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Synthesis of some novel s-triazine derivatives and their potential antimicrobial activity

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ABSTRACT

Novel series of compounds containing 2-(2-substituted benzylidene hydrazinyl)-4-(4-(4-methoxyphenyl) piperazin-1-yl)-6-(4-tolyl oxy)-1,3,5-triazine (s-triazine) derivatives were synthesized. The formed compounds have been evaluated by physical methods (melting point, TLC, elemental analyses) and upon spectral data (IR and NMR). The newly synthesized compounds were also evaluated for antimicrobial activity against variety of bacterial strains in which some of these derivatives exhibited potential antibacterial and antifungal activity.

Introduction

1,3,5-triazine derivatives represent a widely used leading structure with multitude of interesting applications in numerous fields such as dyes, polymers but pharmacological applications are most important (1).

Several derivatives of s-triazine show antimicrobial (2,3), antibacterial (4, 5) and herbicidal activities (6). They are also used for the treatment of HIV infection (7), malaria (8), cancer (9,10) and photosynthesis inhibiting activity (11). Several investigators found s-triazine nucleus as hormone receptor antagonists. In this present work deals with the synthesized and studies of some novel s-triazine derivatives having the route shown in experimental section.

Experimental

Synthetic methods, analytical and spectral data:

All the melting points reported are uncorrected and were recorded using an electro thermal melting point apparatus. Thin layer chromatography was performed on Merck precoated 60 F_{254} plates. Ethyl acetate:toluene (7.5:2.5) was used as solvent for the TLC and the spots were rendered visible by exposing to UV light. IR spectra were recorded on Shimadzu FT-IR (Japan) instrument. ^{1}H NMR spectra were recorded on Brucker Avance II NMR spectrometer. Chemical shifts (δ ppm) reported are referred to internal reference tetramethyl silane. Elemental analyses were obtained using a Carlo-Erba CHNS-O EA 1108 elemental analyzer. s-triazine was received from Atul limited, India.

Synthetic pathways are depicted in Schemes 1 and 2, physico-chemical and spectral data are given in Tables I and II.

Synthesis of 2-(4-methylphenoxy)-4,6-(dichloro)-s-triazine (1)

To a stirred solution of s-triazine (1.81g, 0.01mol) in acetone (25 mL) at 0-5 ˚C, the solution of 4-methyl phenol (1.09g, 0.01 mol) in acetone (5 mL) was added and pH was maintained neutral by the addition of 10 % sodium carbonate solution. The stirring was continued at 0-5 ˚C for 4 h. After the completion of reaction, mixture was poured on ice water. The solid product obtained was filtered and dried. The crude product was purified by crystallization from ethanol to give 2-(4-methylphenoxy)-4, 6-(dichloro)-s-triazine.

Synthesis of 2-(4-methylphenoxy)-4-[4-(4-methoxyphenyl) piperazine-1-yl]-6-(chloro)-s-triazine (2)

To a stirred solution of 2-(4-methylphenoxy)-4,6-(dichloro)-s-triazine (1) (2.56g, 0.01 mol) in acetone (10 mL) at 30-35 ˚C, the solution of 4-methoxy N-phenyl piperazine (1.92g, 0.01 mol) in acetone (5 mL) was added dropwise maintaining the temperature 35 ˚C. The pH was adjusted neutral by the addition of 10 % sodium carbonate solution. The temperature was gradually raised to 45˚C during 2 h. After the completion of reaction, the resultant content was poured into ice cold water. The solid product obtained was filtered and dried to give 2-(4-methylphenoxy)-4-[4-(4-methoxyphenyl) piperazine-1-yl]-6-(chloro)-s-triazine.

