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SYNTHESIS OF THIAZOLIDINONE AND AZETIDINONE DERIVATIVES OF BENZOXAZOLINONE AS ANTIMICROBIAL AGENTS

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ABSTRACT: The present investigation is concerned with synthesis of new substituted benzoxazolinone derivatives (1-18) with the objective of discovering novel and potent antimicrobial agents. The structure of all the synthesized compounds were elucidated by elemental (C, H, N) and spectral (IR, ^{1}HNMR and mass) analysis. The purity of the synthesized compounds was checked by thin layer chromatography (TLC). The obtained compounds were screened for their antibacterial as well as antifungal activities and compared with reference drugs ciprofloxacin and fluconazole respectively. All the newly synthesized compounds were screened for their antibacterial activity against *S. aureus*, *E. coli*, *K. pneumoniae* and antifungal activity against *A. niger*, *C. albicans*. Compounds 13 and 18 were found to be the most potent members of the present series; they showed maxium antibacterial and antifungal properties much better than the standard drug. The compounds 15, 16 and 17 exhibited good antibacterial activity as well as while antifungal activity.

INTRODUCTION:

Heterocyclic system containing oxazole ring, constitute an important class of potentially bioactive compounds. The earlier workers reported that benzoxazolinone derivatives are associated with a wide spectrum of biological activities viz, antimicrobial [1-5], antiinflammatory [6, 7], anticonvulsant [8], analgesic [9], and anticancer [10] this significance led us to synthesize the titled compounds. Similarly thiazolidinone [11, 12] and azetidinone [13, 14] derivatives have also been found to exhibit antibacterial and antifungal activities. In light of above observation it wa thought worthwhile to synthesized some new substituted benzoxazolinone derivatives by incorporation of thiazolidinone and azetidinone moiety with the hope to get better antibacterial as well as antifungal agents.

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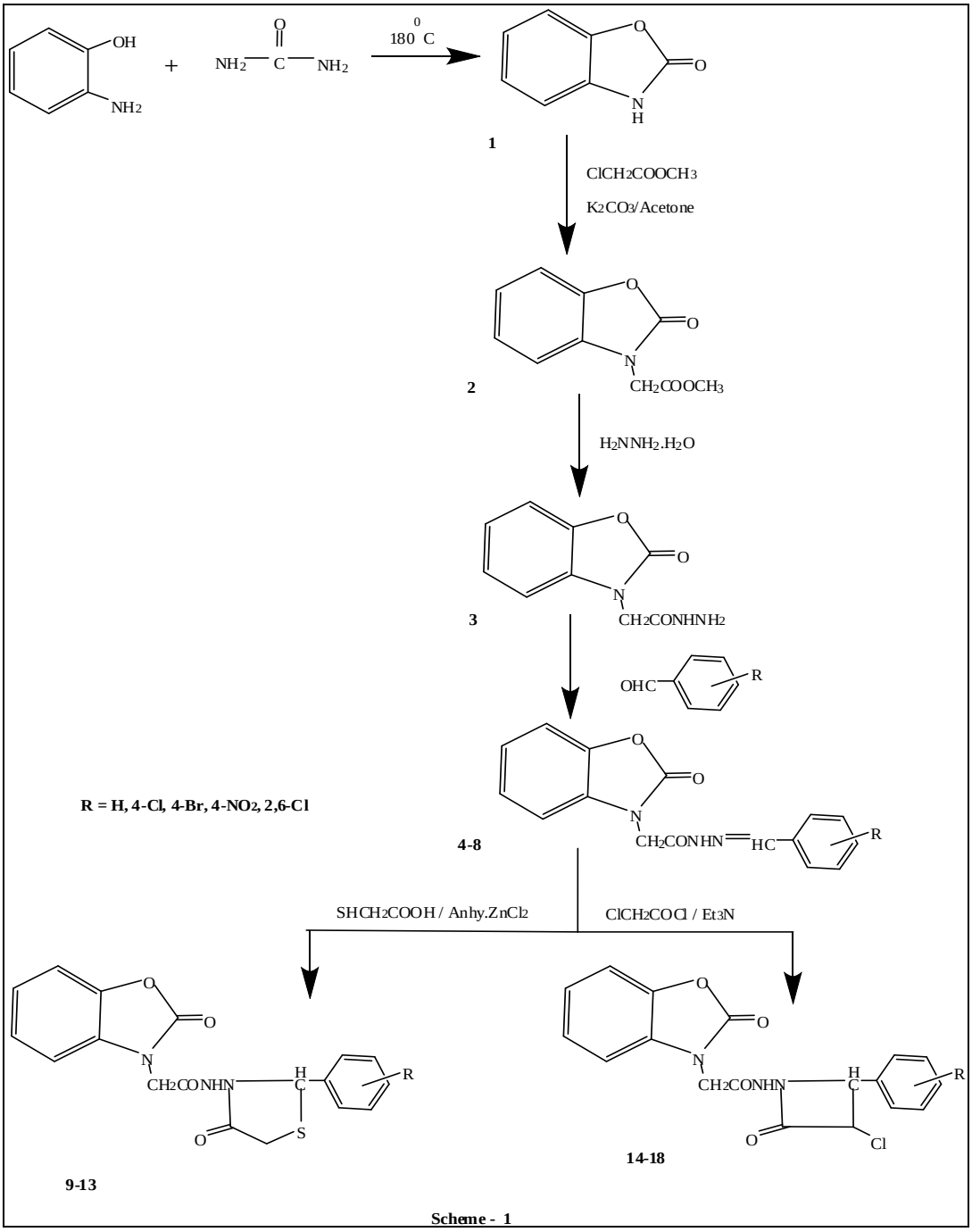
MATERIAL AND METHODS:

