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Original Research Article

Synthesis and Evaluation of Antimicrobial Activity of Some 2-Morpholinomethylamino-4-(7-Unsubstituted/Substituted Coumarin-3-yl)-6-Chlorosubstitutedphenyl Pyrimidines

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Abstract

Purpose: *To prepare some 2-morpholinomethylamino-4-(7-unsubstituted/substitutedcoumarin-3-yl)-6-chlorosubstitutedphenyl pyrimidines as antimicrobial agents.*

Methods: *Some 2-morpholinomethylamino-4-(7-unsubstituted/substitutedcoumarin-3-yl)-6-chloro-substitutedphenyl pyrimidines were prepared by reacting 2-amino-4-(7-substituted/unsubstituted coumarin-3-yl)-6-(chlorosubstitutedphenyl) pyrimidines with morpholine and formaldehyde. The chemical structures of the synthesized compounds were elucidated by their Fourier Transform infra-red (FTIR), ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and mass spectra, as well as by elemental analysis. These compounds were investigated for their antimicrobial activity against ten bacteria and five fungi by serial plate dilution method using the standard drugs, ofloxacin and ketoconazole, respectively, and their minimum inhibitory concentrations (MICs) were determined.*

Results: *A total of eighteen new compounds (1a-18a) were synthesized. Compound 3a (MIC = 75 µg/mL; $p < 0.0001$) and 15a (MIC = 125 µg/mL; $p < 0.001$) produced stronger antifungal activity than the standard drug, ketoconazole (MIC = 25 µg/mL; $p < 0.0001$) against P. citrinum. Compound 4a displayed higher but moderate activity against Gram-positive bacterium, S. aureus (MIC = 100 µg/mL; $p < 0.05$) than the standard drug, ofloxacin (MIC = 25 µg/mL; $p < 0.0001$). Compound 4a also displayed higher but moderate activity against the Gram-negative bacterium, E. coli (MIC = 75 µg/mL; $p < 0.0001$) than the standard drug, ofloxacin (MIC = 12.5 µg/mL; $p < 0.0001$). The structure activity relationship analysis revealed that the chloro- substitution at position 2 of the phenyl ring along with a chlorobromo-substituted coumarin moiety of the synthesized compounds is critical for activity against Gram-positive bacteria, Gram negative bacteria and fungi.*

Conclusion: *The synthesized compounds are relatively active antifungal agents but are weak antibacterial agents. However, they require further evaluation of their antifungal activity against other fungal strains to ascertain their broad spectrum activity.*

Keywords: *Pyrimidine, Coumarin, Morpholine, Antibacterial, Antifungal, Structure-activity relationship*

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INTRODUCTION

Microbial infections have been creating problems for mankind since centuries and scientists have also developed a large number of antimicrobial agents for the treatment of these infections. According to one new report, about 40 new microbial diseases have been identified since 1970s and more than 2 million Americans are suffering from antibiotic resistance, of which

about 23,000 die each year [1]. Because of the development of antibiotic resistance and emergence of new microbial diseases, there is a need to develop new antimicrobial agents for the treatment of microbial infections.

Pyrimidine derivatives have an important place in medicinal chemistry as these are associated with a broad range of biological activities [2-4] including antioxidant activity [5-7] and antimicrobial activity [8-13]. The clinical importance of pyrimidine nucleus is also evident by the marketing of clinically used pyrimidine derivatives as well as fused pyrimidine derivatives, for example, as antineoplastic agent (Tegafur), as vasodilator (Dipyridamole), as expectorant (Tasuldine) and as antibacterial agent (Trimethoprim, Piromidic Acid, Tetroxoprim, Metioprim), as antifungal agent (Flucytosine), and as antiviral agent (Broxuridine, Idoxuridine) [14]. Recently, the significance and biological importance of pyrimidine derivatives including their clinical applications in the microbial world has been reviewed [15]. The antimicrobial activity of pyrimidine derivatives against broad range of microbes makes it an important skeleton in medicinal chemistry and drug development against microbes. Morpholine nucleus is an integral part of linezolid, a clinically used drug for the treatment of infections caused by gram-positive bacteria [16]. A number of morpholine containing chemical compounds have also been reported as antimicrobial agents [17-22]. Encouraged by these observations and also in continuation of our search for potent antimicrobial agents [23,24] including antimicrobial agents having coumarin moiety [25,26], we decided to prepare some 2-morpholinomethylamino-4-(7-unsubstituted/substitutedcoumarin-3-yl)-6-chlorosubstitutedphenyl pyrimidines, herein after the title compounds (1a-18a), as antimicrobial agents.

EXPERIMENTAL

General

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) and ^{1}H-NMR spectra 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. Purity of the compounds was checked on silica gel G plates using iodine vapours as visualizing agent. R_f value of the compounds was determined by using a mixture of benzene and acetone (9:1). All reagents used in the present work were of analytical grade. The synthetic pathway for the preparation of the title compounds (**1a-18a**) is provided in Fig. 1.

The 2-amino-4-(7-substituted/unsubstituted coumarin-3-yl)-6-(chlorosubstitutedphenyl) pyrimidines (**1-18**) prepared according to our previous report [25] were reacted with morpholine and formaldehyde in absolute ethanol to provide the title compounds (**1a-18a**).

General method for the synthesis (1a-18a)

A mixture of 2-amino-4-(7-unsubstituted/ substituted coumarin-3-yl)-6-chlorosubstituted phenyl pyrimidines (0.01 mole), morpholine (0.01 mole) and formaldehyde (0.015 moles) was refluxed in absolute ethanol for 6 to 10 h. The reaction mixture was reduced to half of its volume and poured on crushed ice. The solid separated was filtered, washed with water repeatedly, dried and recrystallized from ethanol.

