.6, 1.2 Hz), 7.26 (ddd, *J* = 8.0, 7.9, 0.5 Hz), 7.33 (td, *J* = 1.6, 0.5 Hz)), 7.69–7.81 (2H, 7.75 (dd, *J* = 8.5, 1.8 Hz), 7.75 (dq, *J* = 8.5, 0.5 Hz)), 7.88 (1H, dt, *J* = 8.8, 0.5 Hz), 8.63 (1H, ddt, *J* = 2.0, 1.8, 0.5 Hz)

Synthesis of 3-chloro-1-(3-mercapto-5-((5-methoxy-1H-indol-1-yl)methyl)-4H-1,2,4-triazol-4-yl)-4-phenylazetidin-2-one

At a temperature of 0 to 20°C, triethylamine (0.01 mol) and chloroacetyl chloride (0.01 mol) were gradually added drop-wise while stirring a solution of compound 5 (0.01 mol) in 25 mL 1,4-dioxane. After 30 minutes at room temperature, the reaction mixture was refluxed for five hours. After the surplus solvent was removed by distillation, the residue was added to ice-cold water. In order to produce colorless crystals, the isolated material was filtered and refined by recrystallization

from DMSO. The solvent system used for the TLC analysis was n-Hexane: Methanol (9:1 v/v). Yield: 59%, Melting point: 152. IR (KBr): 3061 cm^{-1} (Aromatic C-H Stretching), 2920 cm^{-1} (Aliphatic C-H Stretching) 1601 cm^{-1} (C=N Stretching) 1543 cm^{-1} (Aromatic C=CStretching) 1616 cm^{-1} (C=O stretching), 750 cm^{-1} (C-S bending) 619 cm^{-1} (C-Cl); δ 1.17 (3H, t, *J* = 7.1 Hz), 3.59–3.78 (5H, 3.64 (s), 3.64 (s), 3.73 (s)), 4.07–4.19 (2H, 4.13 (q, *J* = 7.1 Hz), 4.13 (q, *J* = 7.1 Hz)), 4.64–4.76 (2H, 4.70 (d, *J* = 6.1 Hz), 4.70 (d, *J* = 6.1 Hz)), 5.50–5.65 (3H, 5.56 (d, *J* = 8.1 Hz), 5.59 (d, *J* = 8.1 Hz), 5.59 (t, *J* = 6.1 Hz)), 6.60 (1H, ddd, *J* = 8.8, 2.0, 0.5 Hz), 6.81–7.01 (3H, 6.88 (ddd, *J* = 8.2, 2.8, 2.1 Hz), 6.92 (ddd, *J* = 2.8, 2.0, 0.5 Hz), 6.94 (dt, *J* = 7.9, 2.0 Hz)), 7.23 (1H, ddd, *J* = 8.2, 7.9, 0.5 Hz), 7.69–7.81 (2H, 7.75 (dd, *J* = 8.5, 1.8 Hz), 7.75 (dq, *J* = 8.5, 0.5 Hz)), 7.88 (1H, dt, *J* = 8.8, 0.5 Hz), 8.63 (1H, ddt, *J* = 2.0, 1.8, 0.5 Hz)

Synthesis of azetidinones

Ethyl chloroacetate (0.01 mol) was added to a compound 6 (0.01 mol) solution in 25 mL of ethanol. The mixture was then agitated for one hour at room temperature and refluxed for four hours while triethylamine (0.01 mol) was present. After that, a solid was produced by removing the solvent at a lower pressure. Ethanol and water (1:2) were used to recrystallize the material. TLC analysis was carried out using n-hexane:Methanol (7:3 v/v) as a solvent system (Figure 1).

- *Ethyl 2-((4-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-((5-methoxy-1H-indol-1-yl)methyl)-4,5-dihydro-1H-1,2,4-triazol-5-yl)thio)acetate (A-I)*

FTIR (KBr): 3243 cm^{-1} (Ar-CH), 1693 cm^{-1} (C=O), 1601 (C-N), 641 cm^{-1} (C-Cl), 742 cm^{-1} (C-S-C); ^{1}H-NMR (DMSO-d6): δ 4.9 (d, *1H*, CH, azetidin-2-one), 7.7–7.9 (m, 8H, ArH), 8.3 (s, *1H*, NH), 3.4 (s, 3H, O-CH3). MS m/z: 526 [M+]. Anal. Calcd for $C_{25}H_{24}ClN_5O_4S$:C, 57.61; H, 5.21; N, 12.92. Found:C, 57.52; H, 5.11; N, 12.81%.