Synthesis of 2-hydrazinyl-4-(4-(4-methoxyphenyl) piperazin-1-yl)-6-(p-tolyloxy)-s-triazine (3) (12)

A mixture of 2-(4-methylphenoxy)-4-[4-(4-methoxy phenyl) piperazine-1-yl] 6-(chloro)-s-triazine (2) (4.27g, 0.01 mol) and hydrazine hydrate (2.50g, 0.05 mol) in ethanol (25 mL) was refluxed in a water bath. The temperature was gradually raised to 80-90 ˚C during 3 h. The pH was adjusted neutral by the addition of 10 % sodium carbonate solution. After the completion of reaction, the refluxed content was added to cold water. The solid product obtained was filtered and dried. The crude product was purified by crystallization from absolute alcohol.

Synthesis of 2-(2-substituted benzylidenehydrazinyl)-4-(4-(4-methoxy phenyl)piperazin-1-yl)-6-(p-tolyloxy)-s-triazine (4a-e) (13)

A mixture of 2-(methylphenoxy)-4-[4-(4-methoxy phenyl) piperazine 1-yl]6-(hydrazino)-s-triazine (3) (4.23g, 0.01 mol) and substituted benzaldehyde (0.01mol) was dissolved in absolute ethanol (25 mL) and add few drops of glacial acetic acid. The reaction mixture was refluxed 8 h in a water bath then

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cooled and poured into crushed ice, solid product obtained was filtered and dried.

Synthesis of 3-chloro-1-(4-(4-(4-methoxyphenyl)piperazin-1-yl)-6-(p-tolyloxy)-s-triazin-2-ylamino)-4-(substituted phenyl)azetidin-2-one (5a-e)

To a solution of 2-(4-methylphenoxy)-4-[4-(4-methoxy phenyl) piperazine 1-yl]6-(benzylidenehydrazino)-s-triazine (4a-e) (0.01 mol) and triethylamine (5-6 drops) as a catalyst in dry benzene (15 mL) was added in chloro acetyl chloride (1.18g, 0.015 mol) at 50 ˚C. The reaction mixture was stirred for 30 min. at room temperature and refluxed for 6-7 h, then cooled it and poured in ice. The solid thus obtained was recrystallization from ethanol to yield desired compounds (5a-e).

Synthesis of 3-(4-(4-(4-methoxyphenyl) piperazin-1-yl)-6-(p-tolyloxy)-1,3,5-triazin-2-ylamino)-2-(substituted phenyl)thiazolidin-4-one (6a-e)

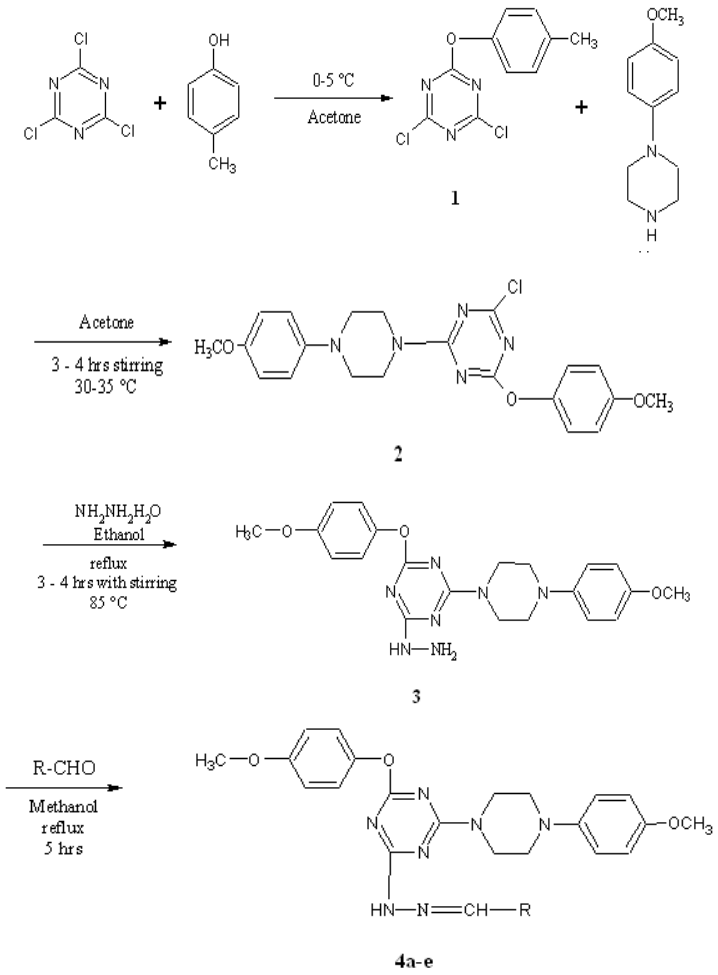
To a solution of 2-(4-methylphenoxy)-4-[4-(4-methoxy phenyl) piperazin-1-yl] 6-(benzylidenehydrazino)-s-triazine (4a-e) (0.01 mol) (0.01 mol) and 1g of anhydrous zinc chloride as a catalyst in dry benzene (15 mL), thioglycolic acid (1.84g, 0.02 mol) was added dropwise with stirring at ambient temperature and refluxed it for 8-9 h, then cooled it and poured in sodium bicarbonate solution (10%) to get neutralized. The solid thus obtained was recrystalized from ethanol to yield desired compounds (6a-e).

