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Original Research Article

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

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Abstract

Purpose: *To prepare some 2-amino-4-(7-substituted/unsubstitutedcoumarin-3-yl)-6-(chlorosubstitute dphenyl) pyrimidines as antimicrobial agents.*
Methods: *Some 2-amino-4-(7-substituted/unsubstitutedcoumarin-3-yl)-6-(chlorosubstitutedphenyl) pyrimidines were prepared by reacting 3-chlorosubstitutedphenyl-1-(7-substituted/unsubstituted coumarin-3-yl)prop-2-ene-1-ones with guanidine carbonate. The chemical structures of the synthesized compounds were elucidated by Fourier transform infra-red spectroscopy (FTIR), ^{1}H-nuclear magnetic resonance (^{1}H-NMR), mass spectrometry and elemental analysis. The synthesized compounds were investigated for their antimicrobial activity against four bacteria and five fungi by serial plate dilution method using ofloxacin and ketoconazole as reference antimicrobial drugs, respectively, and their minimum inhibitory concentrations (MICs) were determined.*
Results: *Compounds 1 ($p < 0.0001$), 2 ($p < 0.0001$), 6 ($p < 0.0001$) and 8 ($p < 0.0001$) were the most active antibacterial agents among the synthesized compounds compared to control and standard agents. Structure-activity relationship revealed that substitution of chlorine atoms at 2- and 6- positions of the phenyl ring are critical for antibacterial activity in the case of dichlorophenyl derivatives, while for monochlorophenyl derivatives, the positions 2 and 4 of the phenyl ring were critical for antibacterial activity. None of the compounds exhibited comparable antifungal activity to the standard antifungal drug, ketoconazole, even at high concentrations.*
Conclusion: *It is evident that the synthesized compounds are relatively very active antibacterial agents but are weak antifungal agents. However, these compounds need further evaluation of their antibacterial activity against other bacterial strains to ascertain their broad spectrum antibacterial activity.*

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

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INTRODUCTION

Microbial infections have been problematic for humans over the centuries. A large number of antimicrobial agents have been developed by scientists to combat microbial infection challenges. However, because of the emergence of new microbial infections and the development of antibiotic resistance, these infections still pose serious threats to patients. According to literature, about 40 new microbial infections have been discovered since 1970s; more than 2

million American people are affected by antibiotic resistance and about 23000 American patients die annually due to the development of antibiotic resistance. Therefore, there is a need to develop new drugs continuously for the treatment of microbial infections [1].

Pyrimidine derivatives have an important place in medicinal chemistry as these are associated with a broad range of biological activities [2,3] including antimicrobial activity [4-7]. Recently, the significance and biological importance of pyrimidine derivatives including their clinical applications in the microbial world has been reviewed [8]. The antimicrobial activity of pyrimidine derivatives against broad range of microbes makes it an important skeleton in medicinal chemistry and drug development against microbes. Encouraged by these observations and also in continuation of our search for potent antimicrobial agents [9-16] including antimicrobial agents having coumarin moiety [16,17], the aim of this study was to prepare some 2-amino-4-(7-substituted/unsubstitutedcoumarin-3-yl)-6-(chlorosubstitutedphenyl) pyrimidines in order to evaluate their antimicrobial activity.

