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ANTIMICROBIAL ACTIVITY EVALUATION OF SOME (Z)-2-(2-OXO-1-((ARYLAMINO)METHYL)INDOLIN-3-YLIDENE)-N-(4-(2-OXO-2*H*-CHROMEN-3-YL)THIAZOL-2-YL)HYDRAZINE-1-CARBOXAMIDES

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Abstract:

*Fourteen(Z)-2-(2-oxo-1-((arylamino)methyl)indolin-3-ylidene)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2 yl)hydrazine-1-carboxamides **4a-4n** were prepared by treating (Z)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-2-(2-oxoindolin-3-ylidene)hydrazine-1-carboxamide **3** with the aryl amines and formaldehyde. The chemical structures of these compounds were elucidated by their physical constants, spectral data and elemental analysis. These compounds were investigated for their antimicrobial activity against five Gram-positive bacteria, namely, S. aureus, E. faecalis, S. epidermidis, B. subtilis and B. cereus; five Gram-negative bacteria, namely, E. coli, P. aeruginosa, K. pneumoniae, B. bronchiseptica and P. vulgaris; and five fungi, namely, C. albicans, A. niger, A. flavus, M. purpureous and P. citrinum by serial plate dilution method using standard drugs, ofloxacin and ketoconazole, respectively, and their minimum inhibitory concentrations (MICs) were also determined. These compounds showed mild to moderate antibacterial activity against Gram positive as well as Gram negative bacteria. However, they exhibited better antifungal activity. The compound **4j** (Ar = 1,2,4-Triazol-4-yl) has been identified as the most promising antifungal agent of this series. There is a possibility that the replacement of triazole ring by other azole ring .e.g. imidazole and 1,2,3-triazole; and / or the presence of halogen substituted coumarin ring may produce promising potent antimicrobial agents that are effective against Gram positive bacteria, Gram negative bacteria and fungi.*

Keywords: *Antibacterial activity, antifungal activity, coumarin, isatin, thiazole.*

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INTRODUCTION:

Currently antimicrobial resistance has become a global problem because in the modern era of travel and trade, resistant organisms rapidly cross the man-made boundaries through humans or the food chain[1,2]. According to the literature, the cause of antibiotic resistance includes the irrational use of antibiotics and failure to discover new antimicrobial agents since the late 1980s. Accordingly, there is a need to develop new antimicrobial agents to combat antimicrobial resistance problems [1,2,3. Coumarinyl thiazole derivatives and istain derivatives have an important place in medicinal chemistry. Coumarinyl thiazole derivatives have been reported to possess different biological activities like antimicrobial activity [4-11], anticancer activity [12], antioxidant activity [13], anticonvulsant activity [14,15], anti-tubercular activity [16,17], anti-inflammatory activity [18-21],analgesic activity[35], and anticholinesterase activity [22]. The isatin derivatives have also been reported to possess antimicrobial activity against a variety of bacteria, fungi and viruses [23-27]. Encouraged by these observations and in continuation of our search for the potent heterocyclic antimicrobial agents [28-32] , we decided to prepare some (Z)-2-(2-oxo-1-((arylamino)methyl)indolin-3-ylidene)-N-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazine-1-carboxamides **4a-4n** as potent antimicrobial agents.

MATERIAL AND METHODS:

Chemistry

Melting points were measured in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FT-IR spectrometer (Browser Morner, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 FT NMR (Bruker, Germany) spectrophotometer using TMS as internal reference (chemical shift in δ ppm). Mass spectra were recorded on a Jeol-JMS-D-300 mass spectrometer (70 eV) (Jeol, Japan). Satisfactory analysis for C, H, and N was obtained for the compounds within ± 0.4% of the theoretical values. The purity of the compounds was checked on silica gel G plates using iodine vapours as visualizing agent. The R_f value of the compounds was determined by using a mixture of toluene, ethyl acetate and formic acid (5:4:1). All the reagents used in the present work were of analytical grade. The synthetic pathway for the preparation of the compounds **4a-4n** is provided in Figure 1.