Antimicrobial screening

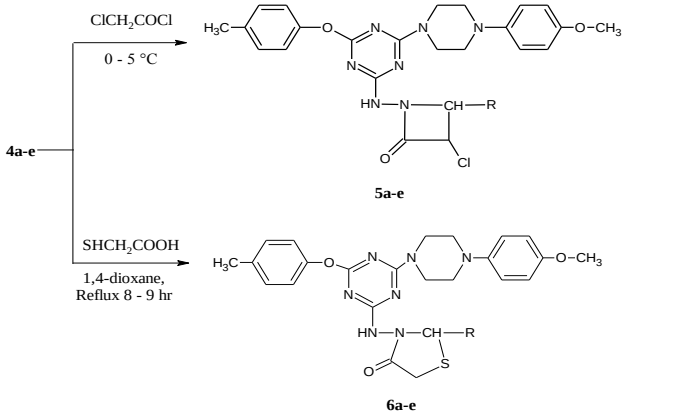
All the synthesized compounds (4 – 6) were screened for their antimicrobial activity by agar disc diffusion method (14, 15). DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. All the tubes not showing visible growth are subcultured and incubated overnight at 37 ˚C. The tubes were then incubated overnight. The MBC and MFC of the control organism were read to check the accuracy of the drug concentrations. Prepared stock solution of antibiotics of concentrations 2000 mg/L, as required. Arrange micro well plate 8×12 well of sterile well in the rack. The lowest concentration inhibiting growth of the organism was recorded as the MBC and MFC. The growth, inhibition is measured and compound is applied in the method to determine the activity in μgmL^{-1} concentration. All the compounds were screened against Gram-positive bacteria [*Staphylococcus aureus* (MTCC-96) and *Streptococcus pyogenus* (MTCC-443)] and Gram-negative bacteria [*Escherichia coli* (MTCC-442) and *Pseudomonas aeruginosa* (MTCC-441)]. The antifungal activity was tested against Gram-positive fungus [*Candida albicans* (MTCC-227)] and Gram-negative fungi [*Aspergillus niger* (MTCC-282) and *Aspergillus clavatus* (MTCC-1323)]. The results were compared with gentamycin and nystatin as a reference drugs. The results are shown in Table III.