Evaluation of antimicrobial activity

The title compounds (**1a-18a**) were tested for their *in vitro* antimicrobial activity by serial plate dilution method [27,28] against Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633) and *Bacillus cereus* (ATCC 9946); Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), *Bordetella bronchiseptica* (ATCC 4617) and *Proteus vulgaris* (ATCC 9920); fungi, *Candida albicans* (ATCC 2091), *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), *Monascus purpureous* (MTCC 369) and *Penicillium citrinum* (NCIM 768).

The microorganisms were obtained from the Department of Microbiology, Majeedia Hospital, New Delhi, India. The Department of Microbiology of Majeedia Hospital obtained some of these microorganisms from the Institute of Genomics and Integrative Biology, New Delhi, India.

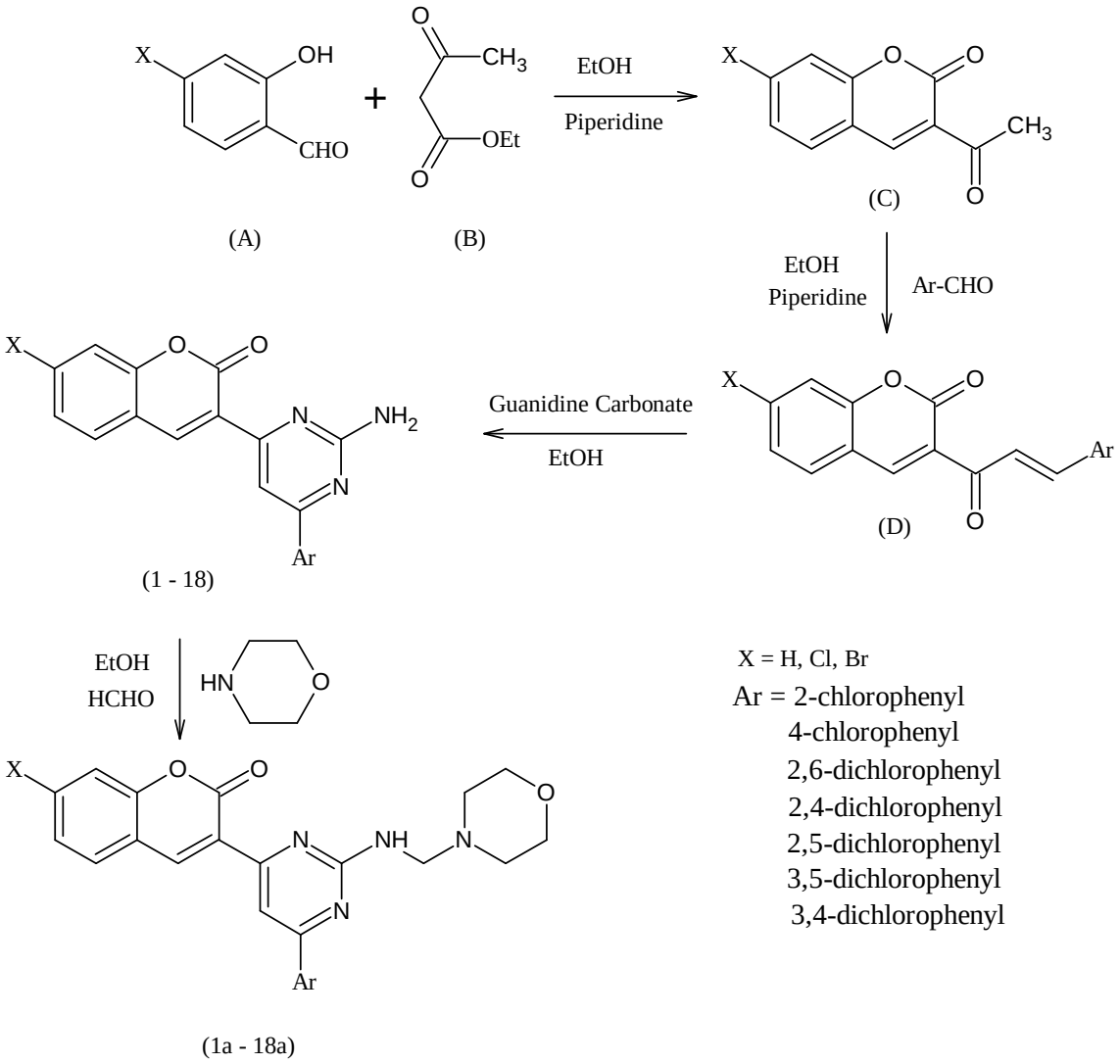

Fig. 1. Synthesis of the title compounds **(1a-18a)**

Nutrient agar medium and Sabouraud dextrose medium were used for antibacterial activity and antifungal activity, respectively. The compounds were tested at concentrations of 200, 175, 150, 125, 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 visible growth of microorganisms on the plate.

Statistical analysis

All antimicrobial activity data are presented as mean ± SEM (n = 6). The data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison Test with respect to control group and standard groups using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). The results were considered significantly different at $p < 0.05$.

