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Synthesis and Evaluation of Novel Indolylthiadiazinoazetidinone Derivatives As Antimicrobial Agent

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ABSTRACT

Some new 3-chloro-(4-substituted aryl)-1-(8-methoxy-[1,3,4]thiadiazino[6,5-b]indol-3-yl)azetidinones (4a-4g) have been synthesized from N-(substituted benzylidene)-8-methoxy-[1,3,4]thiadiazino[6,5-b]indol-3-amine (3a-3g). These newly synthesized compounds were characterized by elemental (C, H, N) and spectral (IR, ^{1}HNMR mass) analysis. Compounds 3a-3g and compounds 4a-4g of the present series have screened for their antibacterial and antifungal activities. Compounds 4f and 4g were found to be the most potent members of the present series; they showed maximum antibacterial and antifungal properties much better than the standard drug. In this series Chloroamphenicol was used as standard drug for antibacterial activity and Fluconazole was used as standard drug for antifungal activity.

Keywords: Indole, thiadiazine, Azetidinone, Antibacterial activity, Antifungal activity

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INTRODUCTION

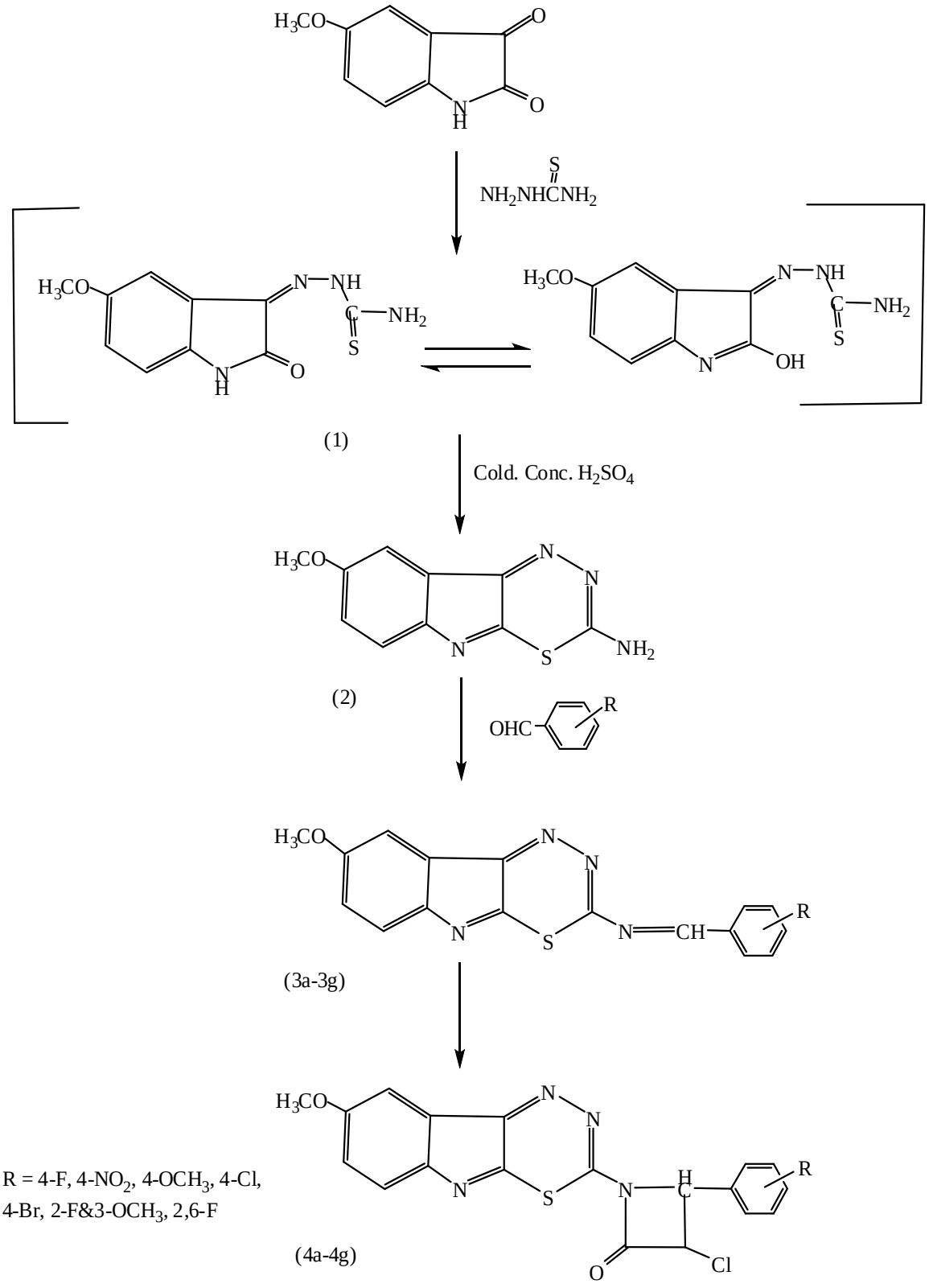
Antibacterial and antifungal disease are very common all over the world. Currently used antimicrobial agents are not very useful due to the resistance developed by the microbes. Over and above there is no permanent structure and activity relationship between the pharmacophore groups and heterocyclic frame work. In continuation to this it is an ongoing effort to synthesize new antimicrobial agents. Thus, various scientist are engaged to synthesize new compounds related to various chemical groups like indole, thiadiazine, azetidinone etc. The indole[1] derivatives are well known in medicinal chemistry due to their diverse biological properties like antimicrobial[2,3], antibacterial[4], antifungal[5], insecticidal[6], antiinflammatory[7] and anticonvalsant[8] etc. Similarly thiadiazine[9,10] and azetidinone[11] derivatives have also been found to exhibit antibacterial and antifungal activities. In the view of these observations, it was thought worthwhile to synthesize new indolilthiadiazinoazetidinone derivatives with the hope to get better antibacterial as well as antifungal activities.