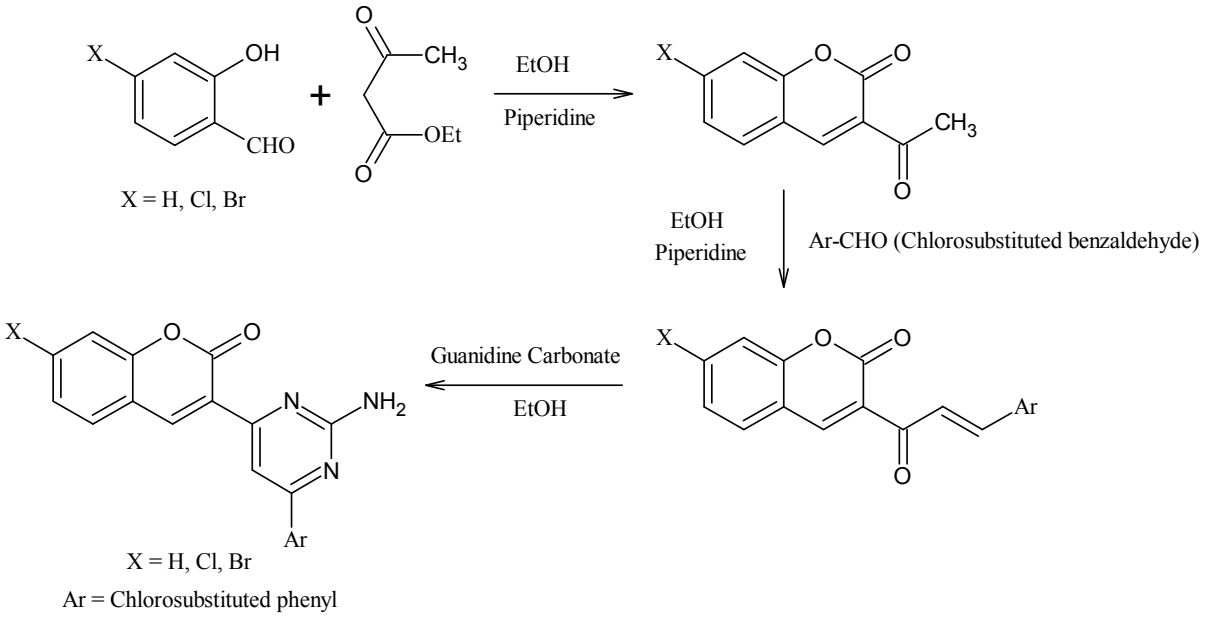
EXPERIMENTAL

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 1H-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. Rf 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 2-amino-4-(7-substituted/unsubstitutedcoumarin-3-yl)-6-(chlorosubstitutedphenyl)pyrimidines (1-18) were prepared according to the method outlined in Scheme 1.

Appropriate salicyldehydes (X = H, Cl, Br) were reacted with ethyl acetoacetate in the presence of piperidine using ethanol as solvent to obtain 7-substituted/unsubstituted-3-acetylcoumarins.

These acetyl coumarins were then reacted with different chlorosubstituted benzaldehydes in the presence of piperidine using absolute ethanol as solvent to obtain 3-chlorosubstitutedphenyl-1-(7-substituted/unsubstitutedcoumarin-3-yl)prop-2-ene-1-ones, .i.e., chalcones. These chalcones were cyclised with guanidine carbonate using ethanol as solvent to obtain the title compounds.



Scheme 1

General method for the synthesis of 7-substituted/unsubstituted-3-acetylcoumarins

A mixture of appropriate salicyldehyde (0.02 moles) and ethyl acetoacetate (0.02 moles) in 30 mL of ethanol was taken in a 100 mL round bottom flask. To this mixture, 3 to 5 drops of piperidine were added. The reaction mixture was refluxed for 1 h to 2 h. The resulting mass was kept at room temperature for 30 min and poured on crushed ice. The solid separated was filtered, dried and recrystallized from ethanol [16,17].

General method for the synthesis of 3-chlorosubstitutedphenyl-1-(7-substituted/unsubstitutedcoumarin-3-yl)prop-2-ene-1-ones

Equimolar quantity of 7-substituted/unsubstituted-3-acetylcoumarin and appropriate chlorosubstituted benzaldehyde was refluxed in absolute ethanol using piperidine as catalyst for 6 h to 10 h. The solution mixture was concentrated, cooled and poured on crushed ice. The compound obtained was filtered at pump, dried and recrystallized from ethanol [16,17].

