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Significant anti-microbial activity of novel Pyrimidine derivatives

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ABSTRACT

A novel series of 6-Chloro-3-(2-morpholino methyl amino)-6-substituted phenyl pyrimidine-4-yl-2H-chromone-2-one (CT_1M–CT_5M), 3-(2-((piperidine-1-yl)methylamino)-6-substituted phenylpyrimidin-4-yl)- 6-Chloro- 2H- chromone -2- one (CT_1P–CT_5P) have been synthesized from 3-(2-amino-6-pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_1-CT_5) which were synthesized from 3-acetyl-6-Chloro-2H-chromen-2-one (3). The structures of the synthesized compounds were elucidated by I. R., ^{1}H NMR spectroscopic techniques. The synthesized compounds were screened for *in-vitro* antimicrobial activity at a 25, 50, 100 and 200 mcg concentrations. Among them, compounds CT_2M, CT_3M, CT_5M, CT_2P, CT_3P and CT_5P exhibited significant antibacterial activity against *P.aeruginosa* and *S.aureus* comparable with standard drug Ampicillin trihydrate. Compounds CT_1M, CT_2M, CT_3M, CT_5M, CT_2P, and CT_5P exhibited significant antifungal activity against *P.chrysogenum* and *A.niger* comparable with standard drug Fluconazole using cup-plate method. Compounds have been further evaluated by measuring zone of inhibition and percent inhibition.

Introduction

Pyrimidines, being an integral part of DNA, and RNA imparts to diverse pharmacological properties as effective bactericide and fungicides (Giovannoni et al., 2003). Nitrogen containing heterocyclic ring such as pyrimidine is a promising structural moiety for drug designing. Pyrimidine derivatives form a component in a number of useful drugs, and are associated with many biological and therapeutically activities (Vogel 1978). Condensed pyrimidine derivatives have been reported as anti-microbial (Desai et al., 2006), analgesic, anti-viral (Patel et al., 2003), anti-inflammatory (Amr et al., 2007), anti-HIV (Fujiwara et al., 2007), anti-tubercular (Ballell et al., 2007), anti-tumor (Wagner et al., 2008), anti-neoplastic (Cordeu et al., 2007), anti-malarial (Gorlitzer et al., 1997), diuretic (Ukrainets et al., 2008), cardiovascular (Kurono et al 1987) agents. Pyrimidine compounds are also used as hypnotic drugs for the nervous system (Wang et al., 2003), calcium-sensing receptor antagonists (Yang et al., 2009) and also for antagonists of the human A_{2A} adenosine receptor (Gillespie et al., 2009). Like pyrimidine, coumarin also exhibits diverse biological properties (Kulkarni et al., 2010, Keri et al 2010, Gupta et al., 2011).

It was envisaged that these two active pharmacophores, if linked together would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. The above-cited applications prompted us to synthesize a series of new compounds reported in this article.

Owing to the importance, here we have described the synthesis of new pyrimidine derivatives from 3-acety-6-chloro-2H-chromen-2-one (3). The compounds were screened for their antimicrobial activity. Thus, we have created new avenues to explore the potent heterocyclic moieties for the pharmacological activities in medicinal chemistry.

When 5-chlorosalicyldehyde (1) is treated with ethylacetoacetate (2) in refluxing ethanol in the presence of piperidine (B), it afforded a single precipitated product that was analyzed correctly for $C_{11}H_7O_3Cl$. The reaction takes 2-3 h for completion. The structure of the precipitated product was identified as 3-acetyl-6-chloro-2H-chromen-2-one (3) based on its spectral data. Formation of compound (3) can be explained on the basis of "Knoevenagel reaction". Here in the first step of this scheme (Mechanism Step: 1), piperidine is working as a proton acceptor which captures the proton from ethylacetoacetate (2), and rendered this as neucleophile (2a). This neucleophile (2a) attack on the aldehyde group of salicyldehyde (1), and made the intermediate (1a). Now again the catalyst piperidine (BH) attack on the intermediate (1a), where this time it works as a proton donor, and results in the intermediate (1b). The intermediate (1b) on dehydration give intermediate (1c), which on the release of one molecule of ethanol, finally gave the 3-acetyl-6-chloro-2H-chromen-2-one (3).

In the next step (Mechanism Step:2), treatment of 3-acetyl-6-Chloro-2H-chromen-2- one (3) with different types of benzaldehydes in the presence of piperidine (B:) for 8-10 h using ethanol as a solvent yielded one precipitated product (CS_1- CS_5) respective of their benzaldehydes. The formation of compounds (CS_1-CS_5) can be explained on the basis of "Claisen-Schmidt condensation". Here again piperidine works as a proton acceptor, and capture the proton from compound (3), and results in the formation of nucleophile (3a). This nucleophile attack on the carbonyl group of benzaldehydes, and forms the intermediate (3b) which is again attacked by piperidine, this time working as a proton acceptor, and results in the formation of intermediate (3c). The intermediate (3c), on dehydration give the product

