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Synthesis towards 4, 6-Disubstituted Pyrimidines via Chalcone Derivatives and Their Biological Evaluation

DS Kundariya*[1], NK Joshi[2] and PK Godhaviya[2]

[1]Department of Chemistry, Tolani College of Arts and Science, Adipur (Kutch),
[2]Department of Chemistry, Maharaja Shree Mahendrasinhji Science College, Morbi, Gujarat (India)

ABSTRACT

Due to the rapidly growing number of resistant strains of bacteria, the search for antimicrobial agents with new modes of action will always remain an important and challenging task. Thus, the reaction of methyl 4-((E)-3-((z)phenyl)acryloyl)-2-methylbenzoate derivatives **4(a-k)** with urea and guanidine hydrochloride in presence of basic catalyst, furnished compounds **5(a-k) and 6(a-k)** respectively. Representative compounds were assigned on the basis of elemental analysis, IR, ^{1}H NMR and mass spectroscopy and further tested for their antimicrobial activity against gram-positive and gram-negative bacteria. Their MICs were then determined. Many showed a broad spectrum of activity while most of the other compounds showed varying antimicrobial activity.

Keywords: Pyrimidines; Pharmacology; IR; NMR; Elemental analysis.

Corresponding author

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INTRODUCTION

The discovery of this class of compounds provides an outstanding case history of modern drug development and also emphasizes the unpredictability of biological activity from structural modification of a prototype drug molecule. Considerable interest has been focused on the pyrimidine structure, which is known to possess a broad spectrum of biological activities.The basic skeleton of chalcones which possess α,β-unsaturated carbonyl group is useful as the starting material for the synthesis of various heterocyclic compounds of physiological importance. The presence of enone functionality in chalcone moiety is the key factor for its biological activity as agrochemical [1], antimalarial [2], antiviral [3], UV absorbers [4] etc. Further, the importance of pyrimidines and analogous compounds in pharmaceutical and biological fields [5, 6] is well known. With the development of clinically useful pyrimidine based antiviral [7] drugs there has been noticeable interest in synthetic manipulations of pyrimidine derivatives. Pyrimidines are associated with various biological activities [8-17] and this ring system is also present in vitamin B_2 and folic acid.

Observations in the literature on the wide spectrum of pharmacological effects of various pyrimidine derivatives prompted us to plan the synthesis of pyrimidines by treating guanidine hydrochloride as well as urea in presence of basic catalyst with various chalcones to obtain a series of substituted pyrimidine derivatives. The presence of nitrogen in the derived compounds was anticipated to impart towards their antinociceptive and antimicrobial activity. In this present work, interest was expressed in synthesizing some novel pyrimidines **5(a-k) and 6(a-k)** for evaluation as antimicrobial agents and against the medically important pathogenic fungi.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass plates. The spots were developed in iodine chamber and visualized under ultraviolet lamp. Infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds in SHIMADZU FTIR 8400 Spectrophotometer and BRUKER Spectrometer (400 MHz) respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analysis was undertaken with Perkin Elmer 2400 instrument and the measured values agreed with the calculated.

Chemistry

The chalcone derivatives have been synthesized by the condensation of methyl 4-acetyl-2-methylbenzoate with different aryl aldehydes in the presence of 40% alcoholic NaOH while pyrimidine derivatives have been synthesized by the cyclocondensation of chalcones with urea/guanidine hydrochloride in the presence of basic catalyst using ethanol as a solvent according to scheme-1.