General method for the synthesis of 2-amino-4-(7-substituted/unsubstitutedcoumarin-3-yl)-6-(chlorosubstitutedphenyl) pyrimidines (1-18)

A mixture of appropriate 3-chlorosubstitute dphenyl-1-(7-substituted/unsubstitutedcoumarin-3-yl) prop-2-ene-1-one (0.01 moles) and guanidine carbonate (0.015 moles) was refluxed in ethanol for 8 - 10 h. The resulting mass was evaporated to dryness and the product obtained was washed with water repeatedly and then recrystallized from ethanol. The purity of the compounds was established on the basis of TLC.

Evaluation of antimicrobial activity

All the synthesized compounds, 2-amino-4-(7-substituted/unsubstitutedcoumarin-3-yl)-6-(chlorosubstitutedphenyl) pyrimidines (1-18), were tested for their *in vitro* antimicrobial activity by serial plate dilution method [18,19] against Gram-positive bacteria, *Staphylococcus aureus* (ATCC-25923); Gram-negative bacteria, *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (ATCC-700603); and 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 synthesized compounds were tested at concentrations of 200, 100, 50, 25 and 12.5 µg/mL. The reference or standard antibiotic, 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 negative control. 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 the data are presented as mean ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). The results were considered significantly different at $p < 0.05$ compared with control group as well as standard drug group.

RESULTS

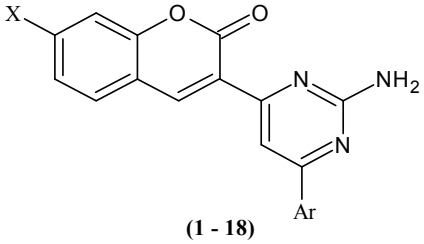
The physical constants of the title compounds are provided in Table 1.

FTIR, ^{1}H-NMR, mass and elemental analysis data of the title compounds (1-18) are presented as follows:

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

IR (KBr) cm^{-1}: 3431 (N-H), 1712 (C=O), 1612 (C=N), 1545 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.38 (bs, 2H, NH_2, exchangable with D_2O), 7.05 (d, J = 12Hz, 2H, Ar-H), 7.26 (m, 3H, Ar-H), 7.48 (t, J = 8Hz, 2H, Ar-H), 7.61 (s, 1H, Ar-H), 7.84 (d, J = 12Hz, 2H, Ar-H); Elemental Analysis ($C_{19}H_{12}N_3O_2Cl$), Found % (Calculated %): C, 65.22 (65.24); H, 3.45 (3.46); N, 12.00 (12.01); Mass (m/z): 349 (M^+), 350 ($M^+ + 1$).

Table 1: Physical constants of the title compounds (1-18)



(1 - 18)

Compd	Ar	X	Molecular formula	Molecular weight	Yield (%)	MP (^{o}C)	R_f value
1	4-Chlorophenyl	H	$C_{19}H_{12}N_3O_2Cl$	349.77	55	165-167	0.74
2	2,6-Dichlorophenyl	H	$C_{19}H_{11}N_3O_2Cl_2$	384.21	48	180-182	0.71
3	2,4-Dichlorophenyl	H	$C_{19}H_{11}N_3O_2Cl_2$	384.21	45	151-153	0.71
4	2-Chlorophenyl	H	$C_{19}H_{12}N_3O_2Cl$	349.77	45	173-175	0.68
5	4-Chlorophenyl	Cl	$C_{19}H_{11}N_3O_2Cl_2$	384.21	50	160-162	0.71
6	4-Chlorophenyl	Br	$C_{19}H_{11}N_3O_2ClBr$	428.66	47	147-149	0.66
7	2,6-Dichlorophenyl	Cl	$C_{19}H_{10}N_3O_2Cl_3$	418.66	52	163-165	0.65
8	2,6-Dichlorophenyl	Br	$C_{19}H_{10}N_3O_2Cl_2Br$	463.11	45	150-152	0.64
9	2,4-Diclorophenyl	Cl	$C_{19}H_{10}N_3O_2Cl_3$	418.66	50	171-173	0.69
10	2,4-Diclorophenyl	Br	$C_{19}H_{10}N_3O_2Cl_2Br$	463.11	46	160-162	0.62
11	2-Chlorophenyl	Cl	$C_{19}H_{11}N_3O_2Cl_2$	384.21	50	187-189	0.72
12	2-Chlorophenyl	Br	$C_{19}H_{11}N_3O_2ClBr$	428.66	50	164-166	0.74
13	2,5-Dichlorophenyl	Cl	$C_{19}H_{10}N_3O_2Cl_3$	418.66	40	141-143	0.66
14	2,5-Dichlorophenyl	Br	$C_{19}H_{10}N_3O_2Cl_2Br$	463.11	43	155-157	0.70
15	3,5-Dichlorophenyl	Cl	$C_{19}H_{10}N_3O_2Cl_3$	418.66	42	172-174	0.68
16	3,5-Dichlorophenyl	Br	$C_{19}H_{10}N_3O_2Cl_2Br$	463.11	48	165-177	0.65
17	3,4-Dichlorophenyl	Cl	$C_{19}H_{10}N_3O_2Cl_3$	418.66	45	149-151	0.69
18	3,4-Dichlorophenyl	Br	$C_{19}H_{10}N_3O_2Cl_2Br$	463.11	40	174-176	0.71