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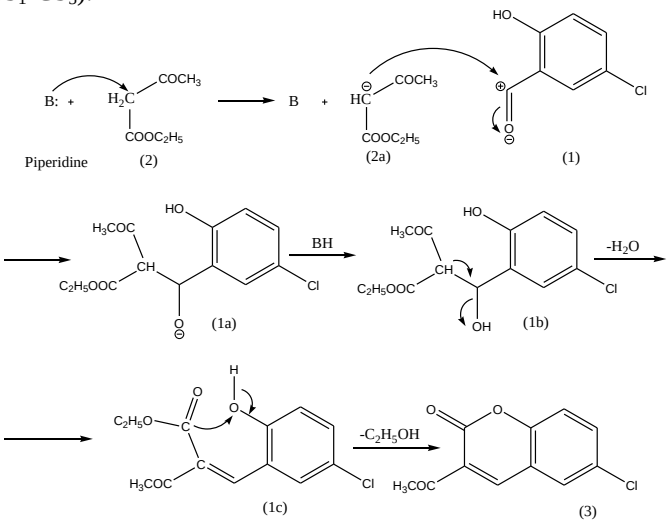
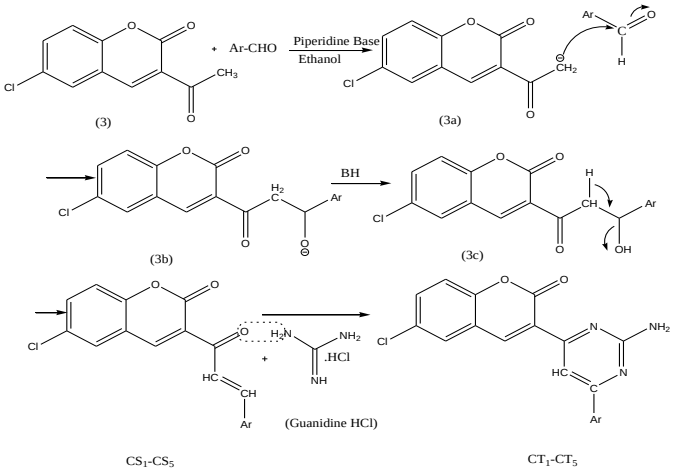
(CS_1-CS_5).



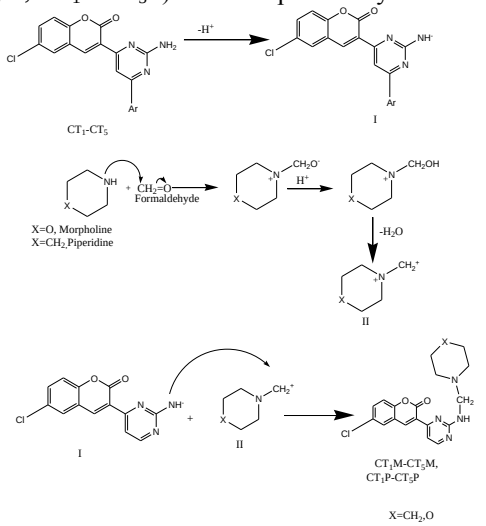
Mechanism Step 1

In the third step, compound (CS_1-CS_5) is refluxed with guanidine HCl, in equimolar quantity in ethanol as a solvent for 8-10 h, which afforded the final compounds (CT_1-CT_5).



Mechanism Step 2

In the fourth step, compound (CT_1-CT_5) is refluxed with Morpholine, and piperidine in equimolar quantity in presence of formaldehyde, and ethanol for 8-10 h, which afforded the final compounds (CT_1M-CT_5M, CT_1P-CT_5P). Formation of (CT_1M-CT_5M, CT_1P-CT_5P) can be explained by "Mannich reaction"



Mechanism step-3

Result and discussion

All the compounds exhibited significant antibacterial and antifungal activity against bacterial stains *P. aeruginosa*, *S. aureous*, and fungi *P. chrysogenum*, *A. niger*. Synthesized pyrimidine compounds CT_2M, CT_5M, CT_4P, and CT_5P showed good inhibitory activity against *P. aeruginosa* while CT_2P, and CT_3P exhibited less antibacterial activity against *P. aeruginosa*. Among the compound tested CT_2M, CT_3M, CT_2P, CT_3P, and CT_5P showed good inhibitory activity against *S. aureous* while other five compounds were found to be less active against *S. aureous*.

The investigation of antifungal data indicate that compounds CT_2M, CT_3M, CT_2P, CT_3P , and CT_5P exerted good antifungal activity against *P. chrysogenum.* Compound CT_4P, CT_4M and CT_5M showed moderate activity where as CT_1P, and CT_1M were found to be less active against *P. chrysogenum*. Compound CT_1M, CT_2M, CT_2P, $CT_5P$and CT_5M were found to be very good antifungal agent against *A. niger.* Compounds CT_3M, CT_1P, CT_3P, and CT_4P exhibited moderate less active against *A. niger* .

The all the antimicrobial compounds possess chloro group on the benzene ring attached to C-4 position of pyrimidine moiety. Significant antimicrobial activity may be attributed to the electronegativity of chloro group which can withdrawn electron more strongly than other group. The compound CT_3M, CT_1M, CT_2M, and CT_5P possess good antimicrobial activity. This may be due to the reason that methoxy group supply its electron more strongly than methyl group as this group contains electronegative oxygen atom.

In conclusion , two new series of compound were synthesized and their antimicrobial activity have seen evaluated, using cup-plate method. All the compounds were found to possess a broad spectrum of antimicrobial activities, specially compounds CT_2M, and CT_5P have shown significant antimicrobial activities against all the pathogenic microorganisms.

Experimental

Chemistry

All reagents and solvents were used as obtained from the supplier. The melting points of the products were determined by open capillaries method, and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL AL300 FTNMR 300 MHz spectrometer in $CDCl_3$ using TMS as an internal standard, with ^{1}H resonance frequency of 300 MHz chemical shift values are expressed in δ ppm. I. R. spectra (KBr) were recorded on FTIR spectrophotometer (Shimadzu FTIR 84005, 4000-400cm^{-1}). Mass spectra were recorded on a 70 eV EI-MS-QP 1000 EX (Schimadzu). The elemental analysis was carried out using Heraus CHN rapid analyzer. The homogeneity of the compounds was described by TLC on alumina silica gel using solvent system "Toluene: Ethylacetate: Formic acid" (5:4:1) and "Benzene: acetone" (9:1) detected by iodine vapours. The physical data of all these compounds are summarized in Table I, Table II, Table III and Table IV.