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 ^{1}H NMR spectra were recorded by Brucker DPX-300 MHz using $CDCl_3$ as solvent. Mass spectra were determiend on VG-70-S instrument. The synthesis of the target compounds was accomplished according to the reaction sequence illustrated in **Scheme 1**.



SCHEME :1

Synthesis of benzo[d]oxazol-2(3H)-one (1):

A mixture of iodine (0.3mol) and urea (0.6mol) was triturated and the reaction mixture transferred into a conical flask containing 2-aminophenol and heated for 8 h. The solid obtained was washed with diethyl ether. Finally, the reaction mixture was poured in ice water. The solid thus obtained was filtered, washed with water, dried and recrystallized from acetone to yield compound 1. Physical, analytical and spectral data are given in table- 1, 2 respectively.

Synthesis of methyl-2-(2-oxobenzo[d]oxazol-3(2H)-yl) acetate (2)

A mixture of compound 1 (0.01 mol), methyl chloroacetate (0.01 mol), anhydrous acetone (90 ml) and K_2CO_3 (8 g) was heated under reflux of 20 hr. After cooling, it was wash with excess of water the solid was filtered and dried, again washed with methanol. The solid was obtained and recrystallized from methanol to yield compound 2. Physical, analytical and spectral data are given in table- 1, 2 respectively.

Synthesis of 2-(2-oxobenzo[d]oxazol-3(2H)-yl) acetohydrazide (3):
A mixture of compound 2 (0.01 mol) and hydrazine hydrate (0.02 mol) in absolute ethanol (80 ml) was refluxed for 20 h. It was then cooled and poured on crushed ice and separated solid was filtered, washed with cold water, dried and recrystallized from ethyl acetone to yield compound 3. Physical, analytical and spectral data are given in table- 1, 2 respectively.

General procedure for the synthesis of N-substituted benzylidene-2-(2-oxobenzo[d]oxazol-3(2H)-yl) acetohydrazide (4-8):
A mixture of compound 3 (0.5 mol) and different substituted benzyldehyde (0.5 mol) in 40 ml of ethanol along with glacial acetic acid (2-3 drops) was refluxed for 12 h. The reaction mixture was cooled. The solid obtained was filtered, washed with water, dried and recrystallized from appropreate solvents to furnish compounds 4-8. Physical, analytical and spectral data are given in table- 1, 2 respectively.

General procedure for the synthesis of N-(2-(substitutedphenyl) - 4 - oxothiazolidin-3-yl) - 2 (2-oxobenzo[d]oxazol-3(2H)-yl)acetamide (9-13):
To ethanolic solution (60 ml) of compounds 4-8 (0.02 mol) thioglycolic acid (0.04mol) was added in the presence of anhydrous zinc chloride respectively. The reaction mixtures were refluxed for 10 h. The excess of solvent was distilled off and separated masses were poured into ice water, filtered and washed with water and recystallized from appropreate solvents to give compounds 9-13. Physical, analytical and spectral data are given in table- 1, 2 respectively.