- *Synthesis of ethyl 2-((4-(3-chloro-4-(4-chlorophenyl)-2-oxoazetidin-1-yl)-3-((5-methoxy-1H-indol-1-yl)methyl)-4,5-dihydro-1H-1,2,4-triazol-5-yl)thio)acetate (A-IX)*

FTIR (KBr): 3160 cm^{-1} (Ar-CH), 1680 cm^{-1} (C=O), 1600 cm^{-1} (C-N), 642 cm^{-1} (C-Cl), 754 cm^{-1} (C-S-C); ^{1}H-NMR (DMSO-d6): δ 4.8 (d, *1H*, CH, azetidin-2-one), 7.6–7.9 (m, 8H, ArH), 8.1 (s, *1H*, NH), 3.5 (s, 3H, O-CH3). MS m/z: 562 [M+]. Anal. Calcd for $C_{25}H_{24}ClN_5O_4S$: C, 53.38; H, 4.48; N, 12.45. Found:C, 53.31; H, 4.38; N, 12.32%.

- *Synthesis of ethyl 2-((4-(3-chloro--4-(4-methoxyphenyl)-2-oxoazetidin-1-yl)-3-((5-methoxy-1H-indol-1-yl)methyl)-4,5-dihydro-1H-1,2,4-triazol-5-yl)thio)acetate (A-X)*

FTIR (KBr): 3142 cm^{-1} (Ar-CH), 1680 cm^{-1} (C=O), 1600 cm^{-1} (C-N), 648 cm^{-1} (C-Cl), 784 cm^{-1} (C-S-C); ^{1}H-NMR (DMSO-d6): δ 4.9 (d, *1H*, CH, azetidin-2-one), 7.5–7.8 (m, 8H, ArH), 8.4 (s, *1H*, NH), 3.9 (s, 3H, O-CH3). MS m/z: 558 [M+]. Anal. Calcd for $C_{26}H_{28}ClN_5O_5S$: C, 55.96; H, 5.06; N, 12.55. Found: C, 55.89; H, 5.01; N, 12.45%.

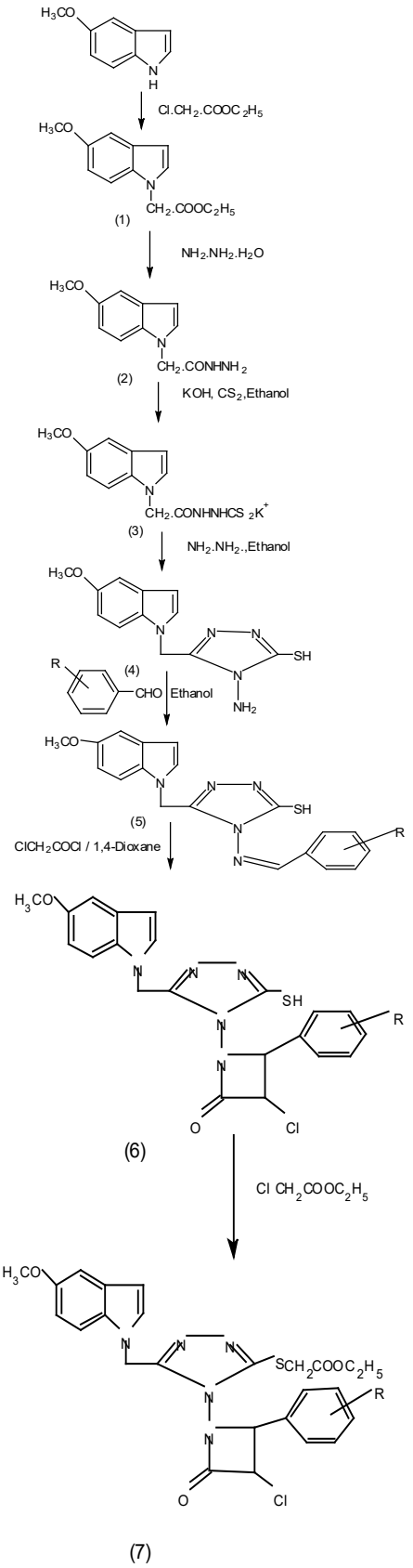

Figure 1: Synthetic route to azetidinones

- *Synthesis of ethyl 2-((4-(3-chloro--4-(3-methoxyphenyl)-2-oxoazetidin-1-yl)-3-((5-methoxy-1H-indol-1-yl) methyl)-4,5-dihydro-1H-1,2,4-triazol-5-yl)thio)acetate (A-XVII)*