Note: *Compd = Compound; MP = melting point*

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

IR (KBr) cm^{-1}: 3431 (N-H), 1717 (C=O), 1611 (C=N), 1545 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.36 (bs, 2H, NH_2, exchangable with D_2O), 7.08 (d, J = 12Hz, 2H, Ar-H), 7.22-7.48 (m, 4H, Ar-H), 7.61 (s, 1H, Ar-H), 7.7 (d, J = 12Hz, 2H, Ar-H); Elemental Analysis ($C_{19}H_{11}N_3O_2Cl_2$), Found % (Calculated %): C, 59.38 (59.39); H, 2.88 (2.89); N, 10.92 (10.94); Mass (m/z): 384 (M^+), 385 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3425 (N-H), 1715 (C=O), 1611 (C=N), 1545 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.34 (bs, 2H, NH_2, exchangable with D_2O), 7.06 (d, J = 12Hz, 2H, Ar-H), 7.26-7.75 (m, 6H, Ar-H), 7.94 (s, IH, Ar-H); Elemental Analysis ($C_{19}H_{11}N_3O_2Cl_2$), Found % (Calculated %): C, 59.37 (59.39); H, 2.88 (2.89); N, 10.93 (10.94); Mass (m/z): 384 (M^+), 385 (M^+ + 1).

2-Amino-4-(coumarin-3-yl)-6-(2-chlorophenyl)pyrimidine (4)

IR (KBr) cm^{-1}: 3430 (N-H), 1718 (C=O), 1613 (C=N), 1539 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.35 (bs, 2H, NH_2, exchangable with D_2O), 7.06 (d, J = 12Hz, 2H, Ar-H), 7.29-7.80 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{12}N_3O_2Cl$), Found % (Calculated %): C, 65.22 (65.24); H, 3.45 (3.46); N, 12.00 (12.01); Mass (m/z): 349 (M^+), 350 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3428 (N-H), 1719 (C=O), 1612 (C=N), 1540 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.32 (bs, 2H, NH_2), 7.10-7.45 (m, 6H, Ar-H), 7.61 (s, 1H, Ar-H), 7.84 (d, J = 12Hz, 2H, Ar-H); Elemental Analysis ($C_{19}H_{11}N_3O_2Cl_2$), Found % (Calculated %): C, 59.38 (59.39); H, 2.88 (2,89); N, 10.93 (10.94); Mass (m/z): 384 (M^+), 385 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3426 (N-H), 1718 (C=O), 1609 (C=N), 1543 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.37 (bs, 2H, NH_2), 7.15-7.48 (m, 6H, Ar-H), 7.69 (s, 1H, Ar-H), 7.80 (d, J = 12Hz, 2H, Ar-H); Elemental Analysis ($C_{19}H_{11}N_3O_2ClBr$), Found % (Calculated %): C, 53.22 (53.24); H, 2.58 (2.59); N, 9.78 (9.80); Mass (m/z): 428 (M^+), 429 (M^+ + 1), 430 (M^+ + 2).