General procedure for the synthesis of N-(3-chloro-2- oxo-4-substitutedphenylazetidin-1-yl) - 2-(2-oxobenzo[d]oxazol-3(2H)-yl) acetamide (14-18):
Compounds 9-13 (0.3 mol), dry dioxane (5 ml) and triethylamine (0.6 mol) were taking in a conical flask. The reaction mixtures were stirred on an ice bath and when the temperature dropped below 5^0C then chloroacetylchloride (0.015mol) was added drop wise with stirring. After completion of addition the stirring was continued for 10 h. at room temperature.

The reaction mixtures were then kept a side for 52 h. Finally the reaction masses were added to ice cold water to obtain the final product. It was filtered washed with water, dried and recrystallized from appropriate solvent to yield compounds 14-18. Physical, analytical and spectral data are given in table- 1, 2 respectively.

Pharmacological Evaluation:
Antibacterial activity:
The compounds 4-18 were tested for their in vitro growth inhibitory activity against different bacteria like *S. aureus, E. coli, K. pnemoniae* and compared with standard drug Ciprofloxacin. The inhabitation zones of synthesized compounds were determines using cup plate methods [15]. 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 37^0C 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 [16]. The following strains of fungi have been used in this study: *C albicans* and *Aspergillus niger*. All cultures were maintained on [Sabouraud-dextrose agar] SDA and incubated at 30^0C. 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 paperwere impregnated with 250µg/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.

RESULTS AND DISCUSSION: All the compounds were screened for their antibacterial activity against pathogenic bacteria such as *S. aureus, E. coli* and *K. pnemoniae* by using the standard drug ciprofloxacin. All the compounds were also screened for their antifungal activity against *C. albicans* and *A. niger* by using the standard drug fluconazole.

The zone of inhibition was measured in mm. The reading are shown in **Table 3**. From the given observations it is clear that compounds 18 was more active against *S. aureus, E. coli, K. pnemoniae* as compared to standard drug. While compound 13 exhibeted more antifungal activity against *C. albicans* and *A. niger* as compared to standard drug. Compound 15, 16 and 17 have shown good antibacterial as well as antifungal activity. Rest of the compounds of this series have shown low to moderate activity against defferent bacteria and fungi.

TABLE 1: PHYSICAL AND ANALYTICAL DATA OF THE COMPOUNDS 1-18

Compounds	R	Recrystalization solvent	Yield%	m.p (^{0}c)	Mol. Formula	Analysis % found (calculated)		
						C	H	N
1	-	Methanol	84	158	$C_7H_5NO_2$	62.23 (62.22	3.75 3.73	10.36 10.37)
2	-	Benzene	82	171	$C_{10}H_9NO_4$	57.94 (57.97	4.36 4.38	6.75 6.76)
3	-	D.M.F.	81	167	$C_9H_9N_3O_3$	52.16 (52.17	4.39 4.38	20.56 20.28)
4	H	Ethanol	79	186	$C_{16}H_{13}N_3O_3$	65.07 (65.08	4.46 4.44	14.25 14.23)
5	4-Cl	Methanol	80	198	$C_{16}H_{12}ClN_3O_3$	58.26 (58.28	4.65 3.67	12.77 12.74)
6	4-Br	D.M.F.	78	191	$C_{16}H_{12}BrN_3O_3$	51.33 (51.36	6.23 3.23	11.26 11.23)
7	4-NO_2	Benzene	75	214	$C_{16}H_{12}N_4O_5$	56.46 (56.47	3.57 3.55	16.44 16.46)
8	2,6-Cl	Ethanol	76	236	$C_{16}H_{11}Cl_2N_3O_3$	52.78 (52.77	3.03 3.04	11.55 11.54)
9	H	Methanol	74	221	$C_{18}H_{15}N_3O_4S$	58.52 (58.53	2.81 4.09	15.74 11.38)
10	4-Cl	Benzene	71	243	$C_{18}H_{14}ClN_3O_4S$	53.54 (53.53	3.46 3.49	10.43 10.41)
11	4-Br	D.M.F.	70	252	$C_{18}H_{14}BrN_3O_4S$	48.24 (48.23	3.13 3.15	9.33 9.37)
12	4-NO_2	Ethanol	67	257	$C_{18}H_{14}N_4O_6S$	52.18 (52.17	3.42 3.41	13.51 13.52)
13	2,6-Cl	Methanol	64	262	$C_{18}H_{13}Cl_2N_3O_4S$	49.32 (49.33	2.97 2.99	9.57 9.59)
14	H	Benzene	65	246	$C_{18}H_{14}ClN_3O_4$	58.19 (58.15	3.83 3.80	11.33 11.30)
15	4-Cl	Ethanol	62	266	$C_{18}H_{13}Cl_2N_3O_4$	53.24 (53.22	3.26 3.23	10.37 10.34)
16	4-Br	D.M.F.	60	271	$C_{18}H_{13}BrClN_3O_4$	47.99 (47.97	2.93 2.91	9.33 9.32)
17	4-NO_2	Methanol	58	278	$C_{18}H_{13}ClN_4O_6$	51.89 51.87	3.17 3.14	13.43 13.44
18	2,6-Cl	Ethanol	55	289	$C_{18}H_{12}Cl_3N_3O_4$	49.03 49.06	2.76 2.74	9.57 9.54