FTIR (KBr): 3151 cm^{-1} (Ar-CH), 2949 cm^{-1} (Aliphatic C-H Stretching), 1693 cm^{-1} (C=O), 1603 cm^{-1}(C-N), 640 cm^{-1} (C-Cl), 831 cm^{-1} (C-S-C); ^{1}*H*-NMR (DMSO-d6): δ 4.8 (d, *1H*, CH, azetidin-2-one), 7.4–7.8 (m, 8H, ArH), 8.7 (s, *1H*, NH), 4.1 (s, 3H, O-CH3). MS m/z: 558 [M+]. Anal. Calcd for $C_{26}H_{28}ClN_5O_5S$: C, 55.96; H, 5.06; N, 12.55. Found :C, 55.89; H, 5.01; N, 12.45%.

- *Ethyl-2-((4-(3-chloro--4-(4-nitrophenyl)- -2-oxoazetidin-1-yl)-3-((5-methoxy-1H-indol-1-yl)methyl)-4,5-dihydro-1H-1,2,4-triazol-5-yl)thio)acetate (A-XVIII)*

FTIR (KBr): 3168 cm^{-1} (Ar-CH), 2983 cm^{-1} (Aliphatic C-H Stretching),1680 cm^{-1} (C=O), 1601 cm^{-1} (C-N), 646 cm^{-1} (C-Cl), 786 cm^{-1} (C-S-C); ^{1}H-NMR (DMSO-d6): δ 4.6 (d, *1H*, CH, azetidin-2-one), 7.5–7.9 (m, 8H, ArH), 8.9 (s, *1H*, NH), 4.4 (s, 3H, O-CH3). MS m/z: 573 [M+]. Anal. Calcd for $C_{25}H_{25}ClN_6O_6S$: C, 52.40; H, 4.40; N, 14.67. Found :C, 52.32; H, 4.31; N, 14.56%.

Antimicrobial Evaluation

The antibacterial studies of azetidinones derivatives were carried out against different microorganisms: *Staphylococcus aureus*, *E. faecalis, Pseudomonas aeruginosa*, and *Escherichia coli*.

Antibacterial activity

Using the Muller Hinton agar medium and the disc diffusion technique, the *in-vitro* antibacterial activity was evaluated under normal operating conditions. Following a 24-hour incubation period at 37°C in nutrient broth, newly produced bacterial cells were placed onto Muller Hinton agar plates in a laminar flow cabinet. Dimethylsulfoxide (DMSO) was used to dissolve the extract before it was soaked onto sterile discs of Whatman filter paper No. 1 (6 mm diameter). After that, the discs were incubated on the surface of the bacterial plates that had been previously produced. The diameter of the zone of inhibition was measured for the extract in millimeters after a 24-hour incubation period at 37°C. A disc impregnated with DMSO was employed as a negative control, and the standard antibiotic Penicillin was utilized to compare the activity. Three times were scheduled for the exams.[15]