General procedures for the preparation of compounds:

Synthesis of 3-acetyl-6-chloro-2H-chromen-2-one (3): General procedure

A mixture of 5-chlorosalicyldehyde (1)(0.02 mol) and ethylacetoacetate (2) (0.03 mol) in ethanol were taken in round bottom flask. To this mixture few drops of piperidine were added and refluxed for 2-3 h. After completion of reaction, the content was poured on crushed ice. The solid separated was

filtered, dried and recrystallized from ethanol. Compound (3) can be explained on the basis of "Knoevenagel reaction". The purity of compound was established on the basis of TLC.

Synthesis of 3-acetyl-6-chloro-2H-chromen-2-one(R=Cl) (3): It was obtained from reaction of 5-chlorosalicyldehyde (2) with ethylacetoacetate in the presence of piperidine. M. P. 115-117ºC; % Yield: 75%; Molecular Formula; $C_{11}H_7BrO_3$; Molecular weight: 267.08; IR (KBr, cm^{-1}): 1735.26 and 1675.61 (C=O), 1549.96 (C=C), 1232.96 (aryl ethers, C-O-C); ^{1}H NMR ($CDCl_3$-d_6, δ, ppm): 2.72 (s, 3H, CH_3), 7.25-7.78 (m, 3H, Ar-H), 8.40(s, 1H, Ar-H).

Synthesis of compounds (CS_1-CS_5,): General procedure

Equimolar quantities of 3-acetyl-6-chloro-2H-chromen-2-one (3) and different substituted benzaldehyde were refluxed in absolute ethanol using piperidine as a catalyst for 8-10 h. The solution mixture was concentrated and poured on to crushed ice. The compound so obtained were filtered at pump, dried and recrystallized from ethanol to get pure crystalline solid. The formation of compounds (CS_1-CS_5,) can be explained on the basis of "Claisen-Schmidt condensation".

Synthesis of 6-chloro-3-((E)-3-(2-chlorophenyl)-acryloyl)-2H-chromen-2-one (CS_1): It was obtained from reaction of compound (3) with 2-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1629.74, and 1604.66 (C=O), 1550.66 (C=C), 1176.50 (C-O-C), 757.97 (C-Cl).

Synthesis of 6-chloro-3-((E)-3-(3-chlorophenyl)-acryloyl)-2H-chromen-2-one (CS_2): It was obtained from reaction of compound (3) with 3-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1679.88 and 1595.02 (C=O), 1571.88 (C=C), 1161.07 (C-O-C), 692.40 (C-Cl).

Synthesis of 6-chloro-3-((E)-3-(4-chlorophenyl)-acryloyl)-2H-chromen-2-one (CS_3): It was obtained from reaction of compound (3) with 4-chlorobenzaldehyde. IR (KBr, cm^{-1}): 1676.03 and 1610.45 (C=O), 1579.59 (C=C), 1174.57 (C-O-C), 757.97 (C-Cl).

Synthesis of 6-chloro-3-((E)-3-(2,4-dichlorophenyl)-acryloyl)-2H-chromen-2-one (CS_4): It was obtained from reaction of compound (3) with 2, 4-dichlorobenzaldehyde. IR (KBr, cm^{-1}): 1637.45 (C=O), 1544.88 (C=C), 1139.85 (C-O-C), 696.25 (C-Cl).

Synthesis of 6-chloro-3-((E)-3-(2, 6-dichlorophenyl)-acryloyl)-2H-chromen-2-one (CS_5): It was obtained from reaction of compound (3) with 2, 6-dichlorobenzaldehyde. IR (KBr, cm^{-1}): 1677.95 and 1634.24 (C=O), 1562.23 (C=C), 1182.28 (C-O-C), 761.83 (C-Cl).

Synthesis of compounds (CT_1-CT_5): General procedure

A mixture of compounds (CS_1-CS_5) (0.01 mol), and guanidine HCl (0.02 mol) was refluxed in the presence of ethanol for 8-10 h. The content was evaporated to dryness and the product so obtained was washed with water repeatedly and recrystallized from ethanol.

Synthesis of 3-(2-amino-6-(2-chlorophenyl)-pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_1): It was obtained from reacting (CS_1) with guanidine HCl. IR (KBr, cm^{-1}): 3440.77 (N-H strech), 1647.10 (C=O), 1596.95 & 1384.79 (C-N strech), 1174.57 (C-O-C), 952.77 (C-N bend), 815.83 (C-Cl), 738.69 (NH_2 bend).

Synthesis of 3-(2-amino-6-(3-chlorophenyl)-pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_2): It was obtained from reacting (CS_2) with guanidine HCl. IR (KBr, cm^{-1}) 3409.91 (N-H strech), 1629.74 (C=O), 1596.00 & 1384.79 (C-N strech), 1174.57 (C-O-C), 854.71 (C-N bend), 831.26 (C-Cl), 779.19 (NH_2 bend).

Synthesis of 3-(2-amino-6-(4-chlorophenyl)-pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_3): It was obtained from reacting (CS_3) with guanidine HCl. IR (KBr, cm^{-1}): 3406.05 (N-H strech), 1681.81 (C=O), 1600.81 & 1361.65 (C-N strech), 1157.21 (C-O-C), 835.12 (C-Cl), 757.97 (NH_2 bend).

Synthesis of 3-(2-amino-6-(2, 4-dichlorophenyl)-pyrimidin-4-yl)-6-chloro-2H-chromen -2-one (CT_4): It was obtained from reacting (CS_4) with guanidine HCl. IR (KBr, cm^{-1}): 3236.33 (N-H strech), 1670.24 (C=O), 1560.30 & 1355.86 (C-N strech), 1186.14 (C-O-C), 838.98 (C-N bend), 817.76 (C-Cl), 775.33 (NH_2 bend).