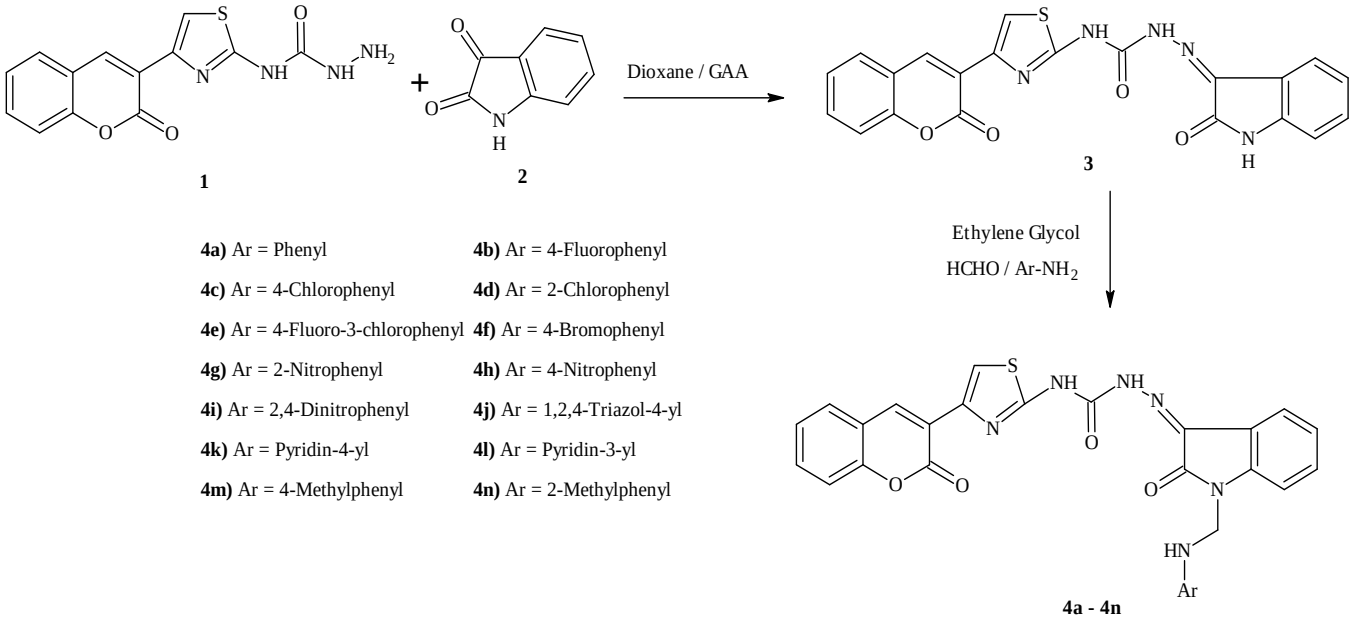

Figure 1. The synthetic pathway for the preparation of the compound**4a-4n**

Synthesis of N-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazinecarboxamide (1)
The compound **(1)** was prepared according to the method provided in the literature [14].

Synthesis of (Z)-N-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-2-(2-oxoindolin-3-ylidene)hydrazine-1-carboxamide (3)
A mixture of the compound **(1)** (0.01mole) and isatin **(2)** (0.01 mole) with 30 ml of dioxane was taken in a 100 mL round bottom flask. To this mixture 5 mL of glacial acetic acid was added. The resulting reaction mixture was refluxed for about 4 hours. The contents were poured on the crushed ice. The solid separated was filtered, dried and recrystallized from dioxane. The purity of the compounds was established on the basis of TLC.

General procedure for the preparation of (Z)-2-(2-oxo-1-((arylamino)methyl)indolin-3-ylidene)-N-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)hydrazine-1-carboxamides (4a-4n)
A mixture of the compound **(3)** (0.01 mole), appropriate aryl amine (0.01 mole) and formaldehyde (0.015 moles) with 30 ml of ethylene glycol was refluxed for 2 to 4 hours in a 100 mL round bottom flask. After completion of the reactions the contents were poured on the crushed ice. The solid separated was filtered, dried and recrystallized from dioxane. The purity of the compounds was established on the basis of TLC.