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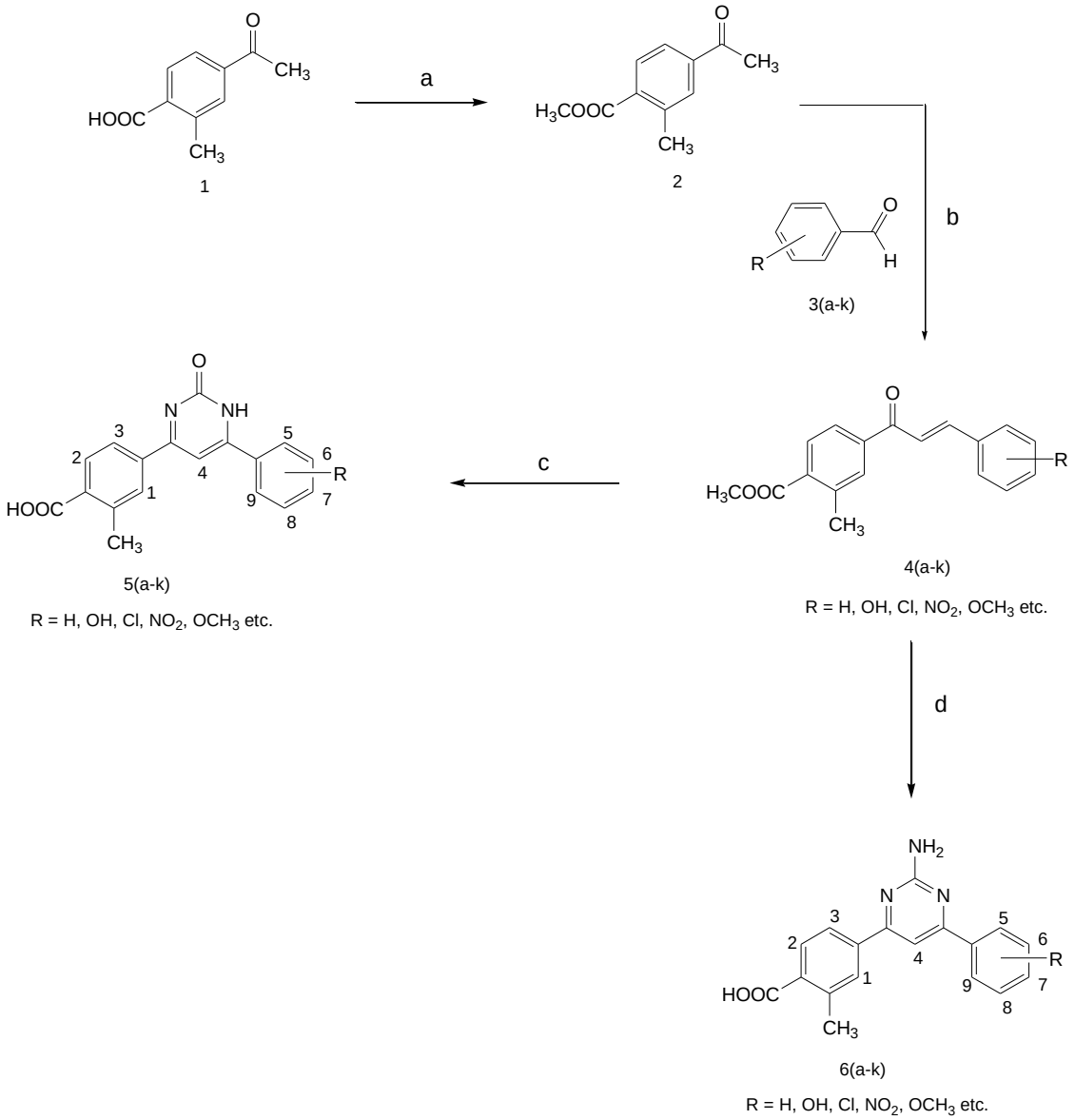

Scheme 1. Synthetic pathway for the compounds 6(a-k)

(**Reagents and conditions:** (a) Methanol, Con. H_2SO_4, reflux 5 h (b) 40% NaOH, Methanol, RT, 3-4.5 h (c) Ethanol, Urea, KOH, reflux 12-14 h (d) Ethanol, Guanidine hydrochloride, KOH, reflux 10-13 h)

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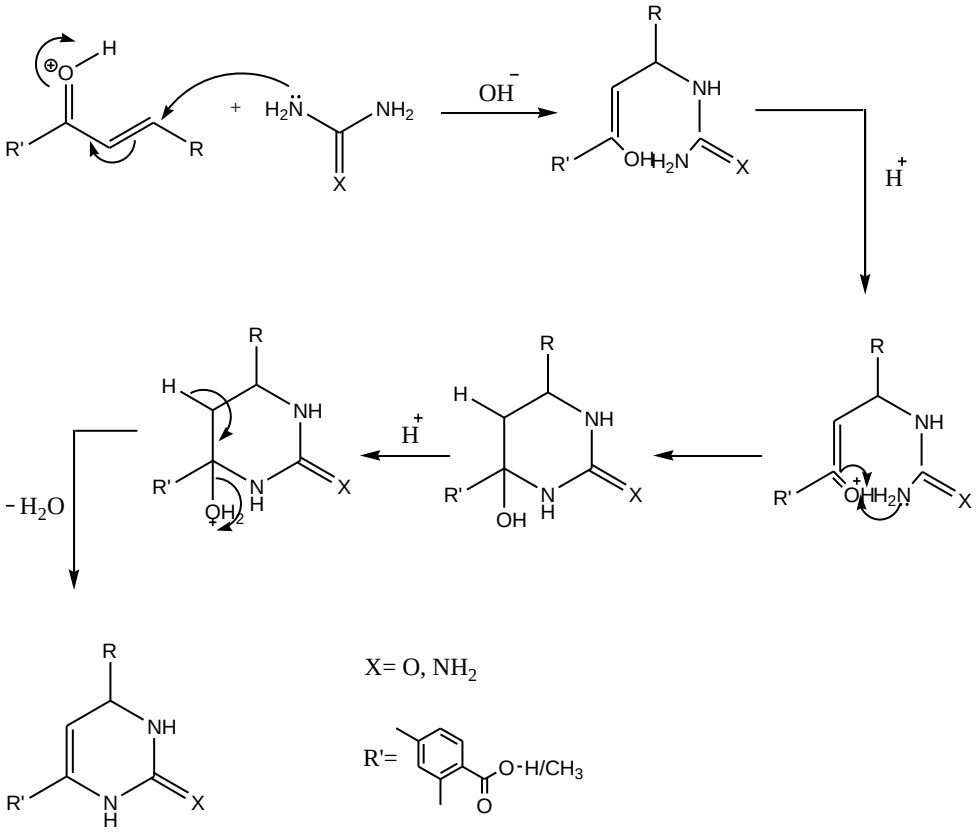
Mechanism



