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Synthesis and Microbiological Evaluation of Some New Phenothiazine Derivatives

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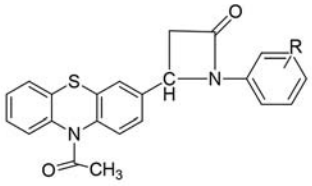
ABSTRACT A new series of 4-(10-acetyl-10*H*-phenothiazine-3-yl)-1-phenylazetidine-2-ones (**6a-g**) was prepared by intermolecular cyclization of Schiff bases (**5a-g**) and chloroacetyl chloride in the presence of base catalyst triethylamine. The microbiological evaluations of the compounds **6a-g** were performed, and the compounds **6a, 6b, 6d, 6f,** and **6g** showed significant antimicrobial activities.



6a-6g

Compounds	Entry
5a, 6a	4-Chloro
5b, 6b	4-Bromo
5c, 6c	4-Nitro
5d, 6d	3,4-Dichloro
5e, 6e	4-Chloro-3-Nitro
5f, 6f	4-Fluoro
5g, 6g	4-Methoxy

KEYWORDS Phenothiazine, Azetidine-2-one, Antimicrobial activity.

INTRODUCTION

Phenothiazine (PTZ) is an organic compound that has the formula $S(C_6H_4)_2NH$ and is related to the thiazine class of heterocyclic compounds. The compound was prepared by Bernthsen in 1883 through the reaction of diphenylamine with sulfur, but more recent methods are based on the cyclization of 2-substituted diphenyl sulfides. In the manufacture of monomers, PTZ is used as a chemical stabilizer or inhibitor to prolong storage and shelf life of products such as acryloyl chloride.[1]

The PTZs as a class and especially chlorpromazine are most widely used is class of neuroleptics.[2] The chemical structure of PTZ considered as an important molecular template for the development of the other agents effective in the treatment of a number of medical conditions. PTZ and related compounds have various biological activities such as the tranquilizer, anti-inflammatory, antimalarial, anti-psychotropic, antimicrobial, antitubercular, antitumor, antihistaminic, and analgesic agents.[3]

Due to the increased importance of these heterocyclic compounds, attempts were made in the synthesis of biologically active new generation of 10H- PTZ. Chlorpromazine was one of the first compounds used as a neuroleptic to treat symptoms of psychosis.[4] 10 (N-10) of the tricyclic ring, with the terminal amine group in the side chain, determine the activity of PHTS against cancer cells, and the activity is more strongly bound to the type of substituents in the PTZ ring than by the nature of the side chain.[5]

Our present study was aimed to synthesize some 4-(10-acetyl-10H-phenothiazin-3-yl)-1-phenylazetidin-2-ones by cyclization of unsymmetrical imines in the presence of chloroacetyl chloride and a base catalyst to search potent

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compounds. The synthesized compounds were tested for antimicrobial activity against different strains of bacteria and fungi.

RESULTS AND DISCUSSION

Synthesis of target compounds **6a-g** was achieved from diphenylamine (**1**) according to the reactions sequence outlined in **Scheme 1**. The Schiff's bases (**5a-g**) were obtained by the reaction between 10-acetyl-10H-phenothiazine-3-carbaldehyde (**4**) and substituted aromatic amines. The intermolecular cyclization of unsymmetrical imines and chloroacetyl chloride gave corresponding azetidine-2-one derivatives (**6a-g**). The reactions occurred in the presence of triethylamine as base catalyst. Tertiary amine counter balances between nucleophilic character and basicity which were considered as the key factor for efficient completion of the reaction.

The physical and analytical data were recorded for synthesized compounds. The IR peaks in KBr pellets recorded at 1775.67-1778.17, 1671.67-1620.73, 1265.13-1260.33, and 1220.11-1200.11 cm^{-1} indicated the presence of C=O, C=N, C-N, and C-C groups in synthesized compounds (**5a-g** and **6a-g**). The characteristic H nuclear magnetic resonance (NMR) peaks at at 7.59–7.58 indicated the presence of CH=N in compounds **5a-g**. Similarly peaks at 4.85–4.79, 3.49–3.40, and 3.24 δ/ppm indicated the presence of azetidine-2- one nucleus in compounds **6a-g**, respectively. The ^{13}C NMR peaks at 171.9–170.9 (C17), 63.9-60.7(C19), and 45.00 (C20) *δ*/ppm also indicated the presence of azetidine-2-one nucleus in compounds **6a-g**. From the ESI-mass spectra, it was found that mass peaks match with a calculated molecular mass of the synthesized compounds **6a-g.**