Antimicrobial Activity
The compounds **4a-4n** were tested for their *in vitro* antimicrobial activity by serial plate dilution method [33,34] (Cruickshank et. al., 1975; Beth et. al., 2000) against five Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633) and *Bacillus cereus* (ATCC 9946); five Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), *Bordetella bronchiseptica* (ATCC 4617) and *Proteus vulgaris* (ATCC 9920); and five fungi, *Candida albicans* (ATCC 2091), *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), *Monascus purpureous* (MTCC 369) and *Penicillium citrinum* (NCIM 768). Nutrient agar medium and Sabouraud dextrose medium were used for antibacterial activity and antifungal activity, respectively. The compounds were tested at concentrations of 200, 150, 100, 75, 50, 25 and 12.5 µg/mL. The reference or standard antibiotics, ofloxacin and ketoconazole were used at 50, 25 and 12.5 µg/mL concentrations for antibacterial activity and antifungal activity, respectively. Sterile dimethyl sulfoxide (DMSO) was used for the preparation of desired concentrations of the synthesized compounds and standard antibiotics. Sterile dimethyl sulfoxide without the synthesized compounds and standard antibiotics served as control group. The minimum inhibitory concentrations (MICs) values of the synthesized compounds, ofloxacin and ketoconazole were also determined. The minimum inhibitory concentration (MIC) has been defined as the lowest concentration of a compound that inhibited the visible growth of microorganisms on the plate.

Statistical Analysis
All data (n = 6) is presented as Mean ± Standard Error Mean (SEM). The data was analyzed by One-Way Analysis of Variance (ANOVA) with Dunnett's Multiple Comparison Test with respect to control group and standard group using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The results were considered significantly different at $p < 0.05$ as compared with control group as well as standard drug groups.

RESULTS:

Chemistry
The title compounds **4a-4n** were prepared according to the method outlined in Figure 1. The compound **1** was prepared according to the method provided in the literature [14]. The compound **1** was reacted with isatin **2** in the presence of dioxane and glacial acetic acid to provide the compound **3**. The compound **3** was further treated with appropriate aryl amine and formaldehyde in the presence of ethylene glycol to obtain the desired compounds **4a-4n**. The physical constant data of the compounds **4a-4n** are provided in Table 1.

Table 1: The physical constant data of the compounds 4a-4n

Compd. No.	Ar	Molecular Formula	M.P. (±2°C)	Yield (%)	R_f Value
4a	Phenyl	$C_{28}H_{20}N_6O_4S$	223-225	45	0.58
4b	4-Fluorophenyl	$C_{28}H_{19}FN_6O_4S$	250-252	40	0.56
4c	4-Chlorophenyl	$C_{28}H_{19}ClN_6O_4S$	221-223	50	0.59
4d	2-Chlorophenyl	$C_{28}H_{19}ClN_6O_4S$	214-216	55	0.59
4e	4-Fluoro-3-chlorophenyl	$C_{28}H_{18}ClFN_6O_4S$	227-229	55	0.57
4f	4-Bromophenyl	$C_{28}H_{19}BrN_6O_4S$	265-267	50	0.59
4g	2-Nitrophenyl	$C_{28}H_{19}N_7O_6S$	257-259	55	0.63
4h	4-Nitrophenyl	$C_{28}H_{19}N_7O_6S$	251-253	60	0.62
4i	2,4-Dinitrophenyl	$C_{28}H_{18}N_8O_8S$	217-219	55	0.64
4j	1,2,4-Triazol-4-yl	$C_{24}H_{17}N_9O_4S$	248-250	55	0.66
4k	Pyridin-4-yl	$C_{27}H_{19}N_7O_4S$	212-214	55	0.64
4l	Pyridin-3-yl	$C_{27}H_{19}N_7O_4S$	220-222	55	0.63
4m	4-Methylphenyl	$C_{29}H_{22}N_6O_4S$	248-250	60	0.59

The FTIR, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis data of representative compounds is provided below.