General procedure for the preparation of methyl 4-acetyl-2-methylbenzoate (2).

To a solution of 4-acetyl-2-methylbenzoic acid (0.01 mol, 1.78 gm) in methanol (20 ml), Con. Sulphuric acid (0.01 mol, 0.53 ml) was added and reflux for 5.0 hrs. During the reaction the progress and the completion of reaction were checked by silica gel-G F_{254} thin layer chromatography using ethyl acetate: hexane (7: 3) as a mobile phase. After the reaction to be completed, excess methanol was distilled off; light yellow color oil obtained was used for the next step, (Scheme 1).

General procedure for the preparation of Chalcones 4(a-k).

To a solution of methyl 4-acetyl-2-methylbenzoate (0.01 mol) and aromatic aldehydes (0.01 mol) in methanol (25 ml) and 40% aq. Solution sodium hydroxide was added till the solution become basic (pH= >10) at room temperature (25-30^{0}C) . The reaction mixture was stirred for 3.0-4.5 hrs at room temperature. During the reaction the progress and the completion of reaction were checked by silica gel-G F_{254} thin layer chromatography using ethyl acetate: hexane (7: 3) as a mobile phase. After completion of reaction the content was poured in to crushed ice. Upon neutralization the solid separated was filtered out and crystallized from ethanol (Scheme 1).

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General procedure for the preparation of Oxopyrimidines 5(a-k).

A mixture of appropriate methyl 4-((E)-3-(Z)acryloyl)-2-methylbenzoate 4(a-k) (0.01 mol) and urea (0.01 mol) in absolute ethanol (20 ml) was refluxed on water bath in presence of alcoholic KOH for 12-14 hrs. During the reaction the progress and the completion of reaction were checked by silica gel-G F_{254} thin layer chromatography using Toluene: Methanol (8 : 2) as a mobile phase. After completion of reaction excess solvent was distilled out and the residue was neutralized with 10% aq. HCl solution, the separated solid was filtered out and crystallized from methanol(3-5 times) (Scheme 1).

4-(1,2-dihydro-6-(2-hydroxyphenyl)-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5a).

Yield 55%, mp. 131 ºC, IR (KBr) cm^{-1}: 3321 (OH), 3045–3053 (CH), 1713 (C=O of acid), 1672 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.50 (s, 3H, CH3), 5.49 (s, 1H, H4), 6.67-6.71 (m, 2H, H6,H8), 6.98–7.01 (m, 2H, H7,H9), 7.32–7.33 (d, 1H, H3), 7.36 (s, 1H, H1), 7.89–7.90 (d, 1H, H2), 8.01 (s, 1H, NH), 11.00 (s, 1H, OH), 11.76 (s, 1H, COOH); Mass (*m/z*): 322, Anal. (%) for C18H14N2O4, Calcd. C, 67.07; H, 4.38; N, 8.69; O, 19.86; Found: C, 67.00; H, 4.45; N, 8.68; O, 19.80.

4-(1,2-dihydro-6-(3-nitrophenyl)-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5b).

Yield 50%, mp. 224 ºC, IR (KBr) cm^{-1}: 3054–3063 (CH), 1718 (C=O of acid), 1677 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.56 (s, 3H, CH3), 5.51 (s, 1H, H4), 7.01-7.05 (m, 1H, H8), 7.49-7.55 (m, 3H, H1,H3,H9), 8.04-8.09 (m, 2H, H2,H7), 8.12 (s, 1H, NH), 8.21 (s, 1H, H5), 11.80 (s, 1H, COOH); Mass (*m/z*): 351, Anal. (%) for C18H13N3O5, Calcd. C, 61.54; H, 3.73; N, 11.96; O, 22.77; Found: C, 61.41; H, 3.83; N, 11.94; O, 22.81.