Table 1: Physical data of synthesized compounds

Compound	*R*	*Molecular formula*	*Formula weight*	*%Yield*	*MP (ºC)*	*Rf value*
A-I	H	$C_{25}H_{24}ClN_5O_4S$	526.00	69	142–146	0.74
A-II	4-OH	$C_{25}H_{24}ClN_5O_5S$	542.00	58	148–151	0.80
A-III	4-Br	$C_{25}H_{23}BrClN_5O_4S$	604.90	52	142–144	0.64
A-IV	3-OH	$C_{25}H_{24}ClN_5O_5S$	542.00	57	120–122	0.88
A-V	2-OH	$C_{25}H_{24}ClN_5O_5S$	542.00	56	128–130	0.79
A-VI	2-Cl	$C_{25}H_{25}Cl_2N_5O_4S$	562.46	61	148–150	0.78
A-VII	4-CH3	$C_{26}H_{28}ClN_5O_4S$	542.04	59	146–148	0.77
A-VIII	2-NO_2	$C_{25}H_{25}ClN_6O_6S$	573.02	58	163–165	0.69
A-IX	4-Cl	$C_{25}H_{25}Cl_2N_5O_4S$	562.46	57	168–170	0.68
A-X	4-OCH_3	$C_{26}H_{28}ClN_5O_5S$	558.04	58	148–150	0.65
A-XI	3-NO_2	$C_{25}H_{25}ClN_6O_6S$	573.02	61	137–139	0.60
A-XII	3,4$(OCH_3)_2$	$C_{27}H_{30}ClN_5O_6S$	588.07	58	150–152	0.69
A-XIII	3,4$(Cl)_2$	$C_{25}H_{24}Cl_3N_5O_4S$	596.91	59	158–161	0.71
A-XIV	4-N$(CH_3)_2$	$C_{27}H_{31}ClN_6O_4S$	571.09	61	142–145	0.81
A-XV	3,4,5$(OCH_3)_3$	$C_{28}H_{32}ClN_5O_7S$	618.10	55	153–155	0.59
A-XVI	2-OCH_3	$C_{26}H_{28}ClN_5O_5S$	558.04	57	112–113	0.64
A-XVII	3-OCH_3	$C_{26}H_{28}ClN_5O_5S$	558.04	59	109–111	0.66
A-XVIII	4-NO_2	$C_{25}H_{25}ClN_6O_6S$	573.02	63	154–156	0.68
A-XIX	4-OH-3-OCH_3	$C_{26}H_{28}ClN_5O_6S$	574.04	55	146–148	0.57
A-XX	3,4$(OH)_2$	$C_{25}H_{26}ClN_5O_6S$	560.02	58	127–130	0.67
A-XXI	2-OH-4-OCH_3	$C_{26}H_{28}ClN_5O_6S$	574.04	56	165–167	0.58
A-XXII	3-Cl	$C_{25}H_{25}Cl_2N_5O_4S$	562.46	59	175–178	0.79
A-XXIII	4-N$(C_2H_5)_2$	$C_{29}H_{35}ClN_6O_5S$	615.14	60	132–134	0.62
A-XXIV	2,4-$(OCH_3)_2$	$C_{27}H_{30}ClN_5O_6S$	588.07	59	154–156	0.68
A-XXV	4(OC_2H_5)	$C_{27}H_{30}ClN_5O_5S$	572.07	61	161–163	0.64

Table 2: Antibacterial activity of tested compound series

S. No.	Azetidinone	-R	Minimum inhibitory concentration (µg/mL)			
			Gram-positive bacteria		Gram-negative bacteria	
			Staphylococcus aureus ATCC6538	*Enterococcus faecalis ATCC 2912*	*Escherichia coli ATCC8739*	*Pseudomonas aeruginosa ATCC9027*
1	A-I	H	25	50	12.50	50
2	A-II	4-OH	12.5	25	12.50	12.50
3	A-III	4-Br	6.25	6.25	6.25	6.25
4	A-IV	3-OH	12.5	50	50	25
5	A-V	2-OH	50	50	50	25
6	A-VI	2-Cl	12.5	12.5	12.5	12.5
7	A-VII	$4CH_3$	50	50	100	50
8	A-VIII	$2\text{-}NO_2$	12.5	12.5	12.5	12.5
9	A-IX	4-Cl	6.25	6.25	6.25	6.25
10	A-X	$4\text{-}OCH_3$	12.5	12.5	12.5	12.5
11	A-XI	$3\text{-}NO_2$	12.5	12.5	12.5	12.5
12	A-XII	$3,4(OCH_3)_2$	12.5	50	100	50
13	A-XIII	$3,4(Cl)_2$	12.5	12.5	12.5	12.5
14	A-XIV	$4\text{-}N(CH_3)_2$	50	100	100	100
15	A-XV	$3,4,5(OCH_3)_3$	25	25	25	50
16	A-XVI	$2\text{-}OCH_3$	50	50	50	25
17	A-XVII	$3\text{-}OCH_3$	12.5	12.5	12.5	12.5
18	A-XVIII	$4\text{-}NO_2$	6.25	6.25	6.25	6.25
19	A-XIX	$4\text{-}OH\text{-}3\text{-}OCH_3$	50	50	50	50
20	A-XX	$3,4(OH)_2$	50	25	50	50
21	A-XXI	$2\text{-}OH\text{-}4\text{-}OCH_3$	25	25	25	12.5
22	A-XXII	3-Cl	6.25	12.5	6.25	12.5
23	A-XXIII	$4\text{-}N(C_2H_5)_2$	50	100	100	50
24	A-XXIV	$2,4\text{-}(OCH_3)_2$	25	25	12.5	12.5
25	A-XXV	$4(OC_2H_5)$	50	100	100	50
26	Std	Penicillin	6.25	6.25	6.25	6.25

Antifungal activity

The conventional disc diffusion technique used potato dextrose agar medium to investigate the *in-vitro* antifungal activity. On the surface of an agar plate infected with a standardized suspension of the microorganisms under test, sterile discs of Whatman filter paper No. 1 (6 mm diameter) containing specified doses of the antifungal agent fluconazole (50 µg/mL) and test compound (100 µg/mL) were inserted. The antifungal activity was assessed by incubating the plates at 28 ± 2°C for a duration of 72 hours. As a negative control, a paper disc impregnated with DMSO was used.[16]