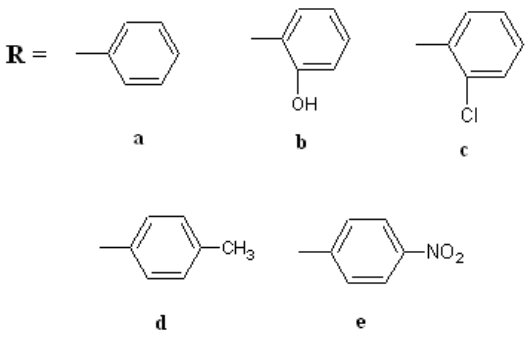
Results and Discussion

The synthetic route of the compounds (4a-e, 5a-e and 6a-e) is outlined in schemes 1 & 2 respectively. The synthesis of 2-(2-substituted benzylidenehydrazinyl)-4-(4-(4-methoxyphenyl) pi perazin-1-yl)-6-(4-tolyl oxy)-1,3,5-triazine (s-triazine) 4 (a-e) as shown in Scheme 1 was carried out from p-cresol and cyanuric chloride. The compound 1 and N-phenyl piperazine was refluxed to give 2 which on refluxed with hydrazine hydrate in ethanol to give 3. The compound 3 was refluxed with different substituted benzaldehyde to give 4 (a-e). The compounds 5 (a-e) was prepared by the reaction of 4 (a-e) with chloro acetyl chloride in presence of base. 6 (a-e) was prepared with thioglycolic acid and 4 (a-e) as shown in scheme 2.



Scheme 1





Scheme 2

The structures of the synthesized compounds were characterized by analytical (Tables I and II) and spectral data (IR and ^{1}H NMR). In the IR spectra, the -N=C bands were observed at 1580, 1365, 815 cm^{-1}. Compounds 4 (a-e) have shown –CH=N band in the range of 1610-1650 cm^{-1}. In 5 (a-e) C-Cl and C=O bands have shown at 790-820 cm^{-1} and 1600-1700 cm^{-1} respectively. In 6 (a-e) C-S-C bands were observed in the range of 650-670 cm^{-1}. In the ^{1}H NMR spectra range 3.80-4.10 ppm and 10.50 ppm (singlet) peaks for all compounds confirmed the availability of -OCH_3 and -NH group respectively. In 5 (a-e) –CH-Cl peaks at 4.39-4.55 ppm (singlet). In 6 (a-e) –S-CH_2 peaks at 3.80-3.95 ppm (singlet). The antimicrobial activities of the synthesized compounds are also shown in the Table III. The antimicrobial activities of these compounds active against variety of bacterial stains in which some of these derivatives exhibited potential antibacterial and antifungal activity.

The antimicrobial activities of the synthesized compounds are shown in the Table III which were found moderate to good active against tested organism. Compounds 4a, 4c, 5a, 5c and 6b showed good activity (25 µg/mL) against *S.aureus* compared to Gentamycin which may be due to unsubstituted phenyl, 2-chloro and 2-hydroxy substituents. All the compounds showed moderate to weak activity against *S.pyogenus* and *E.coli*. Compound 4a showed good activity (25µg/mL) against *P.aeruginosa* which posses due to 2-hydroxy substituent. Compound 4a, 5c and 6b bearing unsubstantiated phenyl, 2-chloro and 2-hydroxy exibited good activity (25µg/mL) against *C.albicans* compared to Nystatin. All the compounds possess moderate to weak activity against *A.niger*. Compound 6e bearing 4-nitro substituent exibited good activity against *A.Clavatus*. Compounds bearing unsubstituted phenyl, 2-hydroxy, 2-chloro and 4-nitro substituents are found active against tested organism.

Conclusion

The antimicrobial activity of the synthesized compounds exhibit that compounds bearing unsubstituted phenyl, 2-hydroxy, 2-chloro and 4-nitro substituents are found active against tested organism. This fact reveals that the activity is not affected due to the electronic properties of the substituents. Compound 4a and 4c schiff's base while 5a and 5c azetidinone were found more active than the cyclized thiazolidinones.