4-(1,2-dihydro-6-(4-hydroxyphenyl)-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5c).

Yield 51%, mp. 124 ºC, IR (KBr) cm^{-1}: 3311 (OH), 3033–3046 (CH), 1710 (C=O of acid), 1675 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.44 (s, 3H, CH3), 5.09 (s, 1H, H4), 6.74-6.78 (m, 2H, H6,H8), 7.11–7.15 (m, 2H, H5,H9), 7.61-763 (m, 1H, H3), 7.67 (s, 1H, H1), 8.05 (s, 1H, NH), 8.10-8.11 (d, 1H, H2), 10.04 (s, 1H, OH), 11.83 (s, 1H, COOH); Mass (*m/z*): 322, Anal. (%) for C18H14N2O4, Calcd. C, 67.07; H, 4.38; N, 8.69; O, 19.86; Found: C, 67.17; H, 4.49; N, 8.69; O, 19.91.

4-(6-(4-(dimethylamino)phenyl)-1,2-dihydro-2-oxopyrimidin-4-yl)-2-methylbenzoicacid (5d).

Yield 55%, mp. 145 ºC, IR (KBr) cm^{-1}: 3041–3053 (CH), 1716 (C=O of acid), 1669 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.42 (s, 3H, CH3), 2.82 (s, 6H, (CH3)2), 4.90 (s, 1H, H4), 6.64-6.67 (m, 2H, H6,H8), 7.11–7.14 (m, 2H, H5,H9), 7.58–7.61 (m, 2H, H1,H3), 8.08 (s, 1H, NH), 8.08-8.10(d, 1H, H2), 10.98 (s, 1H, COOH); Mass (*m/z*): 349, Anal. (%) for C20H19N3O3, Calcd. C, 68.75; H, 5.48; N, 12.03; O, 13.74; Found: C, 69.02; H, 5.49; N, 12.08; O, 13.77.

4-(1,2-dihydro-6-(4-hydroxy-3-methoxyphenyl)-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5e).

Yield 65%, mp. 160 ºC, IR (KBr) cm^{-1}: 3311 (OH), 3067–3072 (CH), 1722 (C=O of acid), 1675 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.38 (s, 3H, CH3), 3.78 (s, 3H, OCH3), 5.12 (s, 1H, H4), 6.53-6.54 (d, 1H, H8), 6.71 (s, 1H, H5), 6.90–6.94 (m, 1H, H9), 7.64–7.69 (m, 2H, H1,H3), 8.00 (s, 1H, NH), 8.11-8.12(d, 1H, H2), 10.08 (s, 1H, OH), 11.35 (s, 1H, COOH); Mass (*m/z*): 352, Anal. (%) for C19H16N2O5, Calcd. C, 64.77; H, 4.58; N, 7.95; O, 22.70; Found: C, 64.62; H, 4.65; N, 7.93; O, 22.72.

4-(6-(4-chlorophenyl)-1,2-dihydro-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5f).

Yield 62%, mp. 114 ºC, IR (KBr) cm^{-1}: 3023–3030 (CH), 1715 (C=O of acid), 1668 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.42 (s, 3H, CH3), 5.00 (s, 1H, H4), 7.12-7.15 (m, 2H, H6,H8), 7.20–7.24 (m, 2H, H5,H9), 7.58-7.61 (m, 1H, H3), 7.65 (s, 1H, H1), 8.03 (s, 1H, NH), 8.12-8.14 (d, 1H, H2), 11.90 (s, 1H, COOH); Mass (*m/z*): 340, Anal. (%) for C18H13ClN2O3, Calcd. C, 63.44; H, 3.85; N, 8.22; O, 14.09; Found: C, 63.40; H, 3.89; N, 8.20; O, 14.13.

4-(1,2-dihydro-6-(4-nitrophenyl)-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5g).

Yield 50%, mp. 198 ºC, IR (KBr) cm^{-1}: 3042–3050 (CH), 1721(C=O of acid), 1672 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.45 (s, 3H, CH3), 5.22 (s, 1H, H4), 7.42-7.45 (m, 2H, H5,H9), 7.62-7.63 (d, 1H, H3), 7.68 (s, 1H, H1), 8.10-8.11 (d, 1H, H2), 8.13 (s, 1H, NH), 8.18-8.22 (m, 2H, H6,H8), 12.11 (s, 1H, COOH); Mass (*m/z*): 351, Anal. (%) for C18H13N3O5, Calcd. C, 61.54; H, 3.73; N, 11.96; O, 22.77; Found: C, 61.50; H, 3.89; N, 11.90; O, 22.80.