2-Amino-4-(7-chlorocoumarin-3-yl)-6-(2,6-dichlorophenyl)pyrimidine (7)

IR (KBr) cm^{-1}: 3426 (N-H), 1716 (C=O), 1608 (C=N), 1544 (C=C), 1134 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.38 (bs, 2H, NH_2), 7.15-7.88 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_3$), Found % (Calculated %): C, 54.50 (54.51); H, 2.40 (2.41); N, 10.03 (10.04); Mass (m/z): 418 (M^+), 419 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3427 (N-H), 1717 (C=O), 1607 (C=N), 1544 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.34 (bs, 2H, NH_2), 7.10-7.85 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_2Br$), Found % (Calculated %): C, 49.27 (49.28); H, 2.17 (2.18); N, 9.05 (9.07); Mass (m/z): 463 (M^+), 464 (M^+ + 1), 465 (M^+ + 2).

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

IR (KBr) cm^{-1}: 3427 (N-H), 1718 (C=O), 1608 (C=N), 1540 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.38 (bs, 2H, NH_2), 7.13-7.83 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_3$), Found % (Calculated %): C, 54.50 (54.51); H, 2.40 (2.41); N, 10.02 (10.04); Mass (m/z): 418 (M^+), 419 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3429 (N-H), 1717 (C=O), 1611 (C=N), 1543 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.38 (bs, 2H, NH_2), 7.13-7.83 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_2Br$), Found % (Calculated %): C, 49.27 (49.28); H, 2.17 (2.18); N, 9.06 (9.07); Mass (m/z): 463 (M^+), 464 (M^+ + 1), 465 (M^+ + 2).

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

IR (KBr) cm^{-1}: 3427 (N-H), 1718 (C=O), 1607 (C=N), 1542 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.34 (bs, 2H, NH_2), 7.19-7.90 (m, 9H, Ar-H); Elemental Analysis ($C_{19}H_{11}N_3O_2Cl_2$), Found % (Calculated %): C, 59.38 (59.39); H, 2.88 (2.89); N, 10.92 (10.94); Mass (m/z): 384 (M^+), 385 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3425 (N-H), 1718 (C=O), 1608 (C=N), 1545 (C=C), 1130 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.36 (bs, 2H, NH_2), 7.15-7.84 (m, 9H, Ar-H); Elemental Analysis ($C_{19}H_{11}N_3O_2ClBr$), Found % (Calculated %): C, 53.22 (53.24); H, 2.58 (2.59); N, 9.78 (9.80); Mass (m/z): 428 (M^+), 429 (M^+ + 1), 430 (M^+ + 2).

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

IR (KBr) cm^{-1}: 3435 (N-H), 1715 (C=O), 1610 (C=N), 1535 (C=C), 1132 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.33 (bs, 2H, NH_2), 7.16-7.88 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_3$), Found % (Calculated %): C, 54.50 (54.51); H, 2.40 (2.41); N, 10.03 (10.04); Mass (m/z): 418 (M^+), 419 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3434 (N-H), 1718 (C=O), 1611 (C=N), 1531 (C=C), 1136 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.35 (bs, 2H, NH_2), 7.10-7.81 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_2Br$), Found % (Calculated %): C, 49.27 (49.28); H, 2.16 (2.18); N, 9.06 (9.07); Mass (m/z): 463 (M^+), 464 (M^+ + 1), 465 (M^+ + 2).

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

IR (KBr) cm^{-1}: 3433 (N-H), 1713 (C=O), 1616 (C=N), 1534 (C=C), 1133 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.37 (bs, 2H, NH_2), 7.15-7.89 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_3$), Found % (Calculated %): C, 54.50 (54.51); H, 2.40 (2.41); N, 10.03 (10.04); Mass (m/z): 418 (M^+), 419 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3429 (N-H), 1719 (C=O), 1612 (C=N), 1540 (C=C), 1136 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.34 (bs, 2H, NH_2), 7.15-7.81 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_2Br$), Found % (Calculated %): C, 49.27 (49.28); H, 2.16 (2.18); N, 9.06 (9.07); Mass (m/z): 463 (M^+), 464 (M^+ + 1), 465 (M^+ + 2).