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Table I. Physical data and elemental analysis for synthesized compounds

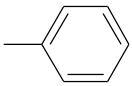
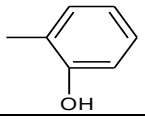
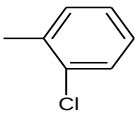
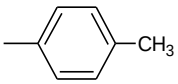
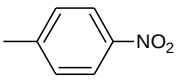
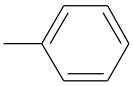
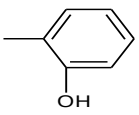
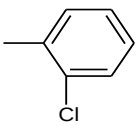
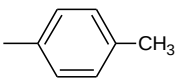
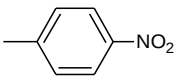
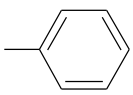
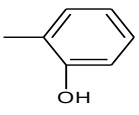
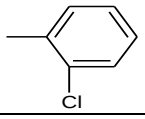
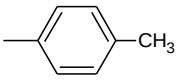
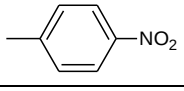
Comp. No.	R	Mol. Formula (Mw)	Solubility	Yield (%)	M. P. (˚C)	Elemental analysis % (Calcd. / found)		
						C	H	N
4a		$C_{28}H_{29}O_2N_7$ (495.57)	Ethanol	70	154-156	67.86 (67.79)	5.90 (5.79)	19.78 (19.90)
4b		$C_{28}H_{29}O_3N_7$ (511.57)	Ethanol	73	203-205	65.74 (65.69)	5.71 (5.80)	19.17 (19.26)
4c		$C_{28}H_{28}O_2N_7Cl$ (530.0)	Ethanol	68	136-138	63.45 (63.33)	5.32 (5.21)	18.50 (18.41)
4d		$C_{29}H_{31}O_2N_7$ (509.6)	Ethanol	74	178-180	68.35 (68.29)	6.13 (6.22)	19.24 (19.31)
4e		$C_{28}H_{28}O_4N_8$ (540.57)	Ethanol	72	168-170	62.21 (62.13)	5.22 (5.14)	20.73 (20.84)
5a		$C_{30}H_{30}O_3N_7Cl$ (572.05)	Ethanol	74	159-161	62.99 (62.86)	5.29 (5.20)	17.14 (17.02)
5b		$C_{30}H_{30}O_4N_7Cl$ (588.05)	Ethanol	71	170-173	61.27 (61.36)	5.14 (5.21)	16.67 (16.58)
5c		$C_{30}H_{29}O_3N_7Cl_2$ (606.5)	Ethanol	76	131-133	59.41 (59.53)	4.82 (4.62)	16.17 (16.28)
5d		$C_{31}H_{32}O_3N_7Cl$ (586.1)	Ethanol	67	98-100	63.53 (63.60)	5.50 (5.37)	16.73 (16.81)
5e		$C_{30}H_{29}O_5N_8Cl$ (617.05)	Ethanol	70	171-173	58.39 (58.43)	4.74 (4.79)	18.1 (18.20)
6a		$C_{30}H_{31}O_3N_7S$ (569.67)	Ethanol	75	161-163	63.25 (63.35)	5.48 (5.39)	17.21 (17.32)
6b		$C_{30}H_{31}O_4N_7S$ (585.67)	Ethanol	65	198-200	61.52 (61.61)	5.34 (5.41)	16.74 (16.68)
6c		$C_{30}H_{30}O_3N_7SCl$ (604.12)	Ethanol	70	149-151	59.64 (59.56)	5.01 (5.14)	16.23 (16.15)
6d		$C_{31}H_{33}O_3N_7S$ (583.70)	Ethanol	77	120-122	63.79 (63.68)	5.70 (5.59)	16.80 (16.92)
6e		$C_{30}H_{30}O_5N_8S$ (614.67)	Ethanol	76	137-139	58.62 (58.74)	4.92 (4.83)	18.23 (18.14)