It has been reported that 2-azetidinones possess antimicrobial activities.[6] Based on this fact, the present study was aimed to investigate the antimicrobial property of some novel PTZ derivatives, containing beta-lactam rings. The antimicrobial activity was determined by paper disc diffusion technique against different strains of bacteria and fungi at 100µg, 150µg, and 250µg/ml shown in **Table 1**. From the antimicrobial data (zone of inhibition), it was found that compounds **6a**, **6b**, **6d**, **6f**, and **6g** were showing more antimicrobial activity compare to standards. The reported compounds were able to exhibit antibacterial activity probably by inhibition of cell wall synthesis due to the presence of beta-lactam rings.[7] Antifungal activity was associated due to azole nucleus by inhibiting ergosterol biosynthesis.[8]

EXPERIMENTAL SECTION

General

All the chemicals used for the synthesis were reagent grade of Sigma-Aldrich and SD fine chemicals. The solvents were purified by standard laboratory procedure and free from atmospheric oxygen. The melting points were determined by the open capillary method and were not corrected. The infrared (IR) spectra in KBr pellets on a Shimadzu 8201 PC Fourier transform-IR (FTIR) spectrophotometer both ^{1}H and ^{13}C NMR were recorded in dimethyl sulfoxide (DMSO) -d_6 using Bruker 500 MHz-NMR spectrophotometers using tetramethylsilane as an internal standard. The masses of the compound have been analyzed by the ESI-mass method using Thermo Finnigan mass spectrophotometer. Elemental analyses were recorded using Thermo Finnigan FLASH EA 1112 CHN analyzer. Thin-layer chromatography was performed on the pre-coated plastic sheet coated with silica gel g/ultraviolet (UV)-254 of 0.2 mm thickness.

Synthetic procedures

Preparation of 10H- PTZ (2)

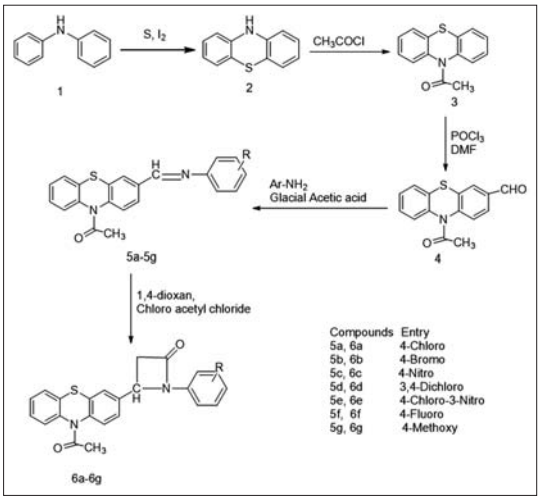
10*H*- PTZ was prepared using diphenylamine, sulfur, and iodine.[9] Yield: 83%, mp found (reported): 184°C (185°C),

Preparation of N-Acetylphenothiazine (3)

To 1 mmole of 10-*H* PTZ was added an equivalent quantity of glacial acetic acid. 10-H PTZ is stirred in solvent for 1 hr to get clear solution. To the resultant solution, 10 mL of acetyl chloride was added. During the addition of acetyl chloride, the internal temperature was maintained at 5°C. The resulting mixture was refluxed for 1 h. The mixture was kept for overnight; the formation of white crystals was taken place. Filter the residue and recrystallized from hot ethanol.[10] Yield: 93%; m.p.: 172°C; IR (KBr, υ_{max}/cm^{-1}): 2899.19 (CH_3, str), 1363.51 (C-N of PTZ ring, str), 1655(C=O, str). ^{1}H NMR (DMSO-d_6, 500 MHz, *δ*/ppm): 8.20–8.18 (d, 2H, J = 5Hz, Ar-H), 7.42–7.40 (d, 2H, J = 5Hz, Ar-H), 7.21–7.19 (d, 2H, J = 5Hz, Ar-H), 6.50-6.48 (d, 2H, J = 5Hz, Ar-H), 3.189 (s, 3H, CH_3).

Preparation of 10-Acetyl-10H- PTZ -3-carbaldehyde (4)

Compound **4** was prepared by Vilsmeier-Haack Reaction[11] using 1mmole of N-acetylphenothiazine. To that (Phosphorous oxychloride) $POCl^3$ and (Dimethyl formamide) DMF were added in 1:3, the mixture was refluxed for 6 h. Then, sodium hydroxide was added and cooled for overnight. On addition of crushed ice, the formation of solid took place. The resulting solid product was recrystallized from hot ethanol. Yield: 93%; m.p: 312°C; IR (KBr, υ_{max}/cm^{-1}): 2891.11 (-CH_3, str), 1700.11 (C=O for CHO, str), 1170.43(C-N of amine, str). ^{1}H NMR (DMSO-d_6, 500 MHz, *δ*/ppm): 9.81 (s,1H, -CHO), 8.21–8.79 (d, 1H, J = 5 Hz,