MATERIAL AND METHODS:

All reagents and solvents were of analytical grade and used directly. Reactions were routinely performed in oven-dried borosil glassware. The melting points of compounds were determined in open capillaries with the help of thermonic melting point apparatus and were uncorrected. The homogeneity of all newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G plates and spots were located by using iodine chamber. Elemental analysis (C, H, N) of all the synthesized compounds were determined by perkin-Elmer 2400 elemental analyzer, and results were found within the ± 0.4% of theoretical values. The IR spectra were recorded on a Beckman Acculab-10 spectrometer (ν□ max in cm^{-1}) and the ^1H NMR spectra were recorded by Brucker DPX-300 MHz using $CDCl_3$ as solvent. Mass spectra were determined on VG-70-S instrument.

Synthesis of (Z)-2-(5-methoxy-2-oxoindolin-3-ylidene)hyrazinecarbothiomide 1

To the methanolic solution of 5-methoxyindolin-2,3-dione (0.01 mol), thiosemicarboazide (0.01 mol) was added. This reaction mixture was refluxed for 4 h, and then, the excess of solvent has been removed by distillation. After this, the residue was allowed to cool then it was poured onto crushed ice, filtered, and washed with water. The solid mass obtained was recrytallized from ethanol to give compound 1. Physical, analytical and spectral data are given in table- 1,2 respectively.