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

IR (KBr) cm^{-1}: 3432 (N-H), 1720 (C=O), 1611 (C=N), 1537 (C=C), 1134 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.37 (bs, 2H, NH_2), 7.13-7.86 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_3$), Found % (Calculated %): C, 54.51 (54.51); H, 2.40 (2.41); N, 10.04 (10.04); Mass (m/z): 418 (M^+), 419 (M^+ + 1).

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

IR (KBr) cm^{-1}: 3433 (N-H), 1717 (C=O), 1614 (C=N), 1533 (C=C), 1134 (C-O-C); ^{1}H-NMR ($CDCl_3$, DMSO-d_6) δ ppm: 5.35 (bs, 2H, NH_2), 7.14-7.88 (m, 8H, Ar-H); Elemental Analysis ($C_{19}H_{10}N_3O_2Cl_2Br$), Found % (Calculated %): C, 49.28 (49.28); H, 2.17 (2.18); N, 9.07 (9.07); Mass (m/z): 463 (M^+), 464 (M^+ + 1), 465 (M^+ + 2).

Antimicrobial activity

Antimicrobial activity of the title compounds was investigated against one Gram-positive bacteria, *S. aureus*; three Gram-negative bacteria, *E. coli, P. aeruginosa*, and *K. pneumoniae*; and five fungi, *C. albicans, A. niger, A. flavus, M. purpureous* and *P. citrinum*. The antibacterial activity and antifungal activity data are presented in Tables 2 and 3, respectively.

DISCUSSION

A total of eighteen 2-amino-4-(7-substituted/ unsubstitutedcoumarin-3-yl)-6-(chlorosubstatute dphenyl) pyrimidines (1-18) were prepared as antimicrobial agents. The chemical structures of these compounds were confirmed by IR, ^{1}H-NMR, mass spectrometry and elemental analysis data. Cyclisation of the chalcones to the title compounds was confirmed by the appearance of IR absorption peaks from 3425 to 3435 cm^{-1} due to the stretching vibration of N-H of -NH_2 group and the appearance of IR absorption peaks from 1607 to 1616 cm^{-1} due to the stretching vibration of C=N group in the title compounds. The presence of -NH_2 group in the title compounds was supported by the appearance of broad singlets at δ (ppm) values from 5.33 to 5.38 in the ^{1}H-NMR spectrum.

Table 2: Antibacterial activity data of the title compounds (1-18)