4-(1,2-dihydro-2-oxo-6-phenylpyrimidin-4-yl)-2-methylbenzoic acid (5h).

Yield 61%, mp. 130 ºC, IR (KBr) cm^{-1}: 3023–3033 (CH), 1718 (C=O of acid), 1666 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.35 (s, 3H, CH3), 5.02 (s, 1H, H4), 7.10-7.16 (m, 3H, H6,H7,H8), 7.22-7.25 (m, 2H, H5,H9), 7.60 (s, 1H, H1), 7.66-7.67 (d, 1H, H3), 8.03 (s, 1H, NH), 8.12-8.13 (d, 1H, H2), 11.01 (s, 1H, COOH); Mass (*m/z*): 306, Anal. (%) for C18H14N2O3, Calcd. C, 70.58; H, 4.61; N, 9.15; O, 15.67; Found: C, 70.50; H, 4.52; N, 9.10; O, 15.70.

4-(1,2-dihydro-6-(4-methoxyphenyl)-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5i).

Yield 69%, mp. 119 ºC, IR (KBr) cm^{-1}: 3020-3030 (CH), 1717 (C=O of acid), 1665 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.32 (s, 3H, CH3), 3.75 (s, 3H, OCH3), 4.94 (s, 1H, H4), 6.87-6.91 (m, 2H, H6,H8), 7.10–7.14 (m, 2H, H5,H9), 7.55-7.56 (d, 1H, H3), 7.60 (s, 1H, H1), 8.07 (s, 1H, NH), 8.14-8.15 (d, 1H, H2), 11.88 (s, 1H, COOH); Mass (*m/z*): 336, Anal. (%) for C19H16N2O4, Calcd. C, 67.85; H, 4.79; N, 8.33; O, 19.03; Found: C, 67.80; H, 4.79; N, 8.30; O, 19.13.

4-(6-(2-chlorophenyl)-1,2-dihydro-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5j).

Yield 68%, mp. 105 ºC, IR (KBr) cm^{-1}: 3025–3033 (CH), 1717 (C=O of acid), 1668 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.42 (s, 3H, CH3), 5.29 (s, 1H, H4), 6.90-6.94 (m, 2H, H6,H9), 7.01–7.05 (m, 2H, H7,H8), 7.29–7.30 (d, 1H, H3), 7.41 (s, 1H, H1), 7.87–7.88 (d, 1H, H2), 8.06 (s, 1H, NH), 11.79 (s, 1H, COOH); Mass (*m/z*): 340, Anal. (%) for C18H13ClN2O3, Calcd. C, 63.44; H, 3.85; N, 8.22; O, 14.09; Found: C, 63.30; H, 3.80; N, 8.27; O, 14.10.

4-(6-(4-fluorophenyl)-1,2-dihydro-2-oxopyrimidin-4-yl)-2-methylbenzoic acid (5k).

Yield 71%, mp. 110 ºC, IR (KBr) cm^{-1}: 3033–3038 (CH), 1710 (C=O of acid), 1665 (C=O), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.52 (s, 3H, CH3), 5.50 (s, 1H, H4), 7.22-7.24 (t, 2H, H6,H8), 7.34–7.38 (m, 2H, H5,H9), 7.40-7.43 (d, 1H, H3), 7.47 (s, 1H, H1), 7.78-7.88 (m, 2H, H2 & NH), 12.91 (s, 1H, COOH); Mass (*m/z*): 324, Anal. (%) for C18H13FN2O3, Calcd. C, 66.66; H, 4.04; N, 8.64; O, 14.80; Found: C, 66.53; H, 4.00; N, 8.63; O, 14.83.

General procedure for the preparation of 2-aminopyrimidines 6(a-k).

A mixture of methyl 4-((E)-3-(4-flourophenyl)acryloyl)-2-methylbenzoate 4(a-k) (0.01 mol) and guanidine hydrochloride (0.01 mol) in ethanol (20 ml) was refluxed on water bath in presence of alcoholic KOH for 10 hrs. During the reaction the progress and the completion of reaction were checked by silica gel-G F_{254} thin layer chromatography using Toluene: Methanol (7 : 3) as a mobile phase. After completion of reaction excess solvent was distilled off and the residue was neutralized with 10% HCl, the separated solid was filtered out and crystallized from ethanol (5-6 times) (Scheme 1).

4-(2-amino-6-(2-hydroxyphenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6a).