R = 4-F, 4-NO_2, 4-OCH_3, 4-Cl, 4-Br, 2-F&3-OCH_3, 2,6-F

Scheme-1

Synthesis of 8-methoxy-[1,3,4]thiadiazino[6,5-b]indol-3-amine 2

Table 1 Physical and analytical data of the compounds 1, 2, 3a-3g and 4a-4g

compounds	R	Recrystalization solvent	Yield%	m.p (^{0}c)	Mol. Formula	Analysis % found (calculated)		
						C	H	N
1	-	Ethanol	86	135	$C_{10}H_{10}N_4O_2S$	47.97 (47.99	4.06 4.03	22.36 22.39)
2	-	Methanol	87	147	$C_{10}H_8N_4OS$	51.74 (51.71	3.48 3.47	24.15 24.12)
3a	4-F	D.M.F.	85	147	$C_{17}H_{11}FN_4OS$	60.36 (60.34	3.29 3.28	16.57 16.56)
3b	4-NO_2	Benzene	84	155	$C_{17}H_{11}N_5O_3S$	55.87 (55.88	3.06 3.03	19.15 19.17)
3c	4-OCH_3	Methanol	82	157	$C_{18}H_{14}N_4O_2S$	61.72 (61.70	4.05 4.03	15.97 15.99)
3d	4-Cl	Ethanol	83	165	$C_{17}H_{11}ClN_4OS$	57.53 (57.55	6.13 3.12	15.76 15.79)
3e	4-Br	Benzene	82	170	$C_{17}H_{11}BrN_4OS$	51.17 (51.14	2.77 2.78	14.01 14.03)
3f	2-F, 3-OCH_3	D.M.F.	81	179	$C_{18}H_{13}FN_4O_2S$	58.68 (58.69	3.53 3.56	15.25 15.21)
3g	2,6-F	Methanol	80	195	$C_{17}H_{10}F_2N_4OS$	57.32 (57.30	2.81 2.83	15.74 15.72)
4a	4-F	Ethanol	77	197	$C_{19}H_{12}ClFN_4O_2S$	55.03 (55.01	2.94 2.92	13.53 13.51)
4b	4-NO_2	Benzene	75	188	$C_{19}H_{12}ClN_5O_4S$	51.68 (51.65	2.73 2.74	15.83 15.85)
4c	4-OCH_3	Ethanol	73	186	$C_{20}H_{15}ClN_4O_3S$	56.28 (56.27	3.52 3.54	13.11 13.12)
4d	4-Cl	Methanol	70	202	$C_{19}H_{12}Cl_2N_4O_2S$	52.92 (52.91	2.82 2.80	12.97 12.99)
4e	4-Br	D.M.F.	65	195	$C_{19}H_{12}BrClN_4O_2S$	47.99 (47.97	2.53 2.54	11.76 11.78)
4f	2-F, 3-OCH_3	Ethanol	60	226	$C_{20}H_{14}ClFN_4O_3S$	54.02 (54.00	3.16 3.17	12.57 12.59)
4g	2,6-F	Benzene	61	211	$C_{19}H_{11}ClF_2N_4O_2S$	52.70 (52.72	2.57 2.56	12.93 12.94)

Table 2 spectral data of compounds 1, 2, 3a-3g and 4a-4g

Compound No.	$[M]^+$ m/z	IR (KBr) ν max in Cm^{-1}	^{1}H-NMR ($CDCl_3$+$DMSOd_6$)δ in ppm
1	250.28	3370 (NH_2), 3245 (NH), 3032 (C-H aromatic), 2910 (C-H aliphatic), 1670 (C=O), 1612 (C=N), 1531 (C-C of aromatic ring), 1510 (C-N-C), 1202 (C=S), 1180 (C-N), 1020 (N-N)	8.60 (s, 1H, NH of indole, exchangeable with D_2O), 7.14 (d, 3H, indole), 6.10 (bs, 2H, NH_2, exchangeable with D_2O), 5.85 (s, 1H. NH, exchangeable with D_2O), 3.25 (s, 3H, OCH_3)
2	232.26	3372 (NH_2), 3035 (C-H aromatic), 2913 (C-H aliphatic), 1672	7.96 (s, 2H, NH_2, exchangeable with D_2O), 7.10 (s, 3H, Indole), 3.30 (s, 3H,