Scheme 1: Synthesis of 6a-g

Table 1: *In vitro* antimicrobial study by disc diffusion technique of compounds 6a-g

Compound no	Zone of inhibitions (mm)											
	Gram-positive bacteria				Gram-negative bacteria				Fungus			
	S. aureus		*B. subtilis*		*E. coli*		*E. cloacae*		*A. niger*		*C. albicans*	
	150 µg/ml	250 µg/ml	150 µg/ml	250 µg/ml	150 µg/ml	250 µg/ml	150 µg/ml	250 µg/ml	150 µg/ml	250 µg/ml	150 µg/ml	250 µg/ml
6a	13	17	13	15	20	20.5	13	19	10.5	17	12	17
6b	16	18	13.5	16	19	21	13	18	13	16	14	18
6c	13	14	13.1	14	22	23.2	19	12	10	12	18	14
6d	18	20	15	19	22	24	18	20	17	18.3	17	20
6e	14	15	13	17	13.6	15	13	15	14	17	14	15
6f	18	20	15	20	19	21	12	18	10.5	18	18	20
6g	18	20	15	20	22	23.2	13	16	13	19	13	20
Amoxicillin	12	14.1	14	17.1	18	22.1	14	17.1	–	–	–	–
Streptomycin	13	16	13	16	21	22	13	16	–	–	–	–
Nystatin	–	–	–	–	–	–	–	–	13.5	16	13.5	16

S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, E. cloacae: Enterobacter cloacae, A. niger: Aspergillus niger, C. albicans: Candida albicans

Ar-H), 7.66–7.64(d, 1H, J = 5Hz, Ar-H), 7.52–7.51 (1H, d, J = 5Hz, Ar-H), 7.43–7.42 (d, 1H, J = 5Hz, Ar-H), 7.39–7.37 (d,1H, J = 5Hz, Ar-H), 7.17–7.14 (d, 1H, J = 5Hz, Ar-H), 7.01-6.96 (m,1H, Ar-H), 2.187 (s,3H, CH_3).

Preparation of 1-(3-((Phenylimino) methyl)-10H-phenothiazin-10-yl)ethanones (5a-g)

The reaction mixture of 1 mole of 10-acetyl-10H- PTZ -3-carbaldehyde (4) in 50 mL of ethanol was stirred for 30 min under nitrogen atmosphere. Added 1 mL of glacial acetic acid followed by addition of 2 g of substituent aromatic amines and refluxed for 1 h. On addition of crushed ice, precipitation was taken place and then filtered, dried at room temperature and then recrystallized from hot ethanol.[12]

1-(3-(((4-Chlorophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5a)

Yield: 85%, mp.170°C; IR (KBr, υ_{max}/cm^{-1}): 2954.34(-CH_3, str), 1681.11 (C=O ketnic, str), 1671.67(C=N of imine, str), 1450.14(-CH_3, def), 664.21 (C-Cl, str). ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.21–8.79 (d, 1H, J = 5 Hz, Ar-H), 7.66–7.64 (d, 1H, J = 5Hz, Ar-H), 7.59 (s, 1H,-N=CH- of imines), 7.52–7.51 (1H, d, J = 5Hz, Ar-H), 7.43–7.42 (d,1H, J = 5Hz, Ar-H), 7.39-7.37 (d,1H, J = 5Hz, Ar-H), 7.17–7.14 (d, 1H, J = 5Hz, Ar-H), 7.01–6.96 (m,1H, Ar-H), 6.91-6.89 (d, 2H, J = 5Hz, Ar-H), 6.82–6.80 (d, 2H, J = 5Hz, Ar-H), 2.187 (s,3H, CH_3).

1-(3-(((4-Bromophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5b)

Yield: 85%, mp.165°C; Yield: 92%; m.p.: 83-85ºC; IR (KBr, υ_{max}/cm^{-1}): 2950.52 (-CH_3,str), 1679.11 (C=O ketnic, str), 1624.73 (C=N for imine, str), 1469.35 (-CH_3, def), 621.93 (C-Br,str); ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.20–8.19 (d, 1H, J = 5 Hz, Ar-H), 7.64–7.63 (d, 1H, J = 5Hz, Ar-H), 7.49 (s, 1H,-N=CH- of imines), 7.47–7.45 (1H, d, J = 5Hz, Ar-H), 7.43–7.42 (d,1H, J = 5Hz, Ar-H), 7.29–7.27 (d,1H, J = 5Hz, Ar-H), 7.16–7.14 (d, 1H, J = 5Hz, Ar-H), 6.99–6.95 (m,1H, Ar-H); 6.91–6.89(d, 2H, J = 5Hz, Ar-H); 6.72–6.70 (d, 2H, J = 5Hz, Ar-H), 2.187 (s,3H, CH_3).