RESULTS

The title compounds (**1a-18a**) were prepared according to the method outlined in Fig 1. The characterization data of the intermediates, (C), (D) and (**1-18**) of the Fig. 1 were in line with our previously published data [25,26]. The structures of the title compounds (**1a-18a**) were confirmed on the basis of their IR, ^{1}H-NMR, Mass and elemental analysis data. The appearance of the

IR absorption peaks from 3290 cm^{-1} to 3280 cm^{-1} confirmed the stretching vibration of N-H group of –NH-CH_2- moiety; from 1710 cm^{-1} to 1705 cm^{-1} confirmed the stretching vibration of C=O group of the coumarin moiety; from 1611 cm^{-1} to 1604 cm^{-1} confirmed the stretching vibration of C=N group of the pyrimidine ring; from 1545 cm^{-1} to 1540 cm^{-1} confirmed the stretching vibration of C=C group of aromatic C=C bond; and from 1135 cm^{-1} to 1130 cm^{-1} confirmed the stretching vibration of C-O-C group of coumarin moiety and morpholine moieties present in the title compounds (**1a-18a**). The appearance of the signals in the ^{1}H-NMR spectra of the title compounds (**1a-18a**) at δ (ppm) values from 2.63 to 2.72 confirmed four protons of –CH_2-N-CH_2- portion of the morpholine moiety; from 3.50 to 3.58 confirmed four protons of –CH_2-O-CH_2- portion of the morpholin moiety; from 4.27 to 4.42 confirmed two methylene protons of –NH-CH_2- moiety; from 6.90 to 7.75 confirmed the number of aromatic protons; and from 7.80 to 789 confirmed the secondary amino group (exchangeable with D_2O) of –NH-CH_2- moiety of the title compounds (**1a-18a**). The elemental analysis and molecular ion peaks of the title compounds (**1a-18a**) were also consistent with the assigned structures. The detailed physical constants, FTIR, ^{1}H-NMR, mass and elemental analysis data of the title compounds (**1a-18a**) are presented as follows.

2-(Morpholinomethylamino)-4-(coumarin-3-yl)-6-(4-chlorophenyl) pyrimidine (1a)

Yield: 55 %; m.p.: 147-149 ^{o}C; R_f: 0.76; IR (KBr) cm^{-1}: 3289 (N-H), 1706 (C=O), 1607 (C=N), 1544 (C=C), 1131 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.66 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.52 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.27 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.91-7.68 (m, 10H, Ar-H), 7.86 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{21}N_4O_3Cl$), Found% (Calculated%): C, 64.20 (64.21); H, 4.72 (4.72); N, 12.47 (12.48); Mass (m/z): 448 (M^+, $C_{24}H_{21}N_4O_3Cl$), 449 (M^++1), 164 (100%, C_9H_7NCl).

2-(Morpholinomethylamino)-4-(coumarin-3-yl)-6-(2,6-dichlorophenyl)pyrimidine (2a)

Yield: 55%; m.p.: 140-142 ^{o}C; R_f: 0.74; IR (KBr) cm^{-1}: 3287 (N-H), 1707 (C=O), 1611 (C=N), 1543 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.69 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.57 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.32 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.93-7.73 (m, 9H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{20}N_4O_3Cl_2$), Found% (Calculated%): C, 59.62 (59.64); H, 4.17 (4.17); N, 11.58 (11.59).

2-(Morpholinomethylamino)-4-(coumarin-3-yl)-6-(2,4-dichlorophenyl)pyrimidine (3a)

Yield: 50 %; m.p.: 155-157 ^{o}C; R_f: 0.71; IR (KBr) cm^{-1}: 3287 (N-H), 1706 (C=O), 1607 (C=N), 1542 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.66 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.50 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.02-7.75 (m, 9H, Ar-H), 7.84 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{20}N_4O_3Cl_2$), Found% (Calculated%): C, 59.63 (59.64); H, 4.17 (4.17); N, 11.57 (11.59); Mass (m/z): 483 (M^+, $C_{24}H_{20}N_4O_3Cl_2$), 484 (M^++1), 199 (100%, $C_9H_6NCl_2$).

2-(Morpholinomethylamino)-4-(coumarin-3-yl)-6-(2-chlorophenyl)pyrimidine (4a)

Yield: 55 %; m.p.: 147-149 ^{o}C; R_f: 0.77; IR (KBr) cm^{-1}: 3286 (N-H), 1706 (C=O), 1605 (C=N), 1545 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.68 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.55 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.02-7.74 (m, 10H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{21}N_4O_3Cl$), Found% (Calculated%): C, 64.20 (64.21); H, 4.71 (4.72); N, 12.47 (12.48).

2-(Morpholinomethylamino)-4-(7-chlorocoumarin-3-yl)-6-(4-chlorophenyl)pyrimidine (5a)

Yield: 55 %; m.p.: 172-174 ^{o}C; R_f: 0.69; IR (KBr) cm^{-1}: 3280 (N-H), 1705 (C=O), 1607 (C=N), 1544 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.69 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.53 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.90-7.71 (m, 9H, Ar-H), 7.85 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{20}N_4O_3Cl_2$), Found% (Calculated%): C, 59.62 (59.64); H, 4.16 (4.17); N, 11.57 (11.59); Mass (m/z): 483 (M^+, $C_{24}H_{20}N_4O_3Cl_2$), 484 (M^++1), 164 (100%, C_9H_7NCl).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(4-chlorophenyl)pyrimidine (6a)

Yield: 60 %; m.p.: 177-179 ^{o}C; R_f: 0.66; IR (KBr) cm^{-1}: 3287 (N-H), 1710 (C=O), 1608 (C=N), 1542 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.70 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.54 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.39 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.90-7.69 (m, 9H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{20}N_4O_3ClBr$),

Found % (Calculated%): C, 54.60 (54.62); H, 3.82 (3.82); N, 10.60 (10.62).