N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)hydrazinecarboxamide (1)

M.P. 171-173 °C; R_f Value: 0.74; Yield: 55%; IR (KBr) cm^{-1}: 1675 and 1710 (C=O), 3420, 3335 and 3251 (N-H), 1620 (C=N), 1555 (C=C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 4.30 (bs, 2H, -NH_2, exchangeable with D_2O), 7.35-7.75 (m, 4H, Ar-H), 8.05 (s, 1H, H-4 of Coumarin ring). 8.55 (s, 1H, -NH-NH_2, exchangeable with D_2O), 8.90 (s, 1H, H-5 of thiazole ring), 9.99 (s, 1H, -NH-CO-, exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 111.3, 114.1, 118.7, 123.2, 125.7, 127.1, 127.2, 142.7, 144.1, 151.0, 151.6, 160.1, 160.7.

(Z)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-2-(2-oxoindolin-3-ylidene)hydrazine-1-carboxamide (3)

M.P. 215-217 °C; R_f Value: 0.66; Yield: 65%; IR (KBr) cm^{-1}: 1660, 1685 and 1710 (C=O), 3340, 3310 and 3265 (N-H), 1615 (C=N), 1565 (C=C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 7.38-7.85 (m, 8H, Ar-H), 8.08 (s, 1H, H-4 of Coumarin ring), 8.90 (s, 1H, H-5 of thiazole ring), 9.45 (s, 1H, -NH-CO-NH-N=, exchangeable with D_2O), 9.45 (s, 1H, -CO-NH-N=, exchangeable with D_2O), 9.99 (s, 1H, -NH- of isatin moiety); ^{13}C-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 111.3, 114.0, 115.5, 117.2, 118.7, 122.2, 123.2, 125.5, 126.1, 127.1, 127.2, 130.2, 132.5, 140.1, 142.7, 144.1, 150.0, 151.0, 160.1, 160.7, 165.5.

(Z)-2-(1-(((4H-1,2,4-triazol-4-yl)amino)methyl)-2-oxoindolin-3-ylidene)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)hydrazine-1-carboxamide (4j)

IR (KBr) cm^{-1}: 1665, 1680 and 1715 (C=O), 3335, 32900 and 3260 (N-H), 1605, 1615 and 1620 (C=N), 1560 (C=C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 4.44 (bs, 2H, -CH_2-NH-), 6.03 (s, 1H, -CH_2-NH-, exchangeable with D_2O), 7.35-7.88 (m, 8H, Ar-H), 8.08 (s, 1H, H-4 of Coumarin ring), 8.25 (s, 2H, H-3 and H5 of triazole), 9.49 (s, 1H, -NH-CO-NH-N=, exchangeable with D_2O), 9.65 (s, 1H, -CO-NH-N=, exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 62.1, 113.1, 114.5, 115.1, 116.4, 120.1, 123.3, 123.4, 125.5, 126.1, 127.4 (2C), 130.1 (2C), 142.5, 143.7 (2C), 144.0, 145.4, 151.2, 151.8, 155.9, 160.2, 161.1; Mass (m/z): 527 (M^+, 100%); Elemental Analysis ($C_{24}H_{17}N_9O_4S$), Found % (Calculated %): C, 54.55 (54.65); H, 3.19 (3.25); N, 23.86 (23.90).

Antimicrobial activity

The antimicrobial activity data of the compounds **4a-4n** at different concentrations against Gram positive bacteria, Gram negative bacteria and fungi obtained by the serial plate dilution method is provided in Table 2, Table 3 and Table 4, respectively. The zone of inhibition produced by the MIC of standard drugs, ofloxacin and ketoconazole, has been considered as 100 % for comparing the antibacterial activity and antifungal activity data of the compounds **4a-4n** respectively.