1-(3-(((4-Nitrophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5c)

Yield: 65%, mp.168°C; Yield: 92%; m.p.: 83-85ºC; IR (KBr, υ_{max}/cm^{-1}): 2952.52 (-CH_3,str), 1678.21 (C=O ketnic, str), 1620.73 (C=N for imine, str), 1540.02 (C-NO_2, str), 1461.25 (-CH_3, def); ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.21–8.19 (d, 1H, J = 5 Hz, Ar-H), 7.67–7.65 (d, 1H, J = 5Hz, Ar-H), 7.51 (s, 1H,-N=CH- of imines), 7.47–7.45 (1H, d, J = 5Hz, Ar-H), 7.42-7.41 (d,1H, J = 5Hz, Ar-H), 7.29–7.27 (d,1H, J = 5Hz, Ar-H), 7.26–7.24 (d, 1H, J = 5Hz, Ar-H), 6.99–6.95 (m,1H, Ar-H); 6.89–6.87(d, 2H, J=5Hz, Ar-H); 6.72-6.70 (d, 2H, J = 5Hz, Ar-H), 2.187 (s,3H, CH_3).

1-(3-(((3,4-Dichlorophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5d)

Yield: 75%, mp.175°C; Yield: 92%; m.p.: 83–85°C; IR (KBr, υ_{max}/cm^{-1}): 2927.14 (-CH_3,str), 1689.21 (C=O ketnic, str), 1679.67 (C=N for imine, str), 1434.24 (-CH_3, def),764.21 (C-Cl, str). ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.25–8.23 (d, 1H, J = 5 Hz, Ar-H), 7.67–7.65

(d, 1H, J = 5Hz, Ar-H), 7.58 (s, 1H,-N=CH- of imines), 7.49–7.47 (1H, d, J = 5Hz, Ar-H), 7.42–7.41 (d,1H, J = 5Hz, Ar-H), 7.29–7.27 (d,1H, J = 5Hz, Ar-H), 7.16–7.14 (d, 1H, J = 5Hz, Ar-H), 6.99–6.95 (m,1H, Ar-H); 6.89 (s, 1H, Ar-H); 6.72–6.70 (d, 1H, J = 5Hz, Ar-H), 6.66–6.64 (d, 1H, J = 5Hz, Ar-H), 2.187 (s,3H, CH_3).

1-(3-(((4-Chloro-3-nitrophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5e)

Yield: 70%, mp.188°C; Yield: 92%; m.p.: 83-85ºC; IR (KBr, υ_{max}/cm^{-1}): 2926.14 (-CH_3,str), 1687.21 (C=O ketnic, str), 1671.67 (C=N for imine, str), 1541.01 (C-NO_2, str), 1430.24 (-CH_3, def),761.21(C-Cl, str). ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.21-8.79 (d, 1H, J = 5 Hz, Ar-H), 7.66–7.64 (d, 1H, J = 5Hz, Ar-H), 7.59 (s, 1H,-N=CH- of imines), 7.52–7.51 (1H, d, J = 5Hz, Ar-H), 7.43–7.42 (d,1H, J = 5Hz, Ar-H), 7.39-7.37 (d,1H, J = 5Hz, Ar-H), 7.17–7.14 (d, 1H, J = 5Hz, Ar-H), 7.01–6.96 (m, 1H, Ar-H), 6.89 (s, 1H, Ar-H); 6.72–6.70 (d, 1H, J = 5Hz, Ar-H), 6.66–6.64 (d, 1H, J = 5Hz, Ar-H), 2.187 (s,3H, CH_3).

1-(3-(((4-Fluoro-3-nitrophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5f)

Yield: 95%; m.p.: 83–85°C; IR (KBr, υ_{max}/cm^{-1}): 2950.52 (-CH_3,str), 1679.11 (C=O ketnic, str), 1624.73 (C=N for imine, str), 1469.35 (-CH_3, def), 721.93 (C-F,str); ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.20–8.19 (d, 1H, J = 5 Hz, Ar-H), 7.64-7.63 (d, 1H, J = 5Hz, Ar-H), 7.49 (s, 1H,-N=CH- of imines), 7.47–7.45 (1H, d, J = 5Hz, Ar-H), 7.43–7.42 (d,1H, J = 5Hz, Ar-H), 7.29–7.27 (d,1H, J = 5Hz, Ar-H), 7.16–7.14 (d, 1H, J = 5Hz, Ar-H), 6.99–6.95 (m, 1H, Ar-H); 6.91–6.89 (d, 2H, J = 5Hz, Ar-H); 6.72–6.70 (d, 2H, J = 5Hz, Ar-H), 2.187 (s,3H, CH_3).