Yield 54%, mp. 159 ºC, IR (KBr) cm^{-1}: 3410 (NH), 3321 (OH), 3035–3048 (CH), 1708 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.58 (s, 3H, CH3), 6.81-6.84 (m, 2H, H6,H8), 6.89 (s, 1H, H4), 7.05–7.08 (m, 2H, H7,H9), 7.12 (s, 2H, NH2), 7.37–7.38 (d, 1H, H3), 7.41 (s, 1H, H1), 8.03–8.04 (d, 1H, H2), 10.05 (s, 1H, OH), 11.66 (s, 1H, COOH); Mass (*m/z*): 321, Anal. (%) for C18H15N3O3, Calcd. C, 67.28; H, 4.71; N, 13.18; O, 14.94; Found: C, 67.30; H, 4.82; N, 13.22; O, 15.00.

4-(2-amino-6-(3-nitrophenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6b).

Yield 45%, mp. 199 ºC, IR (KBr) cm^{-1}: 3400 (NH), 3041–3044 (CH), 1714 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.59 (s, 3H, CH3), 6.92 (s, 1H, H4), 7.20 (s, 2H, NH2), 7.46-7.51 (m, 2H, H3,H8), 7.52 (s, 1H, H1), 7.96-8.01 (m, 2H, H7,H9), 8.11-8.12 (d, 1H, H2), 8.33 (s, 1H, H5), 12.74 (s, 1H, COOH); Mass (*m/z*): 350, Anal. (%) for C18H14N4O4, Calcd. C, 61.71; H, 4.03; N, 15.99; O, 18.27; Found: C, 61.80 H, 3.95; N, 15.90; O, 18.21.

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4-(2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6c).

Yield 50%, mp. 162 ºC, IR (KBr) cm^{-1}: 3400 (NH), 3314 (OH), 3030–3041 (CH), 1707 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.43 (s, 3H, CH3), 6.70-6.73 (m, 2H, H6,H8), 7.00 (s, 1H, H4), 7.15 (s, 2H, NH2),7.21–7.24 (m, 2H, H5,H9), 7.47 (s, 1H, H1), 7.52-7.53 (d, 1H, H3), 8.05-8.06 (d, 1H, H2), 9.56 (s, 1H, OH), 11.80 (s, 1H, COOH); Mass (*m/z*): 321, Anal. (%) for C18H15N3O3, Calcd. C, 67.28; H, 4.71; N, 13.18; O, 14.94; Found: C, 67.20; H, 4.72; N, 13.20; O, 14.90.

4-(2-amino-6-(4-(dimethylamino)phenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6d).

Yield 57%, mp. 175 ºC, IR (KBr) cm^{-1}: 3390 (NH), 3025–3031 (CH), 1701 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.38 (s, 3H, CH3), 2.80 (s, 6H, (CH3)2), 6.67-6.70 (m, 2H, H6,H8), 6.94 (s, 1H, H4), 7.05 (s, 2H, NH2),7.22–7.26 (m, 2H, H5,H9), 7.43 (s, 1H, H1), 7.48-7.49 (d, 1H, H3), 8.00-8.01 (d, 1H, H2), 11.02 (s, 1H, COOH); Mass (*m/z*): 348, Anal. (%) for C20H20N4O2, Calcd. C, 68.95; H, 5.79; N, 16.08; O, 09.18; Found: C, 68.94; H, 5.87; N, 16.08; O, 09.17.

4-(2-amino-6-(4-hydroxy-3-methoxyphenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6e).

Yield 43%, mp. 151 ºC, IR (KBr) cm^{-1}: 3394 (NH), 3301 (OH), 3051–3060 (CH), 1720 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.30 (s, 3H, CH3), 3.85 (s, 3H, OCH3), 6.67-6.68 (d, 1H, H8), 6.80 (s, 1H, H5), 685–6.88 (m, 1H, H9), 6.97 (s, 1H, H4), 7.09 (s, 2H, NH2),7.47 (s, 1H, H1), 7.52–7.53 (d, 1H, H3), 8.13-8.14(d, 1H, H2), 09.87 (s, 1H, OH), 11.56 (s, 1H, COOH); Mass (*m/z*): 351, Anal. (%) for C19H17N3O4, Calcd. C, 64.95; H, 4.88; N, 11.96; O, 18.21; Found: C, 64.90; H, 4.91; N, 11.93; O, 18. 20.

4-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6f).

Yield 59%, mp. 180 ºC, IR (KBr) cm^{-1}: 3388 (NH), 3030–3038 (CH), 1711 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.39 (s, 3H, CH3), 7.08 (s, 1H, H4), 7.25 (s, 2H, NH2), 7.31-7.35 (m, 2H, H6,H8), 7.38–7.41 (m, 2H, H5,H9), 7.48-7.49 (d, 1H, H3), 7.50 (s, 1H, H1), 8.10-8.11 (d, 1H, H2), 12.03 (s, 1H, COOH); Mass (*m/z*): 339, Anal. (%) for C18H14ClN3O2, Calcd. C, 63.63; H, 4.15; N, 12.37; O, 09.42; Found: C, 63.54; H, 4.19; N, 12.30; O, 09.43.