The antibacterial activity of ofloxacin against Gram positive bacteria revealed that it has a MIC value of 25 µg/mL against *S. aureus*, *E. faecalis* and *S. epidermidis*; and it has a MIC value of 12.5 µg/mL against *B. subtilis* and *B. cereus*. The antibacterial

activity of the compounds **4a-4n** against Gram positive bacteria revealed that the compound **4b** had the highest activity of 61.19% (MIC = 75 µg/mL; $p < 0.05$) against *S. aureus*; 53.47% (MIC = 75 µg/mL; $p < 0.0001$) against *E. faecalis*; 63.48% (MIC = 75 µg/mL; $p < 0.0001$) against *S. epidermidis*; 48.48% (MIC = 75 µg/mL; $p < 0.0001$) against *B. subtilis*; and 61.08% (MIC = 75 µg/mL; $p < 0.0001$) against *B. cereus*.

The antibacterial activity of ofloxacin against Gram negative bacteria revealed that it has a MIC value of 12.5 µg/mL against *E. coli*; *P. aeruginosa K. pneumonia and P. vulgaris*; and it has a MIC value of 25 µg/mL against *B. bronchiseptica.* The antibacterial activity of the compounds **4a-4n** against Gram negative bacteria revealed that the compound **4b** also had the highest activity of 68.45% (MIC = 50 µg/mL; $p < 0.0001$) against *E. coli*; 68.22% (MIC = 50 µg/mL; $p < 0.0001$) against *P. aeruginosa*; 81.94% (MIC = 50 µg/mL; $p < 0.0001$) against *K. pneumonia*; 70.57% (MIC = 50 µg/mL; $p < 0.0001$) against *B. bronchiseptica*; and 76.44% (MIC = 50 µg/mL; $p < 0.0001$) against *P. vulgaris*.

The antifungal activity of ketoconazole against fungi revealed that it has a MIC value of 12.5 µg/mL against *C. albicans, A. niger and M. purpureous;* and it has a MIC value of 25 µg/mL against *A. flavus and P. citrinum.* The antifungal activity of the compounds **4a-4n** against fungi revealed that the compound **4j** had the antifungal activity of 94.92% (MIC = 50 µg/mL; $p < 0.0001$) against *C. albicans*; 110.47% (MIC = 50 µg/mL; $p < 0.05$) against *A. niger*; 107.12% (MIC = 50 µg/mL; $p < 0.0001$) against *A. flavus*; 104.94% (MIC = 50 µg/mL; $p < 0.0001$) against *M. purpureous*; and 110.27% (MIC = 50 µg/mL; $p < 0.05$) against *P. citrinum*. The compound **4b** exhibited activity of 107.57% (MIC = 75 µg/mL; $p < 0.0001$) against *A. niger*; 105.58% (MIC = 75 µg/mL; $p < 0.0001$) against *A. flavus*; and 107.82% (MIC = 100 µg/mL; $p < 0.0001$) against *P. citrinum*. The compound **4c** displayed activity of 100.19% (MIC = 100 µg/mL; $p < 0.05$) against *A. flavus*; and 103.45% (MIC = 100 µg/mL; $p < 0.0001$) against *P. citrinum*. The compound **4f** also produced antifungal activity of 102.77% (MIC = 100 µg/mL; $p < 0.05$) against *A. flavus*; and 105.60% (MIC = 100 µg/mL; $p < 0.0001$) against *P. citrinum*. The compounds **4k** and **4m** also produced antifungal activity of 102.31% (MIC = 100 µg/mL; $p < 0.0001$) and 101.81% (MIC = 75 µg/mL; $p < 0.0001$) against *P. citrinum*, respectively.

Table 2: Antibacterial activity data of the targeted compounds 4a-4n against Gram positive bacteria