		(C=O), 1615 (C=N), 1534 (C-C of aromatic ring), 1513 (C-N-C), 1182 (C-N), 1020 (N-N), 1071 (C-O-C), 670 (C-S-C)	OCH_3)
3a	338.36	3032 (C-H aromatic), 2911 (C-H aliphatic), 1673 (C=O), 1613 (C=N), 1530 (C-C of aromatic ring), 1512 (C-N-C), 1185 (C-N), 1025 (N-N), 1070 (C-O-C), 675 (C-S-C)	8.19 (s, 1H, CH-Ar), 7.88 (m, 4H, Ar-H), 7.16 (s, 3H, Indole), 3.32 (s, 3H, OCH_3)
3b	365.37	3037 (C-H aromatic), 2915 (C-H aliphatic), 1672 (C=O), 1617 (C=N), 1533 (C-C of aromatic ring), 1515 (C-N-C), 1184 (C-N), 1024 (N-N), 1074 (C-O-C), 673 (C-S-C)	8.15 (s, 1H, CH-Ar), 7.87 (m, 4H, Ar-H), 7.17 (s, 3H, Indole), 3.35 (s, 3H, OCH_3)
3c	350.39	3036 (C-H aromatic), 2916 (C-H aliphatic), 1677 (C=O), 1615 (C=N), 1531 (C-C of aromatic ring), 1510 (C-N-C), 1186 (C-N), 1025 (N-N), 1071 (C-O-C), 678 (C-S-C)	8.13 (s, 1H, CH-Ar), 7.85 (m, 4H, Ar-H), 7.10 (s, 3H, Indole), 3.36 (s, 3H, OCH_3), 3.15 (s, 3H, OCH_3)
3d	354.81	3032 (C-H aromatic), 2917 (C-H aliphatic), 1670 (C=O), 1616 (C=N), 1535 (C-C of aromatic ring), 1517 (C-N-C), 1180 (C-N), 1020 (N-N), 1079 (C-O-C), 670 (C-S-C)	8.12 (s, 1H, CH-Ar), 7.83 (m, 4H, Ar-H), 7.13 (s, 3H, Indole), 3.31 (s, 3H, OCH_3)
3e	399.26	3038 (C-H aromatic), 2910 (C-H aliphatic), 1675 (C=O), 1612 (C=N), 1536 (C-C of aromatic ring), 1516 (C-N-C), 1188 (C-N), 1027 (N-N), 1073 (C-O-C), 676 (C-S-C)	8.20 (s, 1H, CH-Ar), 7.84 (m, 4H, Ar-H), 7.16 (s, 3H, Indole), 3.34 (s, 3H, OCH_3)
3f	368.38	3039 (C-H aromatic), 2919 (C-H aliphatic), 1674 (C=O), 1615 (C=N), 1530 (C-C of aromatic ring), 1514 (C-N-C), 1185 (C-N), 1025 (N-N), 1070 (C-O-C), 678 (C-S-C)	8.10 (s, 1H, CH-Ar), 7.80 (m, 4H, Ar-H), 7.10 (s, 3H, Indole), 3.30 (s, 3H, OCH_3), 3.12 (s, 3H, OCH_3)
3g	356.35	3034 (C-H aromatic), 2915 (C-H aliphatic), 1670 (C=O), 1618 (C=N), 1531 (C-C of aromatic ring), 1519 (C-N-C), 1187 (C-N), 1020 (N-N), 1071 (C-O-C), 679 (C-S-C)	8.13 (s, 1H, CH-Ar), 7.85 (m, 3H, Ar-H), 7.13 (s, 3H, Indole), 3.32 (s, 3H, OCH_3)
4a	414.84	3030 (C-H aromatic), 2918 (C-H aliphatic), 1674 (C=O), 1615	8.13 (s, 1H, CH-Ar), 7.805 (m, 4H, Ar-H), 7.10 (s, 3H, Indole), 6.40 (d, 1H, N-