Compound	Zone of inhibition, mm (minimum inhibitory concentration, µg/mL)			
	S. aureus	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
1	22.05±0.13[a] (50)	23.15±0.06[a] (50)	17.08±0.05[a] (25)	20.08±0.05[a] (25)
2	18.03±0.13[a] (25)	17.95±0.10[a] (50)	28.08±0.05[a] (12.5)	19.03±0.05[a] (25)
3	13.95±0.13[a] (25)	20.90±0.08[a] (50)	19.95±0.06[a] (50)	15.95±0.06[a] (100)
4	4.95±0.13[a] (200)	15.98±0.10[a] (50)	16.05±0.06[a] (25)	12.93±0.05[a] (25)
5	19.95±0.13[a] (50)	13.08±0.05[a] (50)	7.95±0.10[a] (200)	8.08±0.05[a] (100)
6	20.08±0.13[a] (25)	24.9±0.08[a] (50)	23.1±0.14[a] (25)	18.95±0.06[a] (25)
7	9.9±0.08[a] (100)	16.95±0.06[a] (50)	18.05±0.06[a] (25)	8.88±0.05[a] (50)
8	21.9±0.08[a] (50)	16.08±0.13[a] (50)	27.95±0.06[a] (25)	17.98±0.05[a] (25)
9	11.03±0.10[a] (100)	5.90±0.05[a] (200)	6.95±0.06[a] (200)	7.13±0.05[a] (200)
10	9.98±0.10[a] (50)	14.90±0.05[a] (25)	14.90±0.05[a] (50)	6.93±0.05[a] (100)
11	12.93±0.05[a] (50)	13.13±0.05[a] (25)	11.0±0.08[a] (50)	6.15±0.06[a] (100)
12	7.15±0.06[a] (200)	7±0.08[a] (200)	6.05±0.06[a] (200)	4.98±0.10[a] (200)
13	5.98±0.10[a] (200)	8.08±0.05[a] (200)	9.88±0.05[a] (100)	8.0±0.08[a] (50)
14	17.98±0.10[a] (50)	13.95±0.10[a] (25)	14.03±0.05[a] (50)	20.0±0.08[a] (25)
15	4±0.08[a] (200)	5.13±0.05[a] (200)	6.95±0.06[a] (200)	4.1±0.08[a] (200)
16	5.05±0.10[a] (200)	6.05±0.06[a] (200)	5.98±0.05[a] (200)	6.03±0.10[a] (200)
17	16.15±0.06[a] (50)	19.05±0.06[a] (25)	9.95±0.06[a] (50)	9.93±0.05[a] (50)
18	12.1±0.08[a] (25)	20.03±0.15[a] (50)	21.95±0.06[a] (25)	18.03±0.05[a] (50)
Ofloxacin	24.08±0.05[a] (25)	27.95±0.06[a] (12.5)	31.05±0.06[a] (12.5)	30.03±0.10[a] (12.5)
Negative control	0.0	0.0	0.0	0.0

The values in parenthesis represent the corresponding MIC (µg/mL); [a]p < 0.0001

Table 3: Antifungal activity data of the title compounds (1-18)

Compound	Zone of inhibition, mm (minimum inhibitory concentration, µg/mL)				
	C. albicans	***A. niger***	***A. flavus***	***M. purpureous***	***P. citrinum***
1	19.13±0.05[a] (25)	18.08±0.05[a] (50)	19.93±0.05[a] (50)	14.05±0.06[a] (50)	13.03±0.05[a] (50)
2	9.95±0.06[a] (100)	14.03±0.05[a] (100)	11.08±0.05[a] (100)	11.03±0.05[a] (100)	18.98±0.05[a] (50)
3	10.05±0.06[a] (100)	6.93±0.05[a] (200)	9.98±0.05[a] (50)	13.98±0.05[a] (50)	11.98±0.05[a] (50)
4	9.9±0.08[a] (50)	13.05±0.06[a] (100)	14.98±0.05[a] (100)	9.93±0.05[a] (100)	11.08±0.05[a] (100)
5	20.05±0.1[a] (50)	16.05±0.06[a] (50)	14.1±0.08[a] (50)	20.95±0.06[a] (50)	19.05±0.06[a] (25)
6	10.93±0.05[a] (100)	20.95±0.06[a] (50)	18.13±0.10[a] (25)	14.03±0.05[a] (50)	17.15±0.06[a] (50)
7	16.1±0.08[a] (25)	14.03±0.05[a] (25)	11.95±0.06[a] (100)	13.05±0.06[a] (50)	15.05±0.06[a] (100)
8	18.03±0.05[a] (50)	20.05±0.06[a] (50)	16.05±0.06[a] (25)	12.93±0.05[a] (100)	10.95±0.06[a] (50)
9	3.95±0.06[a] (200)	17.03±0.05[a] (100)	6.93±0.05[a] (200)	10.98±0.05[a] (100)	10.98±0.05[a] (100)
10	12.98±0.10[a] (50)	18.93±0.05[a] (100)	11.9±0.08[a] (50)	10.93±0.05[a] (100)	13.03±0.10[a] (50)
11	10.1±0.08[a] (50)	12.05±0.06[a] (100)	10.03±0.05[a] (100)	9.95±0.06[a] (50)	15.05±0.06[a] (100)
12	13.93±0.05[a] (50)	14.03±0.05[a] (100)	13.05±0.06[a] (50)	11.98±0.05[a] (50)	18.08±0.05[a] (50)
13	12.08±0.05[a] (50)	19.08±0.05[a] (100)	18.95±0.06[a] (100)	16.95±0.06[a] (50)	14.95±0.06[a] (50)
14	7.03±0.05[a] (200)	5.93±0.05[a] (200)	8.1±0.08[a] (200)	5.08±0.05[a] (200)	8.98±0.05[a] (200)
15	6.93±0.05[a] (200)	13.03±0.05[a] (50)	16.08±0.05[a] (50)	9.05±0.06[a] (200)	11.95±0.06[a] (50)
16	18.9±0.08[a] (50)	11.95±0.06[a] (100)	14.95±0.06[a] (100)	11.93±0.05[a] (100)	16.03±0.05[a] (50)
17	18.98±0.05[a] (50)	12.93±0.05[a] (50)	15.93±0.05[a] (25)	12.05±0.06[a] (50)	12.03±0.05[a] (50)
18	7.08±0.05[a] (200)	5.05±0.06[a] (200)	6.93±0.05[a] (200)	6.95±0.06[a] (200)	8.05±0.06[a] (200)
Ketoconazole	31.08±0.05[a] (12.5)	28.1±0.08[a] (12.5)	27.08±0.10[a] (25)	31.08±0.10[a] (12.5)	25.08±0.05[a] (25)
Negative control	0.0	0.0	0.0	0.0	0.0