Compound	Zone of inhibition in mm and MIC (Minimum Inhibitory Concentration) in µg/mL				
	S. aureus	***E. faecalis***	***S. epidermidis***	***B. subtilis***	***B. cereus***
4a	9.18±0.20[a] (100)	10.58±0.30[a] (75)	10.54±0.41[a] (75)	9.24±0.26[a] (75)	12.40±0.25[a] (75)
4b	18.72±0.37[c] (75)	18.25±0.31[a] (75)	20.97±0.25[a] (75)	18.52±0.27[a] (75)	21.55±0.27[a] (75)
4c	18.50±0.22[a] (75)	17.85±0.25[a] (75)	20.94±0.22[a] (100)	18.42±0.35[a] (75)	20.92±0.36[a] (75)
4d	13.75±0.30[a] (75)	14.39±0.33[a] (75)	15.06±0.17[a] (75)	13.53±0.32[a] (100)	17.51±0.10[b] (75)
4e	14.57±0.27[a] (75)	15.10±0.24[a] (75)	16.26±0.30[a] (75)	14.75±0.25[a] (100)	17.77±0.29[a] (75)
4f	17.30±0.21[a] (75)	16.27±0.35[a] (75)	18.33±0.23[a] (75)	15.32±0.30[a] (50)	20.39±0.19[a] (75)
4g	10.51±0.23[a] (75)	11.62±0.41[a] (75)	13.18±0.33[a] (75)	10.62±0.32[a] (75)	15.04±0.18[a] (75)
4h	9.48±0.28[a] (75)	10.89±0.23[a] (100)	12.23±0.27[a] (100)	10.58±0.26[c] (100)	14.92±0.28[a] (75)
4i	11.36±0.24[c] (75)	11.68±0.28[a] (75)	13.22±0.22[c] (100)	11.11±0.23[a] (75)	15.84±0.26[a] (75)
4j	15.57±0.38[a] (100)	15.22±0.13[a] (75)	16.74±0.27[a] (100)	14.75±0.35[a] (75)	18.32±0.10[a] (100)
4k	12.81±0.31[a] (75)	12.98±0.14[a] (75)	14.43±0.25[a] (75)	13.24±0.21[a] (75)	16.71±0.10[a] (75)
4l	14.36±0.14[a] (75)	14.79±0.22[a] (100)	16.17±0.33[a] (75)	14.45±0.25[a] (75)	17.68±0.24[a] (75)
4m	13.30±0.40[a] (100)	14.18±0.23[a] (75)	14.66±0.22[a] (75)	13.44±0.31[a] (75)	17.00±0.36[a] (75)
4n	8.32±0.37[a] (75)	10.19±0.32[a] (75)	10.14±0.45[a] (50)	7.45±0.36[a] (75)	12.38±0.21[a] (50)
Ofloxacin	30.59±0.50[a] (25)	34.13±0.51[a] (25)	33.03±0.49[a] (25)	38.20±0.49[a] (12.5)	35.28±0.33[a] (12.5)
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The values in brackets represent the MIC in µg/mL of the corresponding compounds; a = p <0.0001 as compared to control and/or standard; b = p <0.0001 as compared to control and p <0.001 as compared to standard; c = p <0.0001 as compared to control and p <0.05 as compared to standard; d = p <0.0001 as compared to control and p > 0.05 as compared to standard.

Table 3: Antibacterial activity data of the targeted compounds 4a-4n against Gram negative bacteria