Synthesis of 3-(2-amino-6-(2, 6-dichlorophenyl)-pyrimidin-4-yl)-6-chloro-2H-chromen -2-one (CT_5): It was obtained from reacting (CS_5) with guanidine HCl. IR (KBr, cm^{-1}): 3382.91 (N-H strech), 1679.88 (C=O), 1595.02 (C-N strech), 1180.35 (C-O-C), 871.76 (C-N bend), 752.40 (C-Cl), 771.33 (NH_2 bend).

Synthesis of compounds (CT_1M-CT_5M): General procedure:

A mixture of compounds (CT_1 - CT_5) (0.01 mol), and morpholine (0.01 mol) and formaldehyde (0.02 mol) was refluxed in the presence of ethanol for 6-10 h. The reaction mixture was reduced to half of its volume and poured on crushed ice. The product so obtained was washed with water repeatedly, dried and recrystallized from ethanol. The formation of compounds (CT_1M–CT_5M) can be explained on the basis of "Mannich reaction".

Synthesis of 6-chloro-3-(6-(2-chlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl) -2H-chromen-2-one (CT_1M): It was obtained from reacting (CT_1) with morpholine and formaldehyde. IR (KBr, cm^{-1}): 3427.27 (N-H stretch), 1670.24 (C=O), 1604.66 and 1384.79 (C=N stretch), 1579.59 (C=C), 1174.57 (C-O-C), 829.33 (C-N,Bend), 640.32 (C-Cl); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 1.7 (s, 1H, NH), 1.2-1.3 (t, 4H, 2 x CH_2), 3.18-3.711 (t, 4H 2 x CH_2) , 4.525 (s, 2H, CH_2), 6.377-7.496 (m, 9H, Ar-H); MS m/z: 481.4, 371.2, 257.0, 204.2; 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).

Synthesis of 6-chloro-3-(6-(3-chlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (CT_2M): It was obtained from reacting (CT_2) with morpholine and formaldehyde. IR (KBr, cm^{-1}): 3456.58 (N-H stretch), 1637.45 (C=O), 1612.38 & 1384.79 (C=N stretch), 1581.52 (C=C), 1155.28 (C-O-C), 823.55 (C-N,Bend), 750.26 (C-Cl); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 2.752 (s, 1H, NH), 2.009-2.690 (t, 4H, 2 x CH_2), 3.328-3.994 (t, 4H 2 x CH_2), 4.410 (s, 2H, CH_2), 6.010-7.773 (m, 9H, Ar-H).

Synthesis of 6-chloro-3-(6-(4-chlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (CT_3M): It was obtained from reacting (CT_3) with morpholine and formaldehyde. IR (KBr, cm^{-1}): 3425.34 (N-H stretch),1629.74 (C=O), 1610.45 (C=N stretch), 1596.85 (C=C), 1197.71 (C-O-C), 829.33 (C-N,Bend), 637.68 (C-Cl); ^{1}H NMR ($CDCl_3$-d, δ, ppm): 3.583 (s, 1H, NH), 1.138-1.1305 (t, 4H, 2 x CH_2), 3.764-3.856 (t,4H, 2 x CH_2), 5.290 (s, 2H, CH_2), 6.980-8.101 (m, 9H, Ar-H); MS m/z: 396.03, 368.7, 246.02(100%), 205.1; elemental analysis ($C_{24}H_{20}N_4O_3Cl_2$), found % (calculated %): C, 59.62 (59.64); N, 11.57 (11.59).

Synthesis of 6-chloro-3-(6-(2, 4-dichlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (CT_4M): It was obtained from reacting (CT_4) with morpholine and formaldehyde. IR (KBr, cm^{-1}): 3415.70 (N-H stretch),

1602.74 (C=O), 1159.14 (C-O-C), 752.19 (C-N, bend), 696.25 (C-Cl); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 2.658 (s, 1H, NH), 1.178-2.628 (t, 4H, 2 x CH_2), 3.323-3.896 (t, 4H, 2 x CH_2), 5.112 (s, 2H, CH_2), 6.756-7.758 (m, 8H, Ar-H).

Synthesis of 6-chloro-3-(6-(2, 6-dichlorophenyl)-2-(morpholinomethylamino) pyrimidin-4-yl)-2H-chromen-2-one (CT_5M): It was obtained from reacting (CT_5) with morpholine and formaldehyde. IR (KBr, cm^{-1}): 3446.56 (N-H stretch), 1608.52 (C=O), 1350.08 (C-O-C); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 3.331 (s, 1H, NH), 1.124-1.300 (t, 4H, 2 x CH_2), 3.569-3.908 (t, 4H 2 x CH_2), 5.290 (s, 2H, CH_2), 6.626-7.786 (m, 8H, Ar-H); MS m/z: 473.2, 386.9, 372.9; elemental analysis ($C_{24}H_{19}N_4O_3Cl_3$), found % (calculated %): C, 55.66 (55.67); N, 10.82 (10.82).

Synthesis of compounds (CT_1P-CT_5P): General procedure

A mixture of compounds (CT_1-CT_5) (0.01 mol) and piperidine (0.01 mol) and formaldehyde (0.02 mol) was refluxed in the presence of ethanol for 6-10 h. The reaction mixture was reduced to half of its volume and poured on crushed ice. The product so obtained was washed with water repeatedly, dried, and recrystallized from ethanol. The formation of compounds (CT_1-CT_5) can be explained on the basis of "Mannich reaction".

Synthesis of 3-(2-((piperidin-1-yl) methylamino)-6-(2-chlorophenyl) pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_1P): It was obtained from reacting (CT_1) with piperidine and formaldehyde. IR (KBr, cm^{-1}): 3418.58 (N-H stretch), 1602.74 (C=O), 1384.79 (C=N stretch), 1546.80 (C=C), 1164.92 (C-O-C), 811.98 (C-N,Bend), 727.11 (C-Cl); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 3.506 (s, 1H, NH), 1.118-2.888 (m, 10H, 5 x CH_2), 5.286 (s, 2H, CH_2), 7.079-8.318 (m, 9H, Ar-H); MS m/z: 299.08, 267.1, 203.1(100%); elemental analysis ($C_{25}H_{22}N_4O_2Cl_2$), found % (calculated %): C, 62.36 (62.38); N, 11.64 (11.64).