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

The presence of IR absorption peaks from 1712 to 1720 cm^{-1} due to the presence of C=O group and IR absorption peaks from 1130 to 1136 cm^{-1} due to the presence of C-O-C group supported the coumarin moiety. The ^{1}H-NMR spectra also confirmed the required number of H-atoms of the title compounds. Furthermore, the elemental analysis and molecular ion peaks of the title compounds were consistent with the assigned structures.

The results of the antibacterial activity revealed that ofloxacin showed its MIC against *S. aureus* at 25 µg/mL concentration, and MIC against *E. coli, P. aeruginosa*, and *K. pneumonia* at 12.5 µg/mL. Most of the title compounds did not display comparable antibacterial activity to ofloxacin at 25 and 12.5 µg/mL concentrations. However, some of the title compounds displayed comparable antibacterial activity to ofloxacin at higher concentrations with high statistically significant results as compared to control group and standard drug group. Compound 1 (X = H; Ar = 4-chlorophenyl; $p < 0.0001$) and compound 8 (X = Br; Ar = 2,6-dichlorophenyl; $p < 0.0001$) showed comparable activity to ofloxacin against *S. aureus* at 50 µg/mL concentration. Compound 6 (X = Br; Ar = 4-chlorophenyl; $p < 0.0001$) showed comparable activity to ofloxacin against *E. coli* at 50 µg/mL concentration. Compound 2 (X = H; Ar = 2,6-dichlorophenyl; $p < 0.0001$) and compound 8 (X = Br; Ar = 2,6-dichlorophenyl; $p < 0.0001$) showed comparable activity to ofloxacin against *P. aeruginosa* at 12.5 and 25 µg/mL concentrations, respectively. None of the compounds exhibited comparable activity to

ofloxacin against *K. pneumonia* even at higher concentrations. The results of antifungal activity revealed that ketoconazole showed MIC of 12.5 µg/mL against *C. albicans, A. niger* and *M. purpureous* at concentration, and of 25 µg/mL against *A. flavus* and *P. citrinum*. None of the title compounds exhibited comparable antifungal activity to ketoconazole even at higher concentrations.

CONCLUSION

It is evident that the synthesized compounds are relatively very active antibacterial agents but display weak antifungal activity. However, these compounds need further evaluation of their antibacterial activity against other bacterial strains to ascertain their broad spectrum antibacterial profile. It is possible too that modification of the compound structures will enhance their antifungal activity, but this requires further studies.

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