Table II. IR, ^{1}H NMR and mass spectra of the synthesized compounds

Compd. No.	IR (ν, cm^{-1})	^{1}H NMR (δ, ppm)
4a	3207 (O-H), 2980, 1439, 1617, 1360, 815, 1570	2.33 (s, 3H, -CH_3), 3.05 (t, 4H, -N-CH_2), 3.92 (t, 4H, -N-CH_2), 3.84 (s, 3H, -OCH_3), 6.60-7.74 (m, 13H, Ar-H), 8.37 (s,1H, -N-CH), 10.45 (s, 1H, -NH)
4b	3210 (O-H), 3605, 2980, 1440, 1610, 1355, 784, 815, 760, 1580	2.37 (s, 3H, -CH_3), 3.08 (t, 4H, -N-CH_2), 3.62 (t, 4H, -N-CH_2), 3.82 (s, 3H, -OCH_3), 6.61-7.71 (m, 12H, Ar-H), 8.51 (s, 1H, -N-CH), 10.41(s, 1H, -NH), 5.34 (s, 1H, -OH)
4c	3205 (O-H), 2980, 1440,1620, 1360, 784, 815, 755, 1585	2.34 (s, 3H, -CH_3), 3.01 (t, 4H, -N-CH_2), 3.80 (t, 4H, -N-CH_2), 3.81 (s, 3H, -OCH_3), 6.80-7.70 (m, 12H, Ar-H), 8.41 (s,1H, -N-CH), 10.43 (s, 1H, -NH)
4d	3190 (O-H), 2985, 1625, 1470, 1350, 784, 815, 645, 1575	2.41 (s, 6H, -CH_3), 3.00 (t, 4H, - N-CH_2), 3.83 (t, 4H, -N-CH_2), 3.75 (s, 3H, -OCH_3), 6.81-7.75 (m, 13H, Ar-H), 8.40 (s,1H, -N-CH), 10.56 (s, 1H, -NH)
4e	3195 (O-H), 2975, 1435, 1630, 1365, 784, 815, 1584, 1480	2.42 (s, 3H, -CH_3), 3.11 (t, 4H, -N-CH_2), 3.89 (t, 4H, -N-CH_2), 3.87 (s, 3H, -OCH_3), 6.71-7.70 (m, 12H, Ar-H), 8.44 (s,1H, -N-CH), 10.47 (s, 1H, -NH)
5a	3207 (O-H), 2980, 1695, 1439, 1617, 1360, 815, 1570, 810	2.30 (s, 3H, -CH_3), 3.30 (t, 4H, -N-CH_2), 3.89 (t, 4H, -N-CH_2), 4.01 (s, 3H, -OCH_3), 6.62-8.52 (m, 13H, Ar-H), 5.65 (s,1H, -N-CH), 9.80 (s, 1H, -NH), 4.39 (s, 1H, -CH-Cl)
5b	3210 (O-H), 2980, 1705, 1440, 1610, 1355, 784, 815, 760, 1580, 775	2.47 (s, 3H, -CH_3), 3.32 (t, 4H, -N-CH_2), 3.81 (t, 4H, -N-CH_2), 3.77 (s, 3H, -OCH_3), 6.60-8.50 (m, 12H, Ar-H), 5.77 (s,1H, -N-CH), 9.69 (s, 1H, -NH), 4.40 (s, 1H, -CH-Cl), 5.29 (s, 1H, -OH)
5c	3205 (O-H), 2980, 1690, 1620, 1440, 1360, 784, 815, 755, 1585, 790	2.41 (s, 3H, -CH_3), 3.03 (t, 4H, -N-CH_2), 3.91 (t, 4H, -N-CH_2), 3.82 (s, 3H, -OCH_3), 6.80-8.40 (m, 12H, Ar-H), 5.81 (s,1H, -N-CH), 9.79 (s, 1H, -NH), 4.54 (s, 1H, -CH-Cl)
5d	3190 (O-H), 2985, 1680, 1625, 1470, 1350, 784, 815, 645, 1575, 795	2.33 (s, 6H, - CH_3), 3.17 (t, 4H, -N-CH_2), 3.77 (t, 4H, -N-CH_2), 3.95 (s, 3H, -OCH_3), 6.85-8.30 (m, 12H, Ar-H), 5.81 (s,1H, -N-CH), 9.68 (s, 1H, -NH), 4.50 (s, 1H, -CH-Cl)