2-(Morpholinomethylamino)-4-(7-chlorocoumrin-3-yl)-6-(2,6-dichlorophenyl)pyrimidine (7a)

Yield: 50 %; m.p.: 185-187 ^{o}C; R_f: 0.69; IR (KBr) cm^{-1}: 3290 (N-H), 1708 (C=O), 1608 (C=N), 1544 (C=C), 1131 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.71 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.53 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.42 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.03-7.73 (m, 8H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_3$), Found % (Calculated%): C, 55.66 (55.67); H, 3.70 (3.70); N, 10.81 (10.82).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(2,6-dichlorophenyl)pyrimidine (8a)

Yield: 55 %; m.p.: 171-173 ^{o}C; R_f: 0.66; IR (KBr) cm^{-1}: 3287 (N-H), 1705 (C=O), 1610 (C=N), 1543 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.72 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.50 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.99-7.69 (m, 8H, Ar-H), 7.86 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_2Br$), Found % (Calculated%): C, 51.25 (51.27); H, 3.40 (3.41); N, 9.95 (9.96); Mass (m/z): 562 (M^+, $C_{24}H_{19}N_4O_3Cl_2Br$), 564 (M^++2), 199 (100%, $C_9H_6NCl_2$).

2-(Morpholinomethylamino)-4-(7-chlorocoumarin-3-yl)-6-(2,4-dichlorophenyl)pyrimidine (9a)

Yield: 55 %; m.p.: 173-175 ^{o}C; R_f: 0.72; IR (KBr) cm^{-1}: 3287 (N-H), 1706 (C=O), 1604 (C=N), 1544 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.70 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.51 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.03-7.74 (m, 8H, Ar-H), 7.89 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_3$), Found % (Calculated%): C, 55.66 (55.67); H, 3.70 (3.70); N, 10.82 (10.82).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(2,4-dichlorophenyl)pyrimidine (10a)

Yield: 60 %; m.p.: 176-178 ^{o}C; R_f: 0.71; IR (KBr) cm^{-1}: 3280 (N-H), 1705 (C=O), 1605 (C=N), 1540 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.71 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.55 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.42 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.04-7.70 (m, 8H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_2Br$), Found % (Calculated%): C, 51.25 (51.27); H, 3.40 (3.41); N, 9.95 (9.96).

2-(Morpholinomethylamino)-4-(7-chlorocoumarin-3-yl)-6-(2-chlorophenyl)pyrimidine (11a)

Yield: 50 %; m.p.: 185-187 ^{o}C; R_f: 0.72; IR (KBr) cm^{-1}: 3280 (N-H), 1705 (C=O), 1605 (C=N), 1542 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.69 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.58 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.37 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.01-7.74 (m, 9H, Ar-H), 7.86 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{20}N_4O_3Cl_2$), Found % (Calculated %): C, 59.63 (59.64); H, 4.16 (4.17); N, 11.58 (11.59).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(2-chlorophenyl)pyrimidine (12a)

Yield: 55 %; m.p.: 176-178 ^{o}C; R_f: 0.76; IR (KBr) cm^{-1}: 3280 (N-H), 1706 (C=O), 1606 (C=N), 1544 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.70 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.56 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 7.02-7.69 (m, 9H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{20}N_4O_3ClBr$), Found % (Calculated %): C, 54.61 (54.62); H, 3.81 (3.82); N, 10.60 (10.62); Mass (m/z): 527 (M^+, $C_{24}H_{20}N_4O_3ClBr$), 529 (M^++2), 164 (100 %, C_9H_7NCl).

2-(Morpholinomethylamino)-4-(7-chlorocoumarin-3-yl)-6-(2,5-dichlorophenyl)pyrimidine (13a)

Yield: 45 %; m.p.: 190-192 ^{o}C; R_f: 0.82; IR (KBr) cm^{-1}: 3288 (N-H), 1707 (C=O), 1609 (C=N), 1541 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.68 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.55 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.95-7.70 (m, 8H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_3$), Found % (Calculated%): C, 55.66 (55.67); H, 3.68 (3.70); N, 10.81 (10.82).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(2,5-dichlorophenyl)pyrimidine (14a)

Yield: 50 %; m.p.: 210-212 ^{o}C; R_f: 0.75; IR (KBr) cm^{-1}: 3283 (N-H), 1709 (C=O), 1611 (C=N), 1544 (C=C), 1134 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.65 (t, J = 8Hz, 4H, -CH_2-N-CH_2-),

3.51 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.29 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.99-7.72 (m, 8H, Ar-H), 7.82 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_2Br$), Found% (Calculated%): C, 51.26 (51.27); H, 3.40 (3.41); N, 9.95 (9.96).

2-(Morpholinomethylamino)-4-(7-chlorocoumarin-3-yl)-6-(3,5-dichlorophenyl)pyrimidine (15a)

Yield: 40 %; m.p.: 185-187 ^oC; R_f: 0.68; IR (KBr) cm^{-1}: 3285 (N-H), 1705 (C=O), 1607 (C=N), 1541 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.63 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.54 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.39 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.97-7.68 (m, 8H, Ar-H), 7.80 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_3$), Found% (Calculated%): C, 55.65 (55.67); H, 3.69 (3.70); N, 10.80 (10.82); Mass (m/z): 517 (M^+, $C_{24}H_{19}N_4O_3Cl_3$), 518 (M^++1), 199 (100%, $C_9H_6NCl_2$).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(3,5-dichlorophenyl)pyrimidine (16a)

Yield: 55 %; m.p.: 173-175 ^oC; R_f: 0.73; IR (KBr) cm^{-1}: 3281 (N-H), 1707 (C=O), 1610 (C=N), 1543 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.64 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.51 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.27 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.96-7.70 (m, 8H, Ar-H), 7.83 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_2Br$), Found % (Calculated %): C, 51.26 (51.27); H, 3.40 (3.41); N, 9.95 (9.96).