Compound	Zone of inhibition in mm and MIC (Minimum Inhibitory Concentration) in µg/mL				
	E. coli	*P. aeruginosa*	*K. pneumonia*	*B. bronchiseptica*	*P. vulgaris*
4a	7.47±0.40^{c} (75)	9.63±0.37^{c} (75)	11.03±0.37^{a} (75)	17.75±0.37^{b} (75)	13.32±0.24^{c} (75)
4b	22.72±0.42^{a} (50)	24.52±0.31^{a} (50)	27.14±0.24^{a}(50)	24.80±0.28^{a} (50)	24.31±0.24^{a} (50)
4c	21.44±0.35^{b} (75)	23.94±0.37^{a} (50)	25.90±0.38^{a} (50)	18.00±0.30^{a} (75)	21.44±0.31^{a} (75)
4d	16.41±0.42^{a} (75)	19.91±0.34^{a} (75)	12.16±0.40^{a} (50)	13.56±0.33^{a} (75)	21.07±0.34^{a} (50)
4e	16.20±0.36^{a} (50)	18.93±0.36^{a} (50)	16.64±0.46^{a} (50)	19.55±0.25^{a} (50)	23.50±0.32^{a} (50)
4f	21.23±0.29^{a} (50)	23.56±0.24^{b} (50)	20.19±0.23^{a} (50)	19.93±0.37^{b} (50)	19.28±0.35^{a} (50)
4g	12.28±0.39^{a} (75)	13.76±0.41^{a} (50)	19.88±0.28^{a} (50)	11.74±0.37^{a} (50)	14.01±0.24^{a} (50)
4h	13.81±0.25^{a} (50)	15.12±0.34^{a} (75)	11.44±0.34^{a} (75)	17.95±0.33^{a} (50)	12.07±0.31^{a} (75)
4i	16.40±0.29^{a} (50)	19.53±0.24^{a} (75)	21.32±0.42^{a} (75)	23.11±0.37^{a} (50)	12.30±0.26^{a} (75)
4j	16.49±0.34^{a} (75)	20.92±0.26^{a} (50)	20.08±0.29^{a} (50)	18.12±0.12^{a} (75)	21.25±0.35^{a} (50)
4k	9.69±0.24^{c} (50)	11.79±0.31^{a} (75)	19.06±0.40^{a} (50)	16.72±0.37^{a} (50)	8.45±0.36^{a} (75)
4l	20.09±0.34^{a} (50)	21.41±0.37^{a} (75)	16.65±0.27^{a} (50)	24.15±0.16^{a} (75)	11.41±0.29^{b} (75)
4m	16.43±0.45^{a} (50)	20.26±0.30^{a} (75)	21.07±0.29^{a} (50)	23.42±0.36^{a} (50)	18.38±0.31^{a} (50)
4n	15.93±0.46^{b} (75)	18.14±0.39^{a} (75)	22.19±0.37^{a} (50)	12.09±0.49^{a} (50)	12.35±0.32^{a} (75)
Ofloxacin	33.19±0.43^{a} (12.5)	35.94±0.14^{a} (12.5)	33.12±0.19^{a} (12.5)	35.14±0.24^{a} (25)	31.80±0.42^{a} (12.5)
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The values in brackets represent the MIC in µg/mL of the corresponding compounds; a = p <0.0001 as compared to control and/or standard; b = p <0.0001 as compared to control and p <0.001 as compared to standard; c = p <0.0001 as compared to control and p <0.05 as compared to standard; d = p <0.0001 as compared to control and p > 0.05 as compared to standard.