		(C=N), 1533 (C-C of aromatic ring), 1517 (C-N-C), 1188 (C-N), 1023 (N-N), 711 (C-Cl), 659 (C-S-C)	CH-Ar), 3.30 (s, 3H, OCH_3)
4b	441.85	3039 (C-H aromatic), 2913 (C-H aliphatic), 1670 (C=O), 1616 (C=N), 1531 (C-C of aromatic ring), 1513 (C-N-C), 1180 (C-N), 1026 (N-N), 714 (C-Cl), 654 (C-S-C)	8.10 (s, 1H, CH-Ar), 7.80 (m, 4H, Ar-H), 7.12 (s, 3H, Indole), 6.43 (d, 1H, N-CH-Ar), 3.30 (s, 3H, OCH_3)
4c	426.88	3032 (C-H aromatic), 2910 (C-H aliphatic), 1670 (C=O), 1612 (C=N), 1531 (C-C of aromatic ring), 1510 (C-N-C), 1180 (C-N), 1020 (N-N), 712 (C-Cl), 659 (C-S-C)	8.13 (s, 1H, CH-Ar), 7.82 (m, 4H, Ar-H), 7.10 (s, 3H, Indole), 6.44 (d, 1H, N-CH-Ar), 3.40 (s, 3H, OCH_3), 3.07 (s, 3H, OCH_3)
4d	431.30	3035 (C-H aromatic), 2912 (C-H aliphatic), 1677 (C=O), 1610 (C=N), 1535 (C-C of aromatic ring), 1518 (C-N-C), 1187 (C-N), 1024 (N-N), 714 (C-Cl), 655 (C-S-C)	8.14 (s, 1H, CH-Ar), 7.83 (m, 4H, Ar-H), 7.15 (s, 3H, Indole), 6.41 (d, 1H, N-CH-Ar), 3.32 (s, 3H, OCH_3)
4e	475.75	3032 (C-H aromatic), 2910 (C-H aliphatic), 1670 (C=O), 1612 (C=N), 1531 (C-C of aromatic ring), 1510 (C-N-C), 1180 (C-N), 1020 (N-N) 712 (C-Cl), 659 (C-S-C), 610 (C-Br)	8.10 (s, 1H, CH-Ar), 7.80 (m, 4H, Ar-H), 7.12 (s, 3H, Indole), 6.40 (d, 1H, N-CH-Ar), 3.30 (s, 3H, OCH_3)
4f	444.87	3037 (C-H aromatic), 2917 (C-H aliphatic), 1675 (C=O), 1613 (C=N), 1530 (C-C of aromatic ring), 1516 (C-N-C), 1186 (C-N), 1027 (N-N) 715 (C-Cl), 650 (C-S-C)	8.11 (s, 1H, CH-Ar), 7.81 (m, 4H, Ar-H), 7.10 (s, 3H, Indole), 6.405 (d, 1H, N-CH-Ar), 3.34 (s, 3H, OCH_3)
4g	432.83	3039 (C-H aromatic), 2919 (C-H aliphatic), 1670 (C=O), 1610 (C=N), 1534 (C-C of aromatic ring), 1513 (C-N-C), 1183 (C-N), 1025 (N-N), 717 (C-Cl), 657 (C-S-C)	8.12 (s, 1H, CH-Ar), 7.84 (m, 4H, Ar-H), 7.13 (s, 3H, Indole), 6.41 (d, 1H, N-CH-Ar), 3.45 (s, 3H, OCH_3), 3.10 (s, 3H, OCH_3)

A mixture of compound 1 (0.05 mol) and concentrated H_2SO_4 (15 ml) was left to react at room temperature for around 16 h. Then, this reaction mixture was poured onto crushed ice, neutralized with liquid ammonia to get a solid mass, which was filtered and washed with water, dried and recrystallized with methanol to yield compound 2. Physical, analytical and spectral data are given in table- 1,2 respectively.

General procedure for the synthesis of N-(substituted benzylidene)-8-methoxy-[1,3,4]thiadiazino[6,5-b]indol-3-amine (3a-3g)

Equimolar amount of compound 2 (0.02 mol) and different aromatic aldehydes (0.02 mol) in methanol (55 ml) were refluxed for 10-12 h in the presence of a few drops of glacial acetic acid. Progress and completion of the reaction were checked by TLC. After refluxing, the excess of solvent was distilled off and the remanent substance was dropped in ice-water, filtered, dried and the solid thus obtained were recrystallized from appropriate solvents to furnish compounds 3a-3g. Physical, analytical and spectral data are given in table- 1,2 respectively.

General procedure for the synthesis of 3-chloro-4-(substitutedphenyl)-1-(8-methoxy-[1,3,4]thiadiazino[6,5-b]indol-3-yl)azetidin-2-ones 4a-4g

To the solution of compounds 3a-3g (0.01 mol) in dioxane (50 ml), chloroacetyl chloride (0.02 mol) was added dropwise with stirring in the presence of triethyl amine (0.01 mol) at 0-5^{0}C. The different reaction mixtures were refluxed for 4-6 h and excess of solvent was then distilled off. The resulting mixtures were poured into crushed ice to afford compounds 4a-4g. Physical, analytical and spectral data are given in table- 1,2 respectively.

Pharmacological Evaluation

Antibacterial activity

The compounds 3a-3g and 4a-4g were tested for their in vitro growth inhibitory activity against different bacteria's like E. coli, B. subtilis and S. aureus and compared with standard drug Chloroamphenicol. The inhabitation zones of synthesized compounds were determines using cup plate methods[12]. In this methods Nutrient agar was poured onto the sterilized Petri dishes (20-25 ml each Petri dish). The poured material was allowed to set (1-1.5 h) and thereafter the "CUPS" (10 mm diameter) were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into these cups the test compound solution was added with the help of sterile syringe. The plates were incubated at 37^{0}C for 48 hr and the results were noted. A solvent control (10% DMSO in methanol) was also run to note the activity of the blank (solvent). The inhibition zones produced by the various synthesized compounds on the microbial growth were measured (diameter in mm).