2-(Morpholinomethylamino)-4-(7-chlorocoumarin-3-yl)-6-(3,4-dichlorophenyl)pyrimidine (17a)

Yield: 45 %; m.p.: 160-162 ^oC; R_f: 0.71; IR (KBr) cm^{-1}: 3286 (N-H), 1705 (C=O), 1606 (C=N), 1541 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.68 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.57 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.33 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.95-7.72 (m, 8H, Ar-H), 7.85 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_3$), Found % (Calculated %): C, 55.66 (55.67); H, 3.69 (3.70); N, 10.81 (10.82).

2-(Morpholinomethylamino)-4-(7-bromocoumarin-3-yl)-6-(3,4-dichlorophenyl)pyrimidine (18a)

Yield: 45 %; m.p.: 191-193 ^oC; R_f: 0.82; IR (KBr) cm^{-1}: 3289 (N-H), 1709 (C=O), 1605 (C=N), 1544 (C=C), 1135 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 2.66 (t, J = 8Hz, 4H, -CH_2-N-CH_2-), 3.56 (t, J = 8Hz, 4H, -CH_2-O-CH_2-), 4.35 (d, J = 12Hz, 2H, -NH-CH_2-N-), 6.95-7.73 (m, 8H, Ar-H), 7.88 (s, 1H, NH, exchangable with D_2O); Elemental Analysis ($C_{24}H_{19}N_4O_3Cl_2Br$), Found % (Calculated %): C, 51.26 (51.27); H, 3.40 (3.41); N, 9.95 (9.96); Mass (m/z): 562 (M^+, $C_{24}H_{19}N_4O_3Cl_2Br$), 564 (M^++2), 199 (100%, $C_9H_6NCl_2$).

Antimicrobial activity

The antimicrobial activity data of the title compounds (**1a-18a**) at different concentrations against Gram positive bacteria, Gram negative bacteria and fungi is provided in Table 1, Table 2 and Table 3, respectively. For the explanation of the antimicrobial activity result this discussion, 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 title compounds (**1a-18a**), 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 title compounds (**1a-18a**) with respect to ofloxacin revealed that the compound **4a** (MIC = 100 µg/mL) displayed highest activity of about 93.98 % with $p < 0.05$ against *S. aureus*; the compound **15a** (MIC = 125 µg/mL) displayed highest activity of about 83.60 % with $p < 0.0001$ against *E. faecalis*; the compound **9a** (MIC = 100 µg/mL) displayed highest activity of about 91.03 % ($p < 0.0001$) against *S. epidermidis*; the compound **14a** (MIC = 150 µg/mL) displayed highest activity of about 88.5 % with $p < 0.0001$ against *B. subtilis*; and the compound **18a** (MIC = 100 µg/mL) displayed highest activity of about 91.45 % with $p < 0.0001$ against *B. cereus*.

Some compounds exhibited good but statistically non-significant antibacterial activity results ($p > 0.05$) with respect to ofloxacin, for example, the compound **13a** (MIC = 100 µg/mL) displayed about 99.88 % activity against *S. aureus*; compound **18a** (MIC = 125 µg/mL) displayed about 94.75 % activity against *S. aureus*; and compounds **15a** (MIC = 175 µg/mL) displayed about 98 % activity against *S. epidermidis*. The antibacterial activity produced by the compounds **4a**, **9a**, **13a**, **14a**, **15a** and **18a** was lower than the antibacterial activity produced by ofloxacin

Table 1: Antibacterial activity data of the title compounds **(1a-18a)** against Gram positive bacteria

Compound	Zone of inhibition (mm ± SD) and corresponding MIC (µg/mL) value in bracket				
	S. aureus	***E. faecalis***	***S. epidermidis***	***B. subtilis***	***B. cereus***
1a	12.28±0.27[a] (50)	17.12±0.26[a] (50)	16.82±0.26[a] (75)	10.50±0.30[a] (50)	18.77±0.24[a] (100)
2a	10.73±0.23[a] (75)	20.67±0.29[a] (100)	9.20±0.23[a] (200)	16.53±0.25[a] (50)	13.97±0.28[a] (150)
3a	17.02±0.32[a] (100)	13.53±0.33[a] (150)	16.55±0.29[a] (100)	18.07±0.31[a] (125)	20.28±0.27[a] (125)
4a	24.20±0.30[c] (100)	20.38±0.30[a] (125)	24.08±0.26[a] (75)	21.05±0.31[a] (100)	24.78±0.32[a] (100)
5a	13.27±0.28[c] (50)	11.62±0.28[a] (75)	19.72±0.33[a] (75)	14.62±0.42[a] (25)	19.92±0.28[a] (50)
6a	5.27±0.26[a] (200)	17.48±0.28[a] (50)	16.12±0.36[a] (150)	22.05±0.29[a] (150)	13.95±0.24[a] (50)
7a	11.08±0.33[a] (150)	14.02±0.16[a] (75)	19.20±0.32[a] (100)	15.05±0.24[a] (75)	22.97±0.22[a] (150)
8a	20.22±0.25[a] (100)	16.42±0.27[a] (150)	11.63±0.51[a] (50)	15.27±0.36[a] (50)	21.38±0.39[a] (75)
9a	21.62±0.41[a] (75)	23.22±0.37[a] (50)	25.08±0.35[a] (100)	19.97±0.33[a] (100)	21.15±0.40[a] (100)
10a	14.80±0.42[a] (50)	12.25±0.34[a] (100)	18.65±0.35[a] (50)	19.50±0.31[a] (125)	9.23±0.39[a] (175)
11a	9.72±0.43[a] (125)	16.65±0.38[a] (75)	14.03±0.31[a] (50)	12.07±0.36[a] (50)	20.02±0.33[a] (100)
12a	20.62±0.33[a] (100)	11.80±0.37[a] (175)	9.28±0.40[a] (175)	17.02±0.42[a] (150)	23.57±0.41[a] (125)
13a	25.72±0.36[d] (100)	21.13±0.36[a] (150)	19.30±0.38[a] (50)	25.17±0.45[a] (100)	25.65±0.30[a] (100)
14a	21.87±0.43[d] (125)	18.83±0.41[a] (100)	24.02±0.26[a] (100)	27.95±0.29[a] (150)	22.80±0.34[a] (100)
15a	20.20±0.35[a] (100)	24.02±0.39[a] (125)	27.00±0.26[d] (175)	16.77±0.40[a] (100)	19.15±0.41[a] (75)
16a	14.98±0.37[a] (50)	18.50±0.25[a] (75)	12.08±0.47[a] (50)	8.48±0.41[a] (175)	18.20±0.35[a] (150)
17a	18.95±0.28[a] (75)	13.98±0.27[a] (150)	21.03±0.27[a] (75)	15.38±0.37[a] (50)	24.27±0.31[a] (125)
18a	24.40±0.36[d] (125)	21.53±0.38[a] (100)	24.05±0.28[a] (150)	4.63±0.39[a] (200)	26.43±0.36[a] (100)
Ofloxacin	25.75±0.43[a] (25)	28.73±0.43[a] (25)	27.55±0.41[a] (25)	31.58±0.41[a] (12.5)	28.90±0.27[a] (12.5)
Negative Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Values in parenthesis represent the corresponding MIC (µg/mL); [a]*p < 0.0001,* [b]*p < 0.001,* [c]*p < 0.05, ,* [d]*p > 0.05*