1-(3-(((4-Methoxy-3-nitrophenyl)imino)methyl)-10H-phenothiazin-10-yl)ethanone (5 g)

Yield: 85%, mp.170°C; IR (KBr, υ_{max}/cm^{-1}): 2954.34 (-CH_3, str), 1681.11 (C=O ketnic, str), 1671.67 (C=N of imine, str), 1450.14 (-CH_3, def). ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 8.21–8.79 (d, 1H, J = 5 Hz, Ar-H), 7.66–7.64 (d, 1H, J = 5Hz, Ar-H), 7.59 (s, 1H,-N=CH- of imines), 7.52–7.51 (1H, d, J = 5Hz, Ar-H), 7.43–7.42 (d,1H, J = 5Hz, Ar-H), 7.39–7.37 (d,1H, J = 5Hz, Ar-H), 7.17–7.14 (d, 1H, J = 5Hz, Ar-H), 7.01–6.96 (m,1H, Ar-H), 6.91–6.89 (d, 2H, J = 5Hz, Ar-H), 6.82–6.80 (d, 2H, J = 5Hz, Ar-H), 3.18 (s, 3H, CH_3), 2.187 (s, 3H, CH_3).

Preparation of 4-(10-Acetyl-10H-phenothiazin-3-yl)-1-phenylazetidin-2-ones (6a-g)

1 mmole of appropriate1-(3-((phenylimino) methyl)-10H-phenothiazin-10-yl)ethanone (**5**) was placed in 100 mL round bottom flask and stirred under nitrogen atmosphere followed by addition of 1, 4- dioxane (20 mL) as solvent. Triethylamine was used as base catalyst (0.5 mL) and stirred for 1 h. The resulting solution was cooled at 0–5°C and added to this was 2 mL of chloroacetyl chloride. During addition of chloroacetyl chloride internal temperature was maintained not more than 8°C. The mixture was refluxed for 8 h, cooled at room temperature for overnight and then neutralized with 5% sodium bicarbonate solution after addition of ice. The separated crude product was washed with cold water and 50% ethyl acetate in petroleum ether and then dried. The purification of compounds was carried out using benzene and chloroform as mobile phase in column chromatographic technique.[13]

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(4-chlorophenyl) azetidin-2-one (6a)

Yield-78%; m.p.: 241°C; Elemental Analysis Calculated (found) $C_{23}H_{17}ClN_2O_2S$: C, 65.63 (65.59); H, 4.07(4.03); N, 6.66 (6.62); IR (KBr, υ_{max}/cm^{-1}): 2959.34 (-CH_3, str), 1775.67 (C=O, str); 1455.14 (-CH_3, def); 1260.33 (C-N, str); 1200.11 (C-C, str), 760.21 (C-Cl, str); ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 7.67–7.65 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.54–7.52 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.51–7.49 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.34–7.32 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.21–7.19 (m, 1H, Ar-H of PTZ ring), 7.15–7.13 (d, 1H, J = 10 Hz, Ar-H of PTZ ring), 6.97-6.95 (m, 1H, Ar-H of PTZ ring), 6.85–6.83 (d, 1H, J = 10Hz, Ar-H of PTZ ring), 6.77 (s, 1H, Ar-H of PTZ ring), 4.85 (s,1H, CH-N of azetidin-2-one); 3.49 (s, 1H, CH_2-C=O of azetidin-2-one), 3.24 (s, 1H, CH_2-C=O of azetidin-2-one), 2.74 (s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, δ/ppm): 170.9 (C19), 169.3 (C15), 138.9 (C6), 137.6 (C22), 133.6 (C12), 133.3 (C29), 132.9 (C5), 130.9 (C3), 128.1 (C9),129.0 (C24,26), 127.7 (C13), 127.3 (C11), 127.2 (C7), 126.7 (C10), 125.6 (C23,27), 122.2 (C8), 118.0 (C2), 110.2 (C14), 60.7 (C17), 45.0 (C20), 22.9 (C21); ESI-MS (m/z): 420.07 (100.0%), 422.07 (37.3%), 421.07 (26.5%).