4-(2-amino-6-(4-nitrophenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6g).

Yield 57%, mp. 233 ºC, IR (KBr) cm^{-1}: 3422 (NH), 3047–3050 (CH), 1720(C=O of acid, ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.48 (s, 3H, CH3), 5.58 (s, 2H, NH2), 7.19 (s, 1H, H4), 7.52-7.53 (d, 1H, H3), 7.55 (s, 1H, H1), 8.13-8.14 (d, 1H, H2), 7.40-7.43 (m, 2H, H5,H9), 8.19-8.22 (m, 2H, H6,H8), 12.20 (s, 1H, COOH); Mass (*m/z*): 350, Anal. (%) for C18H14N4O4, Calcd. C, 61.71; H, 4.03; N, 15.99; O, 18.27; Found: C, 61.70; H, 4.09; N, 15.90; O, 18.20.

4-(2-amino-6-phenylpyrimidin-4-yl)-2-methylbenzoic acid (6h).

Yield 53%, mp. 210ºC, IR (KBr) cm^{-1}: 3400 (NH), 3020–3030 (CH), 1717 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.32 (s, 3H, CH3), 6.91 (s, 1H, H4), 7.10 (s, 2H, NH2), 7.20-7.26 (m, 3H, H6,H7,H8), 7.42-7.46 (m, 2H, H5,H9), 7.49 (s, 1H, H1), 7.52-7.53 (d, 1H, H3), 8.10-8.11 (d, 1H, H2), 11.45 (s, 1H, COOH); Mass (*m/z*): 305, Anal. (%) for C18H15N3O2, Calcd. C, 70.81; H, 4.95; N, 13.76; O, 10.48; Found: C, 70.80; H, 4.92; N, 13.70; O, 10.43.

4-(2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6i)

Yield 62%, mp. 176 ºC, IR (KBr) cm^{-1}: 3400 (NH), 3028-3037 (CH), 1726 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.38 (s, 3H, CH3), 3.73 (s, 3H, OCH3), 6.81-6.84 (m, 2H, H6,H8), 6.90 (s, 1H, H4), 7.00 (s, 2H, NH2), 7.20–7.23 (m, 2H, H5,H9), 7.52-7.53 (d, 1H, H3), 7.56 (s, 1H, H1), 8.11-8.12 (d, 1H, H2), 11.79 (s, 1H, COOH); Mass (*m/z*): 335, Anal. (%) for C19H17N3O3, Calcd. C, 68.05; H, 5.11; N, 12.53; O, 14.31; Found: C, 68.10; H, 5.23; N, 12.50; O, 14.22.

4-(2-amino-6-(2-chlorophenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6j).

Yield 68%, mp. 193ºC, IR (KBr) cm^{-1}: 3420 (NH), 3022–3028 (CH), 1721 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.44 (s, 3H, CH3), 6.99 (s, 1H, H4), 7.09 (s, 2H, NH2), 7.11–7.15 (m, 2H, H7,H8), 7.30-7.34 (m, 2H, H6,H9), 7.44 (s, 1H, H1), 7.49–7.50 (d, 1H, H3), 7.52–7.53 (d, 1H, H2), 11.70 (s, 1H, COOH); Mass (*m/z*): 339, Anal. (%) for C18H14ClN3O2, Calcd. C, 63.63; H, 4.15; N, 12.37; O, 09.42; Found: C, 63.60; H, 4.22; N, 12.27; O, 09.40.

4-(2-amino-6-(4-fluorophenyl)pyrimidin-4-yl)-2-methylbenzoic acid (6k).

Yield 70%, mp. 240 ºC, IR (KBr) cm^{-1}: 3430 (NH), 3021–3028 (CH), 1719 (C=O of acid), ^{1}H–NMR (DMSO-*d*6) δ ppm: 2.61 (s, 3H, CH3), 6.85 (s, 1H, H4), 7.25-7.30 (t, 2H, H6,H8), 7.56 (s, 2H, NH2), 7.78–7.85 (m, 2H, H5,H9), 7.89 (s, 1H, H1), 8.08-8.15 (m, 1H, H3), 8.29-8.33 (m, 1H, H2), 12.87 (s, 1H, COOH); Mass (*m/z*): 323, Anal. (%) for C18H14FN3O2, Calcd. C, 66.87; H, 4.36; N, 13.00; O, 09.90; Found: C, 66.80; H, 4.30; N, 13.03; O, 09.89.