Table 2: Antibacterial activity data of the title compounds **(1a-18a)** against Gram negative bacteria

Compound	Zone of inhibition (mm ± SD and corresponding MIC (µg/mL)				
	E. coli	***P. aeruginosa***	***K. pneumonia***	***B. bronchiseptica***	***P. vulgaris***
1a	16.42±0.42[a] (100)	19.73±0.24[a] (100)	21.73±0.43[a] (75)	23.78±0.38[a] (100)	12.78±0.27[a] (200)
2a	13.05±0.28[a] (150)	14.72±0.27[a] (150)	19.70±0.26[a] (125)	16.67±0.29[a] (50)	14.07±0.27[a] (50)
3a	21.47±0.35[a] (75)	20.47±0.31[a] (75)	27.67±0.25[a] (100)	25.52±0.30[a] (125)	16.42±0.25[a] (50)
4a	25.87±0.27[a] (75)	27.45±0.29[a] (50)	21.67±0.33[a] (75)	23.22±0.27[a] (100)	23.30±0.41[a] (100)
5a	13.70±0.39[a] (175)	11.92±0.32[a] (200)	19.43±0.41[a] (50)	17.20±0.39[a] (150)	8.78±0.38[a] (175)
6a	18.92±0.42[a] (100)	21.60±0.39[a] (75)	16.98±0.32[a] (50)	11.32±0.41[a] (200)	15.97±0.37[a] (75)
7a	7.48±0.41[a] (200)	15.27±0.35[a] (150)	11.67±0.35[a] (150)	18.47±0.34[a] (75)	12.53±0.33[a] (150)
8a	16.22±0.36[a] (50)	20.12±0.35[a] (100)	12.40±0.41[a] (125)	13.95±0.34[a] (175)	21.88±0.36[a] (100)
9a	21.25±0.30[a] (100)	27.53±0.37[a] (100)	23.20±0.35[a] (100)	24.05±0.30[a] (100)	27.80±0.26[a] (75)
10a	24.03±0.28[a] (100)	21.52±0.27[a] (50)	20.25±0.38[a] (100)	27.63±0.29[a] (100)	24.25±0.38[a] (100)
11a	13.83±0.25[a] (150)	11.60±0.45[a] (75)	13.25±2.38[a] (175)	20.08±0.39[a] (150)	17.37±0.45[a] (50)
12a	9.70±0.24[a] (200)	14.53±0.35[a] (50)	8.47±0.38[a] (75)	15.73±0.29[a] (150)	14.05±0.38[a] (50)
13a	16.42±0.30[a] (50)	24.18±0.37[a] (75)	26.40±0.39[a] (75)	20.38±0.32[a] (125)	22.27±0.33[a] (75)
14a	16.45±0.45[a] (50)	18.33±0.40[a] (75)	22.63±0.39[a] (100)	12.45±0.51[a] (50)	12.83±0.34[a] (125)
15a	14.62±0.28[a] (75)	21.63±0.38[a] (100)	16.98±0.28[a] (50)	24.85±0.17[a] (75)	21.20±0.31[a] (100)
16a	20.12±0.34[a] (100)	24.77±0.31[a] (75)	21.48±0.29[a] (100)	24.10±0.37[a] (100)	19.08±0.33[a] (100)
17a	12.30±0.40[a] (125)	9.37±0.41[a] (150)	11.25±0.38[a] (175)	18.27±0.38[a] (125)	13.83±0.25[a] (150)
18a	22.75±0.42[a] (75)	26.37±0.37[a] (100)	28.72±0.29[c] (100)	24.32±0.43[a] (100)	22.95±0.36[a] (100)
Ofloxacin	28.48±0.37[a] (12.5)	31.12±0.14[a] (12.5)	31.55±0.19[a] (12.5)	32.22±0.20[a] (25)	29.98±0.29[a] (12.5)
Negative Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Values in parenthesis represent the corresponding MIC (µg/mL); [a]p < 0.0001, [b]p < 0.001, [c]p < 0.05, [d]p > 0.05

Table 3: Antifungal activity data of the title compounds **(1a-18a)** against fungi