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(4-bromophenyl) azetidin-2-one (6b)

Yield-76%; m.p.: 251°C; Elemental Analysis Calculated (found) $C_{23}H_{17}BrN_2O_2S$: C, 59.36 (59.32), H, 3.68 (3.64), N, 6.02 (5.98). IR (KBr, υ_{max}/cm^{-1}): 2957.34 (-CH_3, str), 1778.17 (C=O, str); 1453.04 (-CH_3, def); 1265.13 (C-N, str); 1220.11 (C-C, str), 630.21 (C-Br, str); ^{1}H NMR (DMSO-d6, 500 MHz, δ/ppm): 7.60–7.58 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.50–7.48 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.42–7.40 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.34–7.32 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.21–7.19 (m, 1H, Ar-H of PTZ ring), 7.13–7.11 (d, 1H, J = 10 Hz, Ar-H of PTZ ring), 6.94-6.92 (m, 1H, Ar-H of PTZ ring), 6.80–6.79 (d, 1H, J = 10Hz, Ar-H of PTZ ring), 6.67 (s, 1H, Ar-H of PTZ ring), 4.83 (s,1H, CH-N of azetidin-2-one); 3.40 (s,1H,CH_2-C=O of azetidin-2-one), 3.24 (s,1H,CH_2-C=O of azetidin-2-one), 2.71 (s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, δ/ppm): 171.9 (C19), 168.3 (C15), 138.9 (C6), 135.1 (C22), 133.6 (C12), 133.3 (C29), 132.9 (C5), 131.9 (C3), 128.1 (C9), 129.0 (C24,26), 127.7 (C13), 127.2 (C7), 127.1 (C11), 126.7 (C10), 125.6 (C23,27), 122.2 (C8), 118.0 (C2), 110.2 (C14), 60.7 (C17), 45.0 (C20), 22.9 (C21); ESI-MS (m/z): 466.02 (100.0%), 464.02 (97.5%), 465.02 (25.8%).

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(4-nitrophenyl) azetidin-2-one (6c)

Yield-58%; m.p.: 281°C; Elemental Analysis Calculated (found) $C_{23}H_{17}N_3O_4S$: C, 64.03 (59.99); H, 3.97 (3.93); N, 9.74 (9.73). IR (KBr, υ_{max}/cm^{-1}): 2959.34 (-CH_3, str), 1775.67 (C=O, str), 1541.23 (str, aromatic, C-NO_2), 1455.14 (-CH_3, def), 1260.33 (C-N, str), 1200.11 (C-C, str). ^{1}H NMR (DMSO-d_6, 500 MHz, δ/ppm): 7.67–7.65 (d, 1H, J = 5Hz,

Ar-H of PTZ ring), 7.54–7.52 (d,1H, J=5Hz, Ar-H of PTZ ring), 7.51–7.49 (d, 2H, J=10 Hz, Ar-H of phenyl), 7.34–7.32 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.21–7.19 (m, 1H, Ar-H of PTZ ring), 7.15–7.13 (d, 1H, J = 10 Hz, Ar-H of PTZ ring), 6.97-6.95 (m,1H, Ar-H of PTZ ring), 6.85-6.83(d, 1H, J=10Hz, Ar-H of PTZ ring), 6.77 (s, 1H, Ar-H of PTZ ring), 4.85 (s, 1H, CH-N of azetidin-2-one); 3.49 (s, 1H, CH_2-C=O of azetidin-2-one), 3.24 (s, 1H, CH_2-C=O of azetidin-2-one), 2.74 (s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, *δ*/ppm): 170.9 (C19), 169.3 (C15), 138.9 (C6), 137.6 (C22), 133.6 (C12), 133.3 (C29), 132.9 (C5), 130.9 (C3), 128.1 (C9), 129.0 (C24,26), 127.7 (C13), 127.3 (C11), 127.2 (C7), 126.7 (C10), 125.6 (C23,27),122.2 (C8), 118.0 (C2), 110.2 (C14), 60.7 (C17), 45.0 (C20), 22.9 (C21); ESI-MS (m/z): 431.09 (100.0%), 432.10 (25.2%), 433.09 (4.8%).

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(3,4-dichlorophenyl)azetidin-2-one (6d)