TABLE: 2 SPECTRAL DATA OF COMPOUNDS 1-18

Compound No.	$[M]^+$ m/z	IR (KBr) ν max in Cm^{-1}	^{1}H-NMR ($CDCl_3$+$DMSOd_6$)δ in ppm
1	135.12	3245 (NH), 3032 (C-H aromatic), 1670 (C=O), 1531 (C-C of aromatic ring), 1180 (C-N), 1071 (C-O-C)	8.80 (s, 1H, NH of benzoxazole, exchangeable with D_2O), 7.14 -8.10 (m, 4H, Ar-H)
2	207.18	3035 (C-H aromatic), 2913 (C-H aliphatic), 1672 (C=O), 1534 (C-C of aromatic ring), 1513 (C-N-C), 1182 (C-N), 1074 (C-O-C)	7.13 -8.11 (m, 4H, Ar-H), 3.80 (s, 2H, CH_2), 3.60 (s, 3H, CH_3)
3	207.19	3032 (C-H aromatic), 2911 (C-H aliphatic), 1673 (C=O), 1530 (C-C of aromatic ring), 1512 (C-N-C), 1185 (C-N), 1070 (C-O-C), 1025 (N-N)	8.74 (s, 1H, NH exchangeable with D_2O), 8.19 (s, 2H, NH_2), 7.18-8.12 (m, 4H, Ar-H), 3.82 (s, 2H, CH_2)
4	295.29	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), 1071 (C-O-C), 1024 (N-N)	8.74 (s, 1H, NH exchangeable with D_2O), 8.50 (s, 1H, N=CH), 7.13-8.15 (m, 9H, CH-Ar), 3.84 (s, 2H, CH_2)
5	329.74	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), 760 (C-Cl)	8.71 (s, 1H, NH exchangeable with D_2O), 8.53 (s, 1H, N=CH), 7.11-8.14 (m, 8H, CH-Ar), 3.85 (s, 2H, CH_2)
6	374.19	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), 610 (C-Br)	8.73 (s, 1H, NH exchangeable with D_2O), 8.55 (s, 1H, N=CH), 7.14-8.16 (m, 8H, CH-Ar), 3.82 (s, 2H, CH_2)
7	340.29	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)	8.75 (s, 1H, NH exchangeable with D_2O), 8.54 (s, 1H, N=CH), 7.12-8.12 (m, 8H, CH-Ar), 3.86 (s, 2H, CH_2)
8	364.18	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), 763 (C-Cl)	8.72 (s, 1H, NH exchangeable with D_2O), 8.57 (s, 1H, N=CH), 7.14-8.15 (m, 7H, CH-Ar), 3.83 (s, 2H, CH_2)
9	369.39	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)	8.74 (s, 1H, NH exchangeable with D_2O), 8.55 (s, 1H, N=CH), 7.13-8.14 (m, 9H, CH-Ar), 3.85 (s, 2H, CH_2), 3.50 (s, 2H, CH_2 of thiazolidinone)
10	403.84	3030 (C-H aromatic), 2918 (C-H aliphatic), 1674 (C=O), 1615 (C=N), 1533 (C-C of aromatic ring), 1517 (C-N-C), 1188 (C-N), 1023 (N-N), 761 (C-Cl), 659 (C-S-C)	8.76 (s, 1H, NH exchangeable with D_2O), 8.58 (s, 1H, N=CH), 7.11-8.16 (m, 8H, CH-Ar), 3.87 (s, 2H, CH_2), 3.52 (s, 2H, CH_2 of thiazolidinone)
11	448.29	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), 654 (C-S-C), 613 (C-Br))	8.77 (s, 1H, NH exchangeable with D_2O), 8.56 (s, 1H, N=CH), 7.15-8.13 (m, 8H, CH-Ar), 3.83 (s, 2H, CH_2), 3.55 (s, 2H, CH_2 of thiazolidinone)
12	414.39	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), 659 (C-S-C)	8.73 (s, 1H, NH exchangeable with D_2O), 8.54 (s, 1H, N=CH), 7.17-8.16 (m, 8H, CH-Ar), 3.89 (s, 2H, CH_2), 3.53 (s, 2H, CH_2 of thiazolidinone)
13	438.28	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)	8.74 (s, 1H, NH exchangeable with D_2O), 8.55 (s, 1H, N=CH), 7.13-8.16 (m, 7H, CH-Ar), 3.85 (s, 2H, CH_2), 3.54 (s, 2H, CH_2 of thiazolidinone)
14	371.77	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)	8.76 (s, 1H, NH exchangeable with D_2O), 8.41 (s, 1H, CH-Ar), 7.13-8.14 (m, 9H, CH-Ar), 4.10 (s, 1H, CH-Cl), 3.55 (s, 2H, CH_2),
15	406.22	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),	8.77 (s, 1H, NH exchangeable with D_2O), 8.43 (s, 1H, CH-Ar), 7.15-8.16 (m, 9H, CH-Ar), 4.12 (s, 1H, CH-Cl),