Compound	Zone of inhibition (mm ± SD and corresponding MIC (µg/mL)				
	C. albicans	***A. niger***	***A. flavus***	***M. purpureous***	***P. citrinum***
1a	18.88±0.33[a] (75)	21.52±0.31[a] (75)	17.30±0.23[a] (50)	15.05±0.38[a] (50)	20.38±0.41[a] (100)
2a	22.77±0.39[a] (100)	14.50±0.34[a] (50)	19.45±0.34[a] (50)	18.43±0.35[a] (50)	19.18±0.40[a] (50)
3a	22.58±0.32[a] (100)	21.95±0.39[a] (75)	25.90±0.33[d] (75)	24.47±0.36[a] (100)	28.13±0.29[a] (75)
4a	19.37±0.44[a] (50)	20.28±0.40[a] (100)	17.53±0.35[a] (50)	18.27±0.38[a] (50)	21.53±0.47[a] (75)
5a	24.17±0.37[a] (75)	27.08±0.26[c] (100)	20.45±0.50[a] (50)	27.70±0.16[a] (50)	23.35±0.38[a] (100)
6a	20.67±0.52[a] (50)	16.92±0.55[a] (50)	23.15±0.34[a] (125)	20.30±0.35[a] (100)	22.13±0.32[a] (100)
7a	23.18±0.34[a] (50)	22.53±0.36[a] (75)	26.20±0.27[d] (75)	27.10±0.43[a] (75)	24.87±0.60[d] (100)
8a	17.25±0.38[a] (125)	24.42±0.40[a] (75)	17.97±3.09[a] (50)	16.97±0.48[a] (50)	16.67±0.41[a] (50)
9a	25.28±0.37[a] (75)	27.50±0.20[d] (100)	25.53±0.30[d] (75)	21.27±0.35[a] (100)	19.87±0.34[a] (125)
10a	27.78±0.44[a] (100)	26.32±0.39[a] (75)	24.85±0.45[d] (125)	27.83±0.32[a] (100)	27.18±0.28[d] (50)
11a	24.62±0.45[a] (75)	22.73±0.42[a] (75)	26.62±0.43[d] (100)	20.75±0.36[a] (75)	24.32±0.33[c] (125)
12a	22.20±0.35[a] (50)	18.80±0.50[a] (50)	17.33±0.40[a] (100)	22.40±0.41[a] (50)	19.28±0.34[a] (50)
13a	16.65±0.40[a] (125)	23.15±0.30[a] (75)	18.22±0.38[a] (50)	14.07±0.38[a] (100)	23.82±0.27[a] (100)
14a	14.13±0.28[a] (150)	19.78±0.43[a] (50)	23.97±0.25[b] (50)	18.98±0.32[a] (75)	21.60±0.32[a] (75)
15a	25.60±0.32[a] (125)	22.22±0.35[a] (75)	20.53±0.44[a] (50)	26.92±0.28[a] (100)	28.17±0.27[b] (125)
16a	24.50±0.34[a] (75)	27.03±0.27[c] (100)	26.90±0.34[d] (75)	22.03±0.41[a] (125)	18.10±0.39[a] (50)
17a	26.32±0.36[a] (100)	27.28±0.25[d] (100)	24.22±0.29[c] (125)	19.60±0.42[a] (50)	20.32±0.45[a] (100)
18a	18.27±0.34[a] (100)	17.83±0.46[a] (50)	21.17±0.34[a] (50)	24.23±0.25[a] (75)	15.78±0.28[a] (50)
Ketoconazole	32.30±0.29[a] (12.5)	28.70±0.38[a] (12.5)	27.97±0.52[a] (25)	31.90±0.34[a] (12.5)	26.07±0.28[a] (25)
Negative Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Values in parenthesis represent the corresponding MIC (µg/mL); [a]$p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.05$

and they also had a higher MIC values than ofloxacin. Other compounds did not produced comparable antibacterial activity against Gram positive bacteria even at higher concentrations with respect to ofloxacin.

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 title compounds (**1a-18a**) with respect to ofloxacin revealed that the compound **4a** (MIC = 75 µg/mL) displayed the highest activity of about 90.83 % with $p < 0.0001$ against *E. coli*; the compounds **4a** (MIC = 50 µg/mL) and **9a** (MIC = 100 µg/mL) displayed highest activity of about 88.20 % and 88.46 %, respectively, with $p < 0.0001$ against *P. aeruginosa*; the compound **18a** (MIC = 100 µg/mL) displayed highest activity of about 91.03 % with $p < 0.05$ against *K. pneumonia*; the compound **10a** (MIC = 100 µg/mL) displayed highest activity of about 85.75 % ($p < 0.0001$); and the compound **9a** (MIC = 75 µg/mL) displayed highest activity of about 92.72 % ($p < 0.0001$) against *P. vulgaris*. The antibacterial activity produced by the compounds **4a**, **9a**, **10a** and **18a** was lower than the antibacterial activity produced by ofloxacin and they also had a higher MIC value than ofloxacin. Other compounds did not produced comparable antibacterial activity against Gram negative bacteria even at higher concentrations with respect to ofloxacin.