Antifungal activity

The newly synthesized compounds and the standard drug, fluconazole were tested for their antifungal activity by employing the standard agar disc diffusion method [13]. The following strains of fungi have been used in this study: Aspergillus niger , Candida albicans, and Candida Krusei. All cultures were maintained on [Sabouraud-dextrose agar] SDA and incubated at 30^{0}C.

To prepare homogeneous suspensions of the above mentioned fungi for the disc assays, they were grown in Sabouraud broth, centrifuged to collect the pellet, and buffered with saline. The fungal pellet was homogenized in a sterile hand-held homogenizer. This suspension was then plated onto SDA using a fungal spreader to obtain an even growth field. Sterile 6 mm Whatmann filter paper were impregnated with 250 µg/mL concentration of the various test compounds and standard drug fluconazole. These disc were then placed in the center of each quadrant of an SDA plate. Each plate had one control disc impregnated with DMSO. The plates were incubated at 30^0C. After 48 h, the plates were removed.

RESULT AND DISCUSSION

The antibacterial activity of compounds 3a-3g, 4a-4g and the standard drug chloroamphenical, was carried out against Escherischia coli ESS 2231, Bacillus subtillis ATCC 1633 and Staphylococcus aureus 209p. Results showed the varying degree of antibacterial and antifungal activity of all the compounds tested (Table 3).

Table-3 Antibacterial and antifungal activity of synthesized compounds 3a-3g and 4a-4g

Comp. No.	R	Bacterial growth inhibition (diameter in mm)			Fungal growth inhibition(diameter in mm)		
		E. coli	B. subtillis	S. aureus	C. albicans	A. niger	C. krusei
3a	4-F	12	10	9	-	12	9
3b	4-NO_2	13	12	14	10	11	10
3c	4-OCH_3	12	14	11	9	9	12
3d	4-Cl	16	13	15	13	10	-
3e	4-Br	15	17	16	15	-	13
3f	2-F, 3-OCH_3	20	22	19	18	16	15
3g	2,6-F	25	23	22	20	19	-
4a	4-F	26	22	21	23	20	14
4b	4-NO_2	24	21	20	-	18	17
4c	4-OCH_3	-	23	-	29	21	19
4d	4-Cl	27	25	24	31	23	20
4e	4-Br	25	21	-	28	22	19
4f	2-F, 3-OCH_3	28	24	23	30	23	21
4g	2,6-F	30	25	24	32	25	20
Chloroamphenicol		26	23	22	-	-	-
Fluconazole					29	22	19

From the results it is clear that compounds 4a, 4f and 4g showed excellent antibacterial activity better than the standard drug and good inhibition zones against all the bacterial strains. Compounds 3g and 4a displayed antibacterial properties equipotent to the reference drug. The other compounds of this series showed a moderate activity as compared to standard drug.

Compounds 3a-3g and 4a-4g along with the reference drug fluconazole were also tested for antifungal activity against candida albicans ATCC 2091, Aspergillus niger ATCC 9029 and candida Krusei ATCC 6518. The results of the antifungal screening revealed that all the tested compounds 3a-3g and 4a-4g showed moderate to good antifungal properties. Out of these compounds tested, compounds 4a, 4f and 4g were found to be more potent antifungal agents against C. albicans , A. niger and C. krusei than reference drug. Compounds 4c and 4e displayed antifungal property equipotent to the reference drug against all three fungal strains. The other compounds of this series were less active compound to the standard drug. Compounds 3a-3e exhibited mild to moderate antibacterial as well as antifungal activities.

CONCLUSION

From this study, we may conclude that: Compounds (4a-4g) containing azetidinone ring exhibited better antibacterial as well as antifungal activity than compounds (3a-3g) having ring. 2-floro,3-methoxy and 2,6-diflorophenyl substituted indolylthiadizine derivatives showed more efficiency due to presence of more electronegative atom.

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