Synthesis of 3-(2-((piperidin-1-yl) methylamino)-6-(3-chlorophenyl) pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_2P): It was obtained from reacting (CT_2) with piperidine and formaldehyde. IR (KBr, cm^{-1}): 3529.49 (N-H stretch), 1637.45 (C=O), 1612.38 (C=N stretch), 1581.52 (C=C), 1199.64 (C-O-C), 906.48 (C-N,Bend), 659.61 (C-Cl); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 3.557 (s, 1H, NH), 1.112-2.653 (m, 10H, 5 x CH_2), 5.290 (s, 2H, CH_2), 6.903-7.824 (m, 9H, Ar-H).

Synthesis of 3-(2-((piperidin-1-yl) methylamino)-6-(4-chlorophenyl) pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_3P): It was obtained from reacting (CT_3) with piperidine and formaldehyde. IR (KBr, cm^{-1}): 3421.48 (N-H stretch), 1610.45 (C=O), 1384.79 (C=N stretch), 754.12 (C-N, bend), 611.39 (C-Cl); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 2.800 (s, 1H, NH), 1.117-2.659 (m, 10H, 5 x CH_2), 5.290 (s, 2H, CH_2), 6.910-7.645 (m, 9H, Ar-H); MS m/z: 355.1, 276.8, 204.2; elemental analysis ($C_{25}H_{22}N_4O_2Cl_2$), found % (Calculated %): C, 62.37 (62.38); N, 11.64 (11.64).

Synthesis of3-(2-((piperidin-1-yl) methylamino)-6-(2, 4-dichlorophenyl) pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_4P): It was obtained from reacting (CT_4) with piperidine and formaldehyde. IR (KBr, cm^{-1}): 3406.05 (N-H stretch), 1604.66 (C=O), 1384.79 (C=N stretch), 1176.50 (C-O-C), 829.33 (C-N, bend), 613.32 (C-Cl); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 2.779 (s, 1H, NH), 1.115-2.762 (m, 10H, 5 x CH_2), 3.783 (s, 2H, CH_2), 6.545-7.586 (m, 8H, Ar-H).

Synthesis of 3-(2-((piperidin-1-yl) methylamino)-6-(2, 6-dichlorophenyl) pyrimidin-4-yl)-6-chloro-2H-chromen-2-one (CT_5P): It was obtained from reacting (CT_5) with piperidine and formaldehyde. IR (KBr, cm^{-1}): 3411.64 (N-H stretch), 1596.96 (C=O), 1155.28 (C-O-C), 833.19 (C-N, bend), 754.12 (C-Cl); ^{1}H NMR ($CDCl_3$-*d*, δ, ppm): 3.2106 (s, 1H, NH), 1.1967-2.914 (m, 10H, 5 x CH_2), 6.564-8.4782 (m, 8H, Ar-H); MS m/z: 479.10, 292.05, 274.0(100%); elemental analysis ($C_{25}H_{21}N_4O_2Cl_3$), found % (calculated %): C, 58.20 (58.21); N, 10.85 (10.86).

Antimicrobial activity

All the synthesized compound were screened *in- vitro* for their antimicrobial activity against bacterial stains *P. aeruginosa*, *S. aureous* and fungi *P. chrysogenum*, *A. niger* at different concentration 25, 50, 100 and 200 mcg by cup plate method. Ampicillin and Fluconzole were used as standard against all bacterial and fungal strains. The incubation time was 24 h at 37°C for bacteria and 48 h at 37°C for fungal strain. All the screened compounds were found to passess moderate to good antimicrobial activity. The cup plate test was performed using agar medium and dextrose agar medium and the medium was autoclaved at 15 lbs pressure (121°C) for 15 minutes then immediately cooled to 50-55°C in a water bath after removing it from autoclave. The cooled medium was poured into sterile petri plates to a uniform depth of 4mm or 25 ml in a 90 mm plate. Once the medium had solidified than the culture was inoculated on the medium by a sterile swab was dipped into the standardized bacteria or fungus suspension or inoculated with 1 ml of the organism suspension. Sterillized 9 mm cork borer was used to make agar wells, than placed 25, 50, 100 and 200 mcg diluted test compound as well as standard compound were placed into each wells and DMSO as a control. The plate were inoculate for 24 h at 37°C for bacteria and 48 h at 37°C for fungal strain and measure zone of inhibition in mm and the percentage (%) of inhibition was calculated by using the formula. The zone of inhibition and % inhibition data of all these compounds are summarized in fig I, II, III, IV and fig V, VI, VII, VIII individually according to stains.

$$\% \text{ of inhibition} = \frac{\text{Diameter of the inhibition zone in mm}}{\text{Diameter of the petri- plates in mm(90)}}$$

Scheme: Schematic diagrams for the synthesis of pyrimidine derivatives (CT_1M-CT_5M, CT_1P-CT_5P).