Yield-66%; m.p.: 261°C; Elemental Analysis Calculated (found) $C_{23}H_{16}Cl_2N_2O_2S$: C, 60.67 (60.64); H, 3.54 (3.50); N, 6.15 (6.11). IR (KBr, υ_{max}/cm^{-1}): 2957.34 (-CH_3, str), 1778.17 (C=O, str); 1453.04 (-CH_3, def); 1265.13(C-N, str); 1220.11 (C-C, str), 782.23(str, C-Cl). ^{1}H NMR (DMSO-d_6, 500 MHz, *δ*/ppm): 7.60-7.58 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.50-7.48 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.42-7.40 (d, 1H, J = 10 Hz, Ar-H of phenyl), 7.38-7.32 (d, 1H, J = 10 Hz, Ar-H of phenyl), 7.26 (s, 1H, Ar-H of phenyl), 7.21-7.19 (m,1H, Ar-H of PTZ ring), 7.13-7.11 (d, 1H, J=10 Hz, Ar-H of PTZ ring), 6.94-6.92 (m,1H, Ar-H of PTZ ring), 6.80-6.79 (d, 1H, J = 10Hz, Ar-H of PTZ ring), 6.67 (s, 1H, Ar-H of PTZ ring), 4.79 (s,1H, CH-N of azetidin-2-one); 3.40 (s, 1H, CH_2-C=O of azetidin-2-one), 3.24 (s, 1H, CH_2-C=O of azetidin-2-one), 2.71 (s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, *δ*/ppm): 170.9 (C19), 169.1 (C15), 142.9 (C6), 138.1 (C22), 136.1 (C12), 133.3 (C29), 132.9 (C5), 131.9 (C3), 129.1 (C9), 128.0 (C24,26), 127.5 (C13), 127.3 (C7), 127.1 (C11), 126.7 (C10), 125.6 (C23,27), 122.2 (C8), 118.0 (C2), 110.2 (C14), 63.7 (C17), 43.0 (C20), 22.9 (C21); ESI-MS (m/z): 454.03 (100.0%), 456.03 (68.8%), 455.03 (26.4%).

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(4-chloro-3-nitrophenyl)azetidin-2-one (6e)

Yield-58%; m.p.: 281°C; Elemental Analysis Calculated (found) $C_{23}H_{16}ClN_3O_4S$: C, 59.29 (59.25); H, 3.46 (3.42); N, 9.02 (8.99). IR (KBr, υ_{max}/cm^{-1}): 2959.34 (-CH_3, str), 1775.67 (C=O, str), 1541.23 (str, aromatic, C-NO_2), 1455.14 (-CH_3, def), 1260.33 (C-N, str), 1200.11 (C-C, str), 751.25 (str, equatorial, C-Cl). ^{1}H NMR (DMSO-d_6, 500 MHz, *δ*/ppm): 7.67–7.65 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.54–7.52 (d, 1H, J = 5Hz, Ar-H of PTZ ring), 7.51–7.49 (d, 1H, J = 10 Hz, Ar-H of phenyl), 7.34–7.32 (d, 1H, J = 10 Hz, Ar-H of phenyl), 7.28 (s, 1H, Ar-H), 7.21–7.19 (m, 1H, Ar-H of PTZ ring), 7.15–7.13 (d, 1H, J = 10 Hz, Ar-H of PTZ ring), 6.97–6.95 (m,1H, Ar-H of PTZ ring), 6.85–6.83 (d, 1H, J = 10Hz, Ar-H of PTZ ring), 6.77 (s, 1H, Ar-H of PTZ ring), 4.85 (s,1H, CH-N of azetidin-2-one); 3.49 (s, 1H, CH_2-C=O of azetidin-2-one), 3.24 (s, 1H, CH_2-C=O of azetidin-2-one), 2.74(s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, *δ*/ppm): 170.9 (C19), 169.3 (C15), 138.9 (C6), 137.6 (C22), 133.6 (C12), 133.3 (C29), 132.9 (C5), 130.9 (C3), 128.1 (C9),129.0 (C24,26), 127.7 (C13), 127.3 (C11), 127.2 (C7), 126.7 (C10), 125.6 (C23,27),122.2 (C8), 118.0 (C2), 110.2 (C14), 60.7 (C17), 45.0 (C20), 22.9 (C21); ESI-MS (m/z): 465.06 (100.0%), 467.05 (36.5%), 466.06 (25.2%).

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(4-flurophenyl) azetidin-2-one (6f)