		1027 (N-N) 715 (C-Cl), 650 (C-S-C)	3.53 (s, 2H, CH_2),
16	450.67	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), 614 (C-Br)	8.74 (s, 1H, NH exchangeable with D_2O), 8.42 (s, 1H, CH-Ar), 7.11-8.13 (m, 8H, CH-Ar), 4.14 (s, 1H, CH-Cl), 3.52 (s, 2H, CH_2),
17	416.77	3035 (C-H aromatic), 2916 (C-H aliphatic), 1673 (C=O), 1615 (C=N), 1532 (C-C of aromatic ring), 1515 (C-N-C), 1188 (C-N), 1026(N-N) 714 (C-Cl), 653 (C-S-C)	8.75 (s, 1H, NH exchangeable with D_2O), 8.45 (s, 1H, CH-Ar), 7.12-8.15 (m, 8H, CH-Ar), 4.16 (s, 1H, CH-Cl), 3.55 (s, 2H, CH_2),
18	440.66	3038 (C-H aromatic), 2913 (C-H aliphatic), 1675 (C=O), 1619 (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)	8.76 (s, 1H, NH exchangeable with D_2O), 8.41 (s, 1H, CH-Ar), 7.13-8.14 (m, 7H, CH-Ar), 4.10 (s, 1H, CH-Cl), 3.55 (s, 2H, CH_2),

TABLE3: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS 4-18

Comp. No.	R	Bacterial growth inhibition (diameter in mm)		Fungalgrowth inhibition (diameter in mm)		
		S. aureus	*E. coli*	*K. pneumoniea*	*C. albicans*	*A. niger*
4	H	7	-	9	8	6
5	4-Cl	10	12	-	10	-
6	4-Br	12	10	11	12	10
7	4-NO_2	-	13	15	13	12
8	2,6-Cl	15	17	14	15	-
9	H	16	-	19	14	16
10	4-Cl	18	16	18	21	19
11	4-Br	17	19	16	20	18
12	4-NO_2	-	20	19	-	20
13	2,6-Cl	24	25	22	32	24
14	H	19	20	-	27	-
15	4-Cl	20	21	20	29	19
16	4-Br	19	20	21	30	20
17	4-NO_2	22	21	20	28	25
18	2,6-Cl	25	24	23	30	27
Ciprofloxacin		20	22	21		
Fluconazole					29	22

CONCLUSION: From this study, we may conclude that Compounds (9-13) containing thiazolidinone ring and compounds (14-18) containing azetidinone ring exhibited better antibacterial as well as antifungal activity than compounds (4-8) having ring. 2, 6-diChlorophenyl substituted benzoxazolinone derivatives showed more efficiency due to presence of more electronegative atom.

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