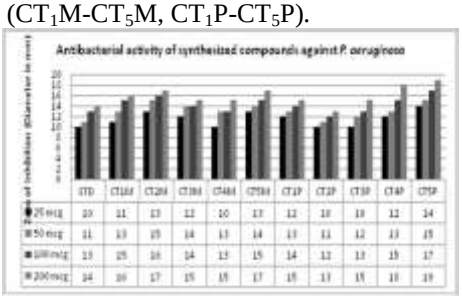


Fig. I: Zone of inhibition of antibacterial activity of synthesized compounds against *P. aeruginosa*

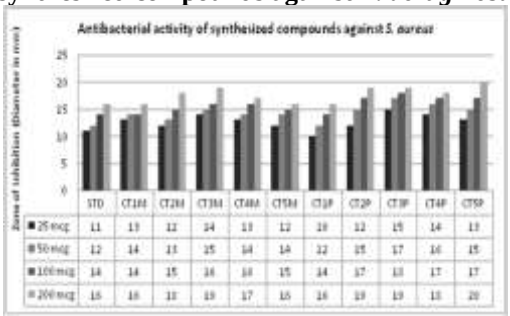
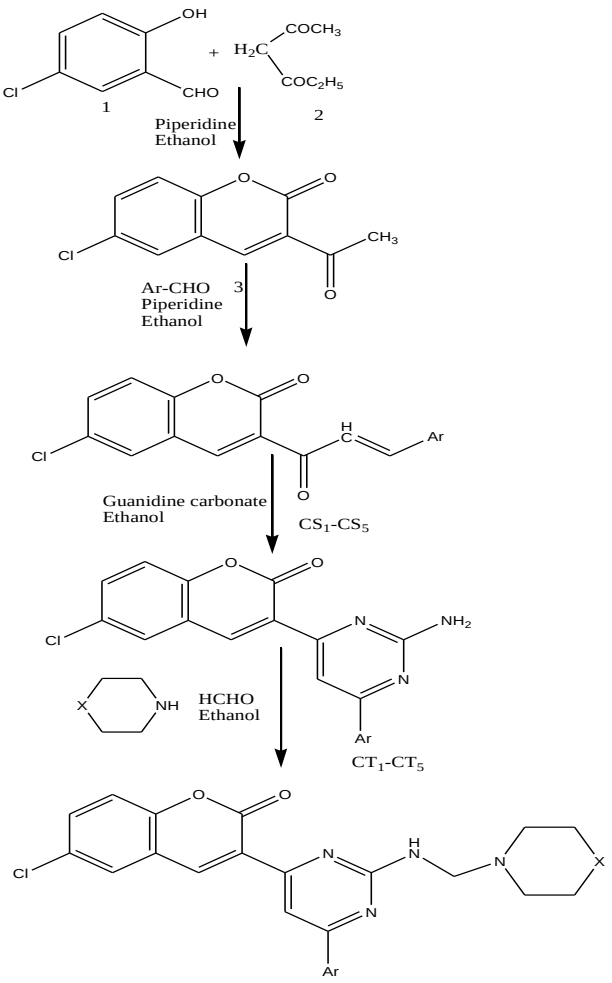


Fig. II: Zone of inhibition of antibacterial activity of synthesized compounds against *S .aureus*



CT_1M-CT_5M, CT_1P-CT_5P

Ar =o-Chloro Benzaldehyde, m-Chloro Benzaldehyde,p-Chloro Benzaldehyde
2,4 dichloro Benzaldehyde
2,6-dichloro Benzaldehyde
X= CH2, O

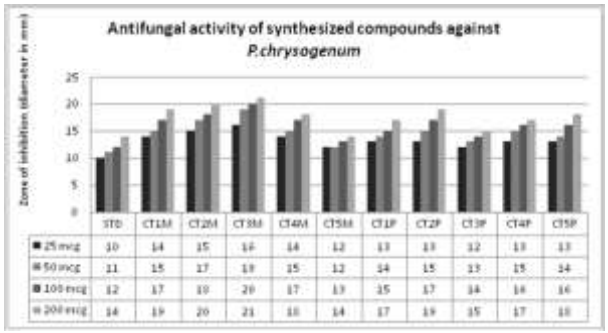


Fig. III: Zone of inhibition of antibacterial activity of synthesized compounds against *P. chrysogenum*

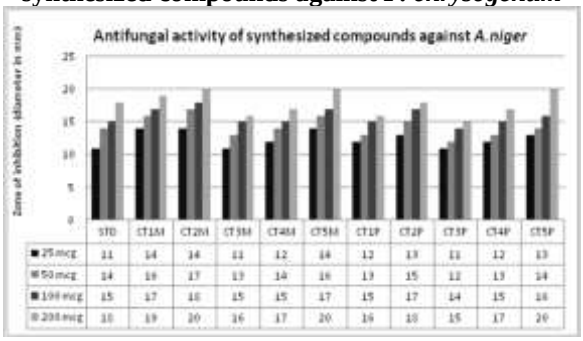


Fig. IV: Zone of inhibition of antibacterial activity of synthesized compounds against *A. niger*

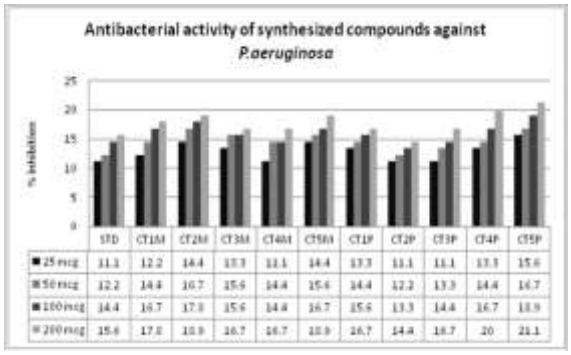


Fig. V: Percentage inhibition of antibacterial activity of synthesized compounds against *P. aeruginosa*

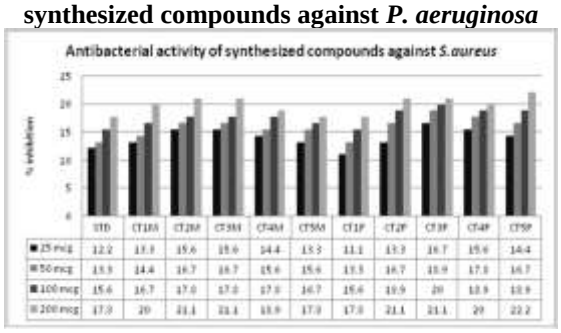


Fig. VI: Percentage inhibition of antibacterial activity of synthesized compounds against *S. aureus*