Yield-78%; m.p.: 241°C; Elemental Analysis Calculated (found) $C_{23}H_{17}FN_2O_2S$: C, 68.30 (68.26); H, 4.24 (4.20); N, 6.93 (6.69). IR (KBr, υ_{max}/cm^{-1}): 2959.34 (-CH_3, str), 1775.67 (C=O, str); 1455.14 (-CH_3, def); 1260.33 (C-N, str); 1200.11 (C-C, str), 761.21 (C-F, str); ^{1}H NMR (DMSO-d_6, 500 MHz, *δ*/ppm): 7.67–7.65 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.54–7.52 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.51–7.49 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.34-7.32 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.21–7.19 (m,1H, Ar-H of PTZ ring), 7.15–7.13 (d, 1H, J = 10 Hz, Ar-H of PTZ ring), 6.97–6.95 (m,1H, Ar-H of PTZ ring), 6.85–6.83 (d, 1H, J = 10Hz, Ar-H of PTZ ring), 6.77 (s, 1H, Ar-H of PTZ ring), 4.85 (s, 1H, CH-N of azetidin-2-one); 3.49 (s, 1H, CH_2-C=O of azetidin-2-one), 3.24 (s, 1H, CH_2-C=O of azetidin-2-one), 2.74 (s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, *δ*/ppm): 170.9 (C19), 169.3 (C15), 138.9 (C6), 137.6 (C22), 133.6 (C12), 133.3 (C29), 132.9 (C5), 130.9 (C3), 128.1 (C9),129.0 (C24,26), 127.7 (C13), 127.3 (C11), 127.2 (C7), 126.7 (C10), 125.6 (C23, 27),122.2 (C8), 118.0 (C2), 110.2 (C14), 60.7 (C17), 45.0 (C20), 22.9 (C21); ESI-MS (m/z): 404.10 (100.0%), 405.10 (26.5%), 406.10 (5.3%).

4-(10-Acetyl-10H-phenothiazin-3-yl)-1-(4-methoxy phenyl) azetidin-2-one (6g)

Yield-76%; m.p.: 251°C; Elemental Analysis Calculated (found) $C_{23}H_{17}BrN_2O_2S$: C, 69.21 (69.18); H, 4.84(4.80); N, 6.73 (6.69). IR (KBr, υ_{max}/cm^{-1}): 2957.34 (-CH_3, str), 1778.17 (C=O, str); 1453.04 (-CH_3, def); 1265.13 (C-N, str); 1220.11 (C-C, str), 630.21 (C-Br, str); ^{1}H NMR (DMSO-d_6, 500 MHz, *δ*/ppm): 7.60–7.58 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.50-7.48 (d,1H, J = 5Hz, Ar-H of PTZ ring), 7.42–7.40 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.34-7.32 (d, 2H, J = 10 Hz, Ar-H of phenyl), 7.21–7.19 (m,1H, Ar-H of PTZ ring), 7.13–7.11 (d, 1H, J = 10 Hz, Ar-H of PTZ ring), 6.94–6.92 (m,1H, Ar-H of PTZ ring), 6.80–6.79 (d, 1H, J = 10Hz, Ar-H of PTZ ring), 6.67 (s, 1H, Ar-H of PTZ ring), 4.83 (s,1H, CH-N of azetidin-2-one); 3.40 (s,1H,CH_2-C=O of azetidin-2-one), 3.24 (s,1H, CH_2-C=O of azetidin-2-one), 2.71 (s, 3H, -CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz, *δ*/ppm): 171.9 (C19), 168.3 (C15), 138.9 (C6), 135.1 (C22), 133.6 (C12), 133.3 (C29), 132.9 (C5), 131.9 (C3), 128.1 (C9), 129.0 (C24,26), 127.7 (C13), 127.2 (C7), 127.1 (C11), 126.7 (C10), 125.6 (C23,27), 122.2 (C8), 118.0 (C2), 110.2 (C14), 60.7 (C17), 55.8 (C13), 45.0 (C20), 22.9 (C21); ESI-MS (m/z): 416.12 (100.0%), 417.12 (27.6%), 418.12 (5.5%).

Biological activity

Test microorganism and medium

For the determination of antimicrobial activity Gram-positive bacteria *Staphylococcus aureus* ATCC12600, *Bacillus subtilis* ATCC 11775, *Enterobacter cloacae* ATCC13047 and the fungi *Candida albicans* ATCC 90028, and *Aspergillus niger* ATCC1027 were used.

Bacterial strains were cultured overnight at 37°C in LB-agar broth the and fungal strains were cultured over night at 30°C in cornmeal agar media. Test strains were suspended in nutrient agar to give final density of 5×10^5 CFU/mL.

Screening for antimicrobial activity (zone of inhibition assay)

Antimicrobial activity of the compounds **6a-g** was determined by disc diffusion method.[14] Sterilized 10% nutrient agar (20 mL) into each sterile Petri dish after mixing the culture of microorganism at the concentration of 150 µL and allowed to solidify the plate. Standard and test compounds were dissolved in DMSO to make a stock solution of 1000µg/ml. From the stock solution concentration of 100, 150, and 200 µg/mL made for amoxicillin, streptomycin, nystatin, and test compounds 6(a-e), respectively. Whatman filter paper was sterilized and dipped in test compounds, standards, and solvent control, respectively. Discs were placed on agar plates and incubated at 37°C for 24 h for bacterial and 30°C for 24 h for fungi indicate and the zone of inhibition match in millimeter. Each study was carried out in triplicate.

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