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 title compounds (**1a-18a**) with respect to ketoconazole revealed that the compound **10a** (MIC = 100 µg/mL) displayed highest activity of about 86 % with $p < 0.0001$ against *C. albicans*; the compounds **5a** (MIC = 100 µg/mL) and **16a** (MIC = 100 µg/mL) displayed highest activity of about 94.35 % and 94.18, respectively, with $p < 0.05$ against *A. niger*; the compound **17a** (MIC = 125 µg/mL) displayed highest activity of about 86.59 % with $p < 0.05$ against *A. flavus*; the compounds **5a** (MIC = 50 µg/mL), **7a** (MIC = 75 µg/mL), and **10a** (MIC = 100 µg/mL) displayed highest activity of about 86.83, 84.95, and 87.24 %, respectively, with $p < 0.0001$ against *M. purpureous*; and the compounds **3a** (MIC = 75 µg/mL) and **15a** (MIC = 125 µg/mL) displayed highest activity of about 107.90 and 108.05 %, respectively, against *P. citrinum*. The antifungal activity produced by the compounds **3a** and **15a** was more than ketoconazole and statistically significant also. However, their MIC was also high. Some compounds exhibited good but statistically non-significant antifungal activity ($p > 0.05$) with respect to ketoconazole, for example, the compound **9a** (MIC = 100 µg/mL) and compound **17a** (MIC = 100 µg/mL), respectively, displayed about 95.81 and 95.05 % activity against *A. niger*; the compounds **3a** (MIC = 75 µg/mL), **7a** (MIC = 75 µg/mL), **9a** (MIC = 75 µg/mL), **11a** (MIC = 100 µg/mL), and **16a** (MIC = 75 µg/mL), respectively, displayed activity of about 92.59, 93.67, 91.27%, 97.17, and 96.17 % against *A. flavus*. The antifungal activity produced by the compounds **3a**, **5a**, **7a**, **9a**, **10a**, **11a**, **16a**, and **17a** was lower than the antifungal activity produced by ketoconazole and they also had a higher MIC value than ketoconazole. Other compounds did not produced comparable antifungal activity against fungi even at higher concentrations with respect to ketoconazole.

DISCUSSION

A total of eighteen new compounds (**1a-18a**) were synthesized, and their structures were confirmed on the basis of their IR, ^{1}H-NMR, Mass and elemental analysis data. The characteristic peaks in ^{1}H-NMR spectra that confirmed the formation of the compounds (**1a-18a**) from the compounds (**1-18**) [25] were the appearance of the signals at δ (ppm) values from 2.63 to 2.72 for the four protons of $–CH_2$-N-CH_2- portion of the morpholine moiety; from 3.50 to 3.58 for the four protons of $–CH_2$-O-CH_2- portion of the morpholin moiety; and from 4.27 to 4.42 for the two methylene protons of $–NH-CH_2$- moiety. The compounds (**1a-18a**) were tested for their *in vitro* antimicrobial activity by serial plate dilution method [27,28] against five Gram-positive bacteria; five Gram-negative bacteria; and five fungi.

Compound **3a** (MIC = 75 µg/mL; $p < 0.0001$) and **15a** (MIC = 125 µg/mL; $p < 0.001$) produced superior antifungal activity than the standard drug, ketoconazole (MIC = 25 µg/mL; $p < 0.0001$) against *P. citrinum*. Compound **4a** (MIC = 100 µg/mL; $p < 0.05$) displayed highest but moderate activity against *S. aureus* with respect to the standard drug, ofloxacin (MIC = 25 µg/mL; $p < 0.0001$). Compound **4a** (MIC = 75 µg/mL; $p < 0.0001$) also exhibited highest but moderate activity against *E. coli* with respect to the standard drug, ofloxacin (MIC = 12.5 µg/mL; $p <$

0.0001). Further, compound **4a** showed less activity than ofloxacin and also had higher MIC values than the standard drug ofloxacin. It is evident from the antimicrobial activity data mentioned in Table 1, Table 2, and Table 3 that the title compounds are better antifungal agents than antibacterial agents. This is contrary to our earlier reported work [25]. This shows that the addition of the morpholine moiety to these type of compounds [25] increases their antifungal activity. Accordingly, there is a possibility that the replacement of the morpholine moiety by its bioisosteres, for example piperidine moiety, in these compounds may also produce promising antifungal compounds. Accordingly, this study may be extended to acquire more information about the structure activity relationships of these type of compounds. It is also believed that the synthesized compounds might be inhibiting the growth of all tested microorganism by same mechanism as earlier reported pyrimidine moiety containing drugs [15].

The structure activity relationship study of the title compounds (**1a-18a**) revealed that the chloro substitution with respect to positions 2, 3 and 5 of the phenyl ring along with a chloro/bromo substituted coumarin moiety are critical for activity against Gram positive bacteria; the chlorosubstitution with respect to positions 2, 3, and 4 of the phenyl ring along with a chloro/bromo substituted coumarin moiety are critical for activity against Gram negative bacteria; the chlorosubstitution with respect to positions 2, 4, and 6 of the phenyl ring along with a chloro substituted coumarin moiety are critical for activity against fungi; the chloro substitution at position 2 of the phenyl ring along with a chloro/bromo substituted coumarin moiety is relatively more critical for activity against Gram positive bacteria, Gram negative bacteria and fungi.

CONCLUSION

It is evident from the antimicrobial activity data of the title compounds (**1a-18a**) that the compounds **3a** and **15a** produced higher antifungal activity than standard drug ketoconazole against *P. citrinum*. However, these compounds produced superior effect at higher concentration, and therefore, are considered to be less potent than ketoconazole. Some of the compounds displayed promising antibacterial activity at higher concentrations. It is also evident that the title compounds are better antifungal agents than as antibacterial agents. These compounds may be modified to achieve more potent antimicrobial activity. Accordingly, further studies to acquire more information about structure activity relationships are in progress in our laboratory.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

Mohd Imran and Abida conceived and designed the study. Abida and Abdulkhaliq J Alsalman performed the practical work. Mohd Imran and Abdulkhaliq J Alsalman analysed the data generated during this work. Mohd Imran and Abida wrote the manuscript which was also reviewed by Abdulkhaliq J Alsalman.

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