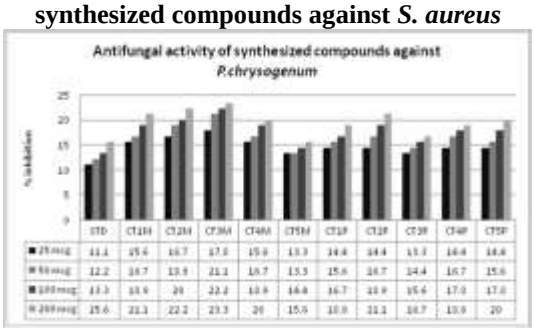


Fig. VII: Percentage inhibition of antibacterial activity of synthesized compounds against *P. chrysogenum*

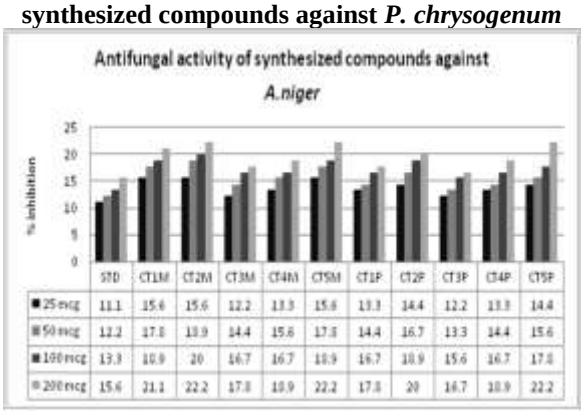


Figure VIII: Percentage inhibition of antibacterial activity of synthesized compounds against *A. niger*.

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The authors are thankful to CDRI, Lucknow, India for providing spectral data and VIMS, Greater noida for providing all facilities to carry out the present work.

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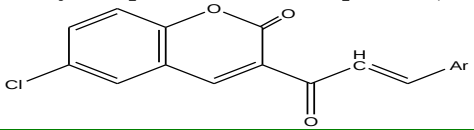
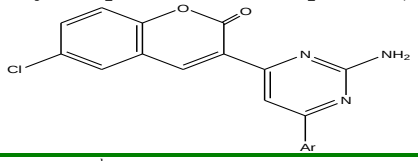
Table I: Physical parameters of compounds (CS_1-CS_5)



Compound [a]	-Ar	Rf value[b]	% Yield[c]	m.p.°C[d]	Molecular formula
CS_1		0.68	49	210-214	$C_{18}H_{11}Cl_2O_3$
CS_2		0.73	40	215-218	$C_{18}H_{11}Cl_2O_3$
CS_3		0.87	52	230-232	$C_{18}H_{11}Cl_2O_3$
CS_4		0.80	43	283-286	$C_{18}H_{10}Cl_3O_3$
CS_5		0.79	41	295-297	$C_{18}H_{10}Cl_3O_3$

[a]Products were characterized by IR, NMR.[b]Rf value, [c]Synthesized yields. [d]M.P. are uncorrected.

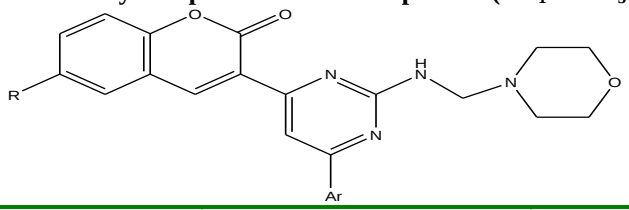
Table II: Physical parameter of compounds (CT_1-CT_5).



Compound [a]	-Ar	Rf value[b]	Yield (%)[c]	Melting point (°c)[d]	Molecular formula
CT_1		0.71	55	181-184	$C_{19}H_{11}Cl_2N_3O_2$
CT_2		0.68	48	195-198	$C_{19}H_{11}Cl_2N_3O_2$
CT_3		0.73	61	192-196	$C_{19}H_{11}Cl_2N_3O_2$
CT_4		0.64	45	200-206	$C_{19}H_{10}Cl_3N_3O_2$
CT_5		0.72	49	202-208	$C_{19}H_{10}Cl_3N_3O_2$

[a]Products were characterized by IR, NMR.[b]Rf value, [c]Synthesized yields. [d]M.P. are uncorrected.

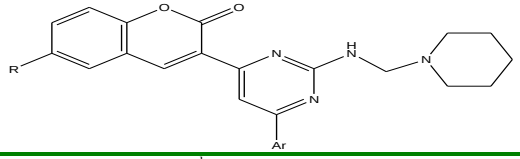
Table III: Physical parameters of compounds (CT_1M-CT_5M)



Compound [a]	-Ar	Rf value[b]	Yield (%)[c]	Melting point (°c)[d]	Molecular formula
CT_1M		0.62	51.7	185-188	$C_{24}H_{20}Cl_2N_4O_3$
CT_2M		0.71	58.4	195-197	$C_{24}H_{20}Cl_2N_4O_3$
CT_3M		0.67	55.6	182-186	$C_{24}H_{20}Cl_2N_4O_3$
CT_4M		0.72	45.3	172-176	$C_{24}H_{19}Cl_3N_4O_3$
CT_5M		0.70	43.6	184-188	$C_{24}H_{19}Cl_3N_4O_3$

[a]Products were characterized by IR, NMR.[b]Rf value, [c]Synthesized yields. [d]M.P. are uncorrected.