Table 4: Antifungal activity data of the targeted compounds 4a-4n against fungi

Compound	Zone of inhibition in mm and MIC (Minimum Inhibitory Concentration) in µg/mL				
	C. albicans	*A. niger*	*A. flavus*	*M. purpureous*	*P. citrinum*
4a	15.63±0.07^{c} (150)	18.31±0.32^{b} (75)	18.54±0.36^{a} (50)	19.53±0.33^{a} (75)	20.83±0.37^{a} (75)
4b	31.32±0.57^{a} (75)	33.79±0.50^{a} (75)	33.08±0.38^{a} (75)	32.00±0.46^{a} (100)	32.10±0.43^{b} (100)
4c	24.63±0.56^{a} (75)	25.82±0.37^{b} (75)	31.39±0.37^{c} (100)	25.63±0.55^{a} (75)	30.80±0.50^{a} (100)
4d	30.74±0.46^{a} (75)	31.14±0.51^{a} (75)	23.61±0.49^{a} (75)	31.62±0.57^{a} (75)	26.82±0.58^{a} (100)
4e	23.24±0.43^{a} (100)	25.21±0.45^{a} (75)	22.71±0.45^{b} (75)	24.10±0.45^{a} (75)	25.53±0.35^{a} (75)
4f	24.01±0.42^{a} (75)	25.39±0.55^{a} (75)	32.20±0.58^{c} (100)	24.82±0.42^{a} (75)	31.44±0.36^{a} (100)
4g	26.29±0.66^{a} (75)	26.04±0.51^{a} (100)	23.79±0.43^{a} (100)	25.78±0.55^{a} (75)	26.89±0.54^{a} (100)
4h	31.16±0.43^{a} (75)	31.35±0.51^{a} (75)	23.29±0.12^{a} (150)	31.69±0.32^{a} (75)	26.23±0.44^{a} (75)
4i	16.89±0.61^{c} (100)	18.61±0.43^{a} (75)	19.18±0.30^{a} (75)	19.68±0.49^{c} (75)	22.00±0.54^{a} (75)
4j	32.16±0.46^{a} (50)	34.70±0.34^{c} (50)	33.56±0.43^{a} (50)	35.21±0.37^{a} (50)	32.83±0.79^{c} (50)
4k	29.48±0.43^{a} (75)	29.72±0.38^{a} (75)	31.05±0.32^{b} (75)	29.30±0.54^{a} (75)	30.46±0.58^{a} (100)
4l	21.93±0.48^{a} (100)	24.14±0.63^{a} (75)	22.56±0.53^{a} (75)	23.89±0.49^{a} (75)	25.45±0.44^{a} (75)
4m	28.96±0.49^{a} (100)	29.18±0.54^{a} (75)	30.0±0.44^{a} (100)	28.81±0.54^{a} (100)	30.31±0.31^{a} (75)
4n	17.97±0.36^{a} (150)	21.72±0.71^{d} (100)	19.34±0.45^{a} (100)	22.08±0.45^{a} (75)	23.25±0.55^{a} (75)
Ketoconazole	33.88±0.41^{a} (12.5)	31.41±0.55^{a} (12.5)	31.33±0.23^{a} (25)	33.55±0.33^{a} (12.5)	29.77±0.44^{a} (25)
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The values in brackets represent the MIC in µg/mL of the corresponding compounds; a = p <0.0001 as compared to control and/or standard; b = p <0.0001 as compared to control and p <0.001 as compared to standard; c = p <0.0001 as compared to control and p <0.05 as compared to standard; d = p <0.0001 as compared to control and p > 0.05 as compared to standard.

DISCUSSION:

A total of fourteen compounds **4a-4n** were synthesized. The structure of the representative compounds were confirmed on the basis of their FTIR, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis data. The compounds **4a-4n** were tested for their *in vitro* antimicrobial activity by the serial plate dilution method [33,34],against five Gram positive bacteria; five Gram-negative bacteria; and five fungi. These compounds showed mild to moderate antibacterial activity against Gram positive as well as Gram negative bacteria. It is also evident from the antimicrobial activity data mentioned in Table 2, Table 3, and Table 4 that the title compounds are better antifungal agents than antibacterial agents. The compound **4j** (Ar = 1,2,4-Triazol-4-yl) has been identified as the most promising antifungal agent. The **4b, 4c, 4f, 4k** and **4m** also produced comparable antifungal activity with respect to the standard drug Ketoconazole, especially against *P. Citrinum*. It is believed that the synthesized compounds might be inhibiting the growth of fungi by same mechanism as other antifungal agents [36]. The structure activity relationship study of the compounds **4a-4n** revealed that the presence of a 1,2,4-triazole ring in the structure of these compounds provide broad spectrum antifungal compounds and the presence of halogen group in the phenyl ring also provide promising antifungal agents.

CONCLUSION:

It is evident from the antimicrobial activity data of the compounds **4a-4n** that these compounds are better antifungal agents than antibacterial agents wherein the compound **4j** (Ar = 1,2,4-Triazol-4-yl) is the most promising antifungal agent of this series of compounds. However, it produced a promising effect at higher MIC value and therefore it is still considered to be less potent than the standard drug ketoconazole. There is a possibility that the replacement of the triazole ring by other azole ring .e.g. imidazole and 1,2,3-triazole; and / or the presence of halogen substituted coumarin ring may produce promising potent antimicrobial agents that are effective against Gram positive bacteria, Gram negative bacteria and fungi. Accordingly, this study may be extended to acquire more information about the structure activity relationships of this series of compounds.

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CONFLICT OF INTEREST:

The author declares that no conflict of interest is associated with this work.

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