5e	3195 (O-H), 2975, 1710, 1630, 1435, 1365, 784, 815, 1584, 1480, 805	2.28 (s, 3H, -CH_3), 3.01 (t, 4H, -N-CH_2), 3.88 (t, 4H, -N-CH_2), 3.64 (s, 3H, -OCH_3), 6.75-8.35 (m, 13H, Ar-H), 5.70 (s,1H, -N-CH), 9.68 (s, 1H, -NH), 4.47 (s, 1H, -CH-Cl)
6a	3207 (O-H), 2980, 1710, 1617, 1439, 1360, 815, 1570, 660	2.38 (s, 3H, -CH_3), 3.1 (t, 4H, -N-CH_2), 3.87 (t, 4H, -N-CH_2), 3.78 (s, 3H, -OCH_3), 6.80-7.45 (m, 13H, Ar-H), 5.81 (s,1H, -N-CH), 9.71 (s, 1H, -NH), 3.91-3.80 (s, 1H, -S-CH_2)
6b	3210 (O-H), 3610, 2980, 1695, 1610, 1440, 1355, 784, 815, 760, 1580, 655	2.29 (s, 3H, -CH_3), 3.34 (t, 4H, -N-CH_2), 3.64 (t, 4H, -N-CH_2), 3.80 (s, 3H, -OCH_3), 6.90-7.55 (m, 12H, Ar-H), 5.88 (s,1H, -N-CH), 9.70 (s, 1H, -NH), 3.94-3.82 (s, 1H, -S-CH_2), 5.39 (s, 1H,-OH)
6c	3205 (O-H), 2980, 1680, 1620, 1440, 1360, 784, 815, 755, 1585, 665	2.48 (s, 3H, -CH_3), 3.21 (t, 4H, -N-CH_2), 3.92 (t, 4H, -N-CH_2), 3.81 (s, 3H, -OCH_3), 6.85-7.75 (m, 12H, Ar-H), 5.84 (s,1H, -N-CH), 9.70 (s, 1H, -NH), 3.90-3.81 (s, 1H, -S-CH_2)
6d	3190 (O-H), 2985, 1625, 1470, 1350, 784, 815, 645, 1575, 650	2.30 (s, 6H, -CH_3), 3.18 (t, 4H, -N-CH_2), 3.90 (t, 4H, -N-CH_2), 3.85 (s, 3H, -OCH_3), 6.70-7.40 (m, 12H, Ar-H), 5.71 (s,1H, -N-CH), 9.81 (s, 1H, -NH), 3.94-3.84 (s, 1H, -S-CH_2)
6e	3195(O-H), 2975, 1702, 1630, 1435, 1365, 784, 815, 1584, 1480, 658	2.31 (s, 3H, -CH_3), 3.00 (t, 4H, -N-CH_2), 3.85 (t, 4H, -N-CH_2), 3.88 (s, 3H, -OCH_3), 6.75-8.35 (m, 12H, Ar-H), 5.76 (s,1H, -N-CH), 9.72 (s, 1H, -NH), 3.95-3.85 (s, 1H, -S-CH_2)

Table III. Antimicrobial activity of the synthesized compounds

Comp. No.	MBC (μg mL^{-1})				MFC (μg mL^{-1})		
	S. aureus	S. pyogenus	E. coli	P. aeruginosa	C. albicans	A. niger	A. clavatus
4a	25	50	25	25	25	50	50
4b	50	200	100	200	50	200	200
4c	25	100	200	200	50	200	50
4d	100	200	50	200	100	25	100
4e	200	25	200	50	50	200	200
5a	25	50	200	200	100	50	200
5b	100	200	100	50	200	50	100
5c	25	100	50	200	25	100	200
5d	100	200	25	200	50	200	200
5e	50	50	25	50	50	100	200
6a	100	100	50	200	100	25	50
6b	25	50	25	50	25	50	50
6c	100	200	50	200	100	50	200
6d	200	50	200	50	50	50	50
6e	50	25	50	100	100	50	25
Gentamycin	25	5	5	25	-	-	-
Nystatin	-	-	-	-	25	5	25

MBC - Minimal Bactericidal Concentration
MFC - Minimal Fungicidal Concentration