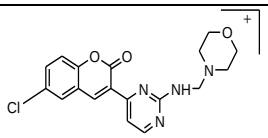
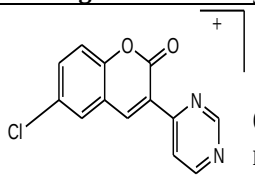
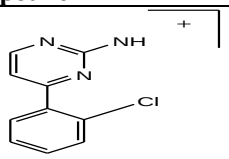
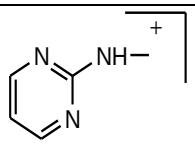
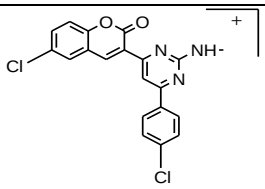
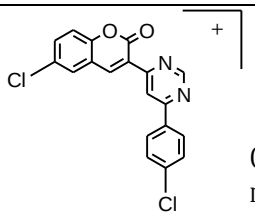
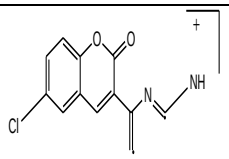
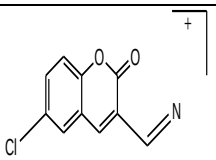
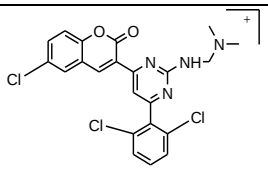
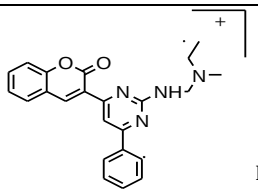
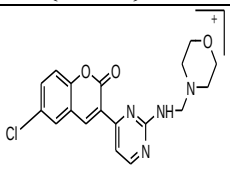
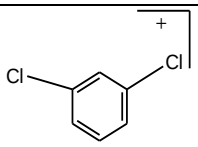
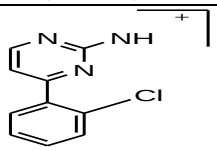
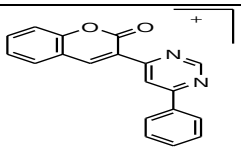
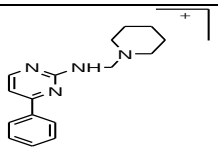
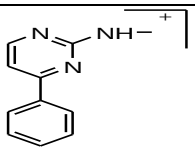
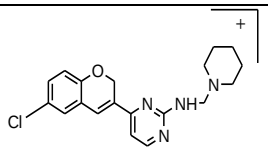
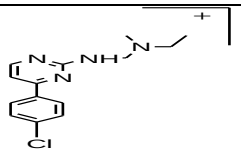
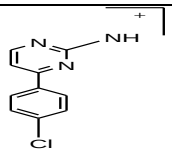
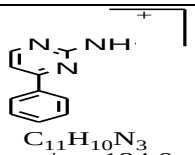
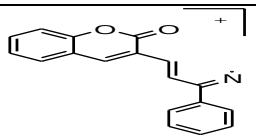
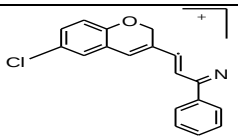
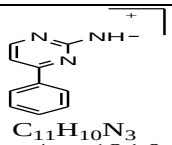
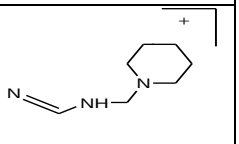
Table IV: Physical parameter of compound (CT_1P-CT_5P)



Compound[a]	-Ar	Rf value[b]	Yield (%)[c]	Melting point (°c)[d]	Molecular formula
CT_1P		0.60	52.7	180-184	$C_{25}H_{22}Cl_2N_4O_2$
CT_2P		0.77	63.9	178-181	$C_{25}H_{22}Cl_2N_4O_2$
CT_3P		0.69	61.2	176-178	$C_{25}H_{22}Cl_2N_4O_2$
CT_4P		0.74	56.5	188-193	$C_{25}H_{21}Cl_3N_4O_2$
CT_5P		0.76	58.4	184-188	$C_{25}H_{21}Cl_3N_4O_2$

[a]Products were characterized by IR, NMR.[b]Rf value, [c]Synthesized yields. [d]M.P. are uncorrected.

Table V: Mass fragmentation of synthesized compound

CT_1M	 $C_{18}H_{16}ClN_4O_3$ m/z= 371.091	 $C_{13}H_6ClN_2O_2$ m/z = 257.0	 $C_{10}H_7ClN_3$ m/z = 204.033	 $C_5H_6N_3$ m/z = 108.0 (100 %)
CT_3M	 $C_{20}H_{12}Cl_2N_3O_2$ m/z = 396.031	 $C_{19}H_9Cl_2N_2O_2$ m/z = 368.1	 $C_{12}H_6ClN_2O_2$ m/z = 245.0 (100 %)	 $C_{10}H_4ClNO_2$ m/z = 205.1
CT_5M	 $C_{22}H_{15}Cl_3N_4O_2$ m/z = 475.3 (100 %)	 $C_{23}H_{21}N_4O_2$ m/z = 385.166	 $C_{18}H_{16}ClN_4O_3$ m/z = 372.9	 $C_6H_3Cl_2$ m/z = 145.0
CT_1P	 $C_{10}H_7ClN_3$ m/z = 203.1 (100 %)	 $C_{19}H_{11}N_2O_2$ m/z = 299.2	 $C_{16}H_{19}N_4$ m/z = 267.1	 $C_{11}H_{10}N_3$ m/z = 185.1
CT_3P	 $C_{19}H_{19}ClN_4O$ m/z = 355.3	 $C_{14}H_{15}ClN_4$ m/z = 276.0 (100 %)	 $C_{10}H_7ClN_3$ m/z = 204.0	 $C_{11}H_{10}N_3$ m/z = 184.0
CT_5P	 $C_{18}H_{11}NO_2$ m/z = 274.0 (100 %)	 $C_{18}H_{11}ClNO$ m/z = 292.1	 $C_{11}H_{10}N_3$ m/z = 184.0	 $C_7H_{14}N_3$ m/z = 140.0