Determination of the minimal inhibitory concentration

The agar streak dilution technique was used to calculate the minimal inhibitory concentration (MIC). Gradient amounts of tested components were combined with a predetermined quantity of sterile molten agar (Muller Hinton agar) to create a stock solution (100 µg/mL) in DMSO. A predetermined amount of the extract-containing medium (40–50°C) was added to a petri dish to a depth of 3 to 4 mm, and the mixture was left to harden. After making the microbial suspension (105 CFU/mL) was added to test plates containing extract in DMSO and incubated for 24 hours at 37°C. Once the incubation time was over, the MIC values were ascertained. Every measurement was made three times, with the average serving as the final reading. A positive control of 100 µg/mL of the common antibiotic penicillin was used, whereas a negative control of 100 mL of DMSO was employed. The MIC was defined as the test substance's concentration at which no observable bacterial or fungal growth could be seen on the plate.[17]

RESULTS AND DISCUSSION

Chemistry

The fourier transform infrared (FTIR) spectra revealed the key properties of the synthesized compounds. Proton nuclear magnetic resonance (^{1}H-NMR) identified the main structural characteristics. The mass spectra reported the base peaks as exactly matching or almost matching their molecular mass. A thorough examination of the mass spectrum identified a few

Table 3: Antifungal activity of tested compound series

S. No.	*Azetidinone Derivative*	*-R*	*Minimum inhibitory concentrations (µg/mL)*	
			Candida albicans ATCC10231	*Aspergillus niger ATCC10231*
1	A-I	H	50	100
2	A-II	4-OH	25	12.5
3	A-III	4-Br	12.5	12.5
4	A-IV	3-OH	50	100
5	A-V	2-OH	50	50
6	A-VI	2-Cl	12.5	25
7	A-VII	$4CH_3$	50	100
8	A-VIII	$2\text{-}NO_2$	12.5	25
9	A-IX	4-Cl	12.5	12.5
10	A-X	$4\text{-}OCH_3$	12.5	12.5
11	A-XI	$3\text{-}NO_2$	12.5	12.5
12	A-XII	$3,4(OCH_3)_2$	12.5	25
13	A-XIII	$3,4(Cl)_2$	12.5	25
14	A-XIV	$4\text{-}N(CH_3)_2$	50	100
15	A-XV	$3,4,5(OCH_3)_3$	25	50
16	A-XVI	$2\text{-}OCH_3$	25	25
17	A-XVII	$3\text{-}OCH_3$	50	50
18	A-XVIII	$4\text{-}NO_2$	12.5	12.5
19	A-XIX	$4\text{-}OH\text{-}3\text{-}OCH_3$	50	100
20	A-XX	$3,4(OH)_2$	25	50
21	A-XXI	$2\text{-}OH\text{-}4\text{-}OCH_3$	25	50
22	A-XXII	3-Cl	12.5	25
23	A-XXIII	$4\text{-}N(C_2H_5)_2$	100	100
24	A-XXIV	$2,4\text{-}(OCH_3)_2$	25	25
25	A-XXV	$4(OC_2H_5)$	100	100
26	Std	Fluconazole	12.5	12.5

important differentiators. Additionally, a number of fragment peaks showed up in various m/z ranges. The percentage of carbon, nitrogen, and hydrogen in the compounds was determined using elemental analysis, and the results were found to be in close agreement with the theoretical value Physical data of synthesized compound given in Table 1.

Antimicrobial Activity

Antibacterial activity

The antibacterial activity results revealed that A-VI, A-IX, A-XVIII displayed equipotent activity compared with the standard drug. The compounds with p-chloro and p-nitro groups exhibited the most potent antimicrobial agent (Table 2).

Antifungal activity

The highest antifungal activity was demonstrated by compounds with electron-withdrawing groups, such as chloro and electron-donating groups, such as nitro (Table 3).

CONCLUSION

In the present study, a highly efficient and simple procedure for the synthesis of ethyl 2-((4-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-((5-methoxy-*1H*-indol-1-yl)methyl)-4,5-dihydro-*1H*-1,2,4-triazol-5-yl)thio)acetate, AI-AXXV, where indolyl and 1,2,4-triazole rings are attached, was developed. The adopted method is simple, inexpensive, and gives good yields. On the basis of antibacterial and antifungal tests, it can be said that most of the compounds are active against bacteria and fungi.

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