RESULTS AND DISCUSSION

Pharmacology

The minimum inhibitory concentrations (MICs) of synthesized compounds were carried out by broth microdilution method as described by Rattan[18]Antibacterial activity was screened against two gram positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenus* MTCC 443) and two gram negative (*Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441) bacteria, ampicillin was used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323, Griseofulvin was used as a standard antifungal agent.
All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and Mueller Hinton broth was used as nutrient media to grow and diluted the drug suspension for

the test. Inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used to dilute to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) were sub cultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted for obtaining 2000 µg/ml concentration, as a stock solution. In primary screening 500 µg/ml, 250 µg/ml and 125 µg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml and 1.56 µg/ml concentrations. The highest dilution showing at least 99 % inhibition is taken as MIC. Results obtained are given in Table 1.

Antibacterial activity

The minimum inhibitory concentrations (MICs) of the tested compounds are shown in Table 1. The different compounds **5(a-k)** and **6(a-k)** were tested for *in vitro* against two gram positive (*S. aureus* MTCC 96, *S. pyogenus* MTCC 443) and two gram negative (*E. coli* MTCC 442, *P. aeruginosa* MTCC 441) bacteria. From the screening data, most of the compounds possessed very good antibacterial activity (MBC, 50-250 µg/ml) against gram positive *S. aureus,* some of them possessed excellent activity compared to ampicillin. Compound **5f**, **5i, 6b** and **6f** showed MBC value in the range between 62.5-100 µg/ml while ampicillin has standard MBC value of 100 µg/ml against gram negative *E. coli* which indicates that this compounds have excellent activity, while other Compound **5c, 5k, 6a** and **6k** possessed MBC value in the range of 200-250 µg/ml against gram negative *E. coli* while **5c and 6b** exhibited very good activity against *P. aeruginosa*. Compounds **5g, 5i, 5k, 6d, 6h** and **6k** displayed moderate activity in the range of 200-250 µg/ml while remaining **5d, 5j, 6d, 6i** and **6k** were equivalent against gram positive *S. aureus* compared with ampicillin. Compound **6h and 6k** have MBC of 50-100 µg/ml which was comparatively good against *S. pyogenus* while compound **5a**, **5g, 5h, 6a, 6d, 6i and 6j** displayed moderate activity in the range of 200-250 µg/ml *S. pyogenus* as compare to ampiciline. The remaining pyrimidine derivatives possessed moderate to poor activity against all four bacterial species.

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Antifungal activity

The minimum inhibitory concentrations (MICs) of the synthesized compounds are shown in Table 1. For *in vitro* antifungal activity, three fungal species *C, albicans* MTCC 227, *A. niger* MTCC 282 and *A. clavatus* MTCC 1323 were used and compared with standard drug griseofulvin. Most of the compounds possessed very good antifungal activity against *C. albicans*; their MFC values were in the range between 100-500 µg/ml. Compounds **5a, 6a** and **6k** showed excellent activity of 200-250 µg/ml; **5b, 5d, 5j, 6c, 6f** and **6j** possessed very good activity of 500 µg/ml which is similar to griseofulvin (500 µg/ml) against *C. albicans* whereas remaining compounds possessed moderate to poor activity against *A. niger* and *A. clavatus* compared with griseofulvin.

Table-1. Antimicrobial activity of Compounds 5(a-k) and 6(a-k)

Comp.	Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
	Gram negative		Gram positive				
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A. niger*	*A. clavatus*
5a	500	1000	500	250	250	500	1000
5b	1000	1000	500	500	500	500	200
5c	200	50	500	500	1000	250	500
5d	500	1000	250	1000	500	500	500
5e	1000	500	1000	500	>1000	1000	500
5f	62.5	500	500	1000	1000	200	250
5g	500	200	1000	250	>1000	500	1000
5h	500	1000	250	200	1000	1000	>1000
5i	100	1000	250	500	1000	>1000	250
5j	1000	250	200	1000	500	500	500
5k	250	250	500	1000	1000	1000	1000
6a	250	500	500	250	200	250	>1000
6b	100	62.5	500	1000	1000	>1000	250
6c	1000	500	1000	500	500	250	500
6d	1000	250	200	200	>1000	200	1000
6e	500	500	1000	1000	1000	500	500
6f	100	100	1000	500	500	>1000	200
6g	500	500	500	500	1000	500	250
6h	500	250	500	50	1000	1000	>1000
6i	1000	1000	200	200	>1000	500	500
6j	500	1000	1000	250	500	500	1000
6k	200	200	200	100	250	1000	500
Ampicillin	100	100	250	100	-	-	-
Griseofulvin	-	-	-	-	500	100	100

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CONCLUSION

From the above screening results, the pyrimidine derivatives which contains fluoro group is more active while compounds containing -OCH_3 are also active against the microbial species. From the above discussions we concluded that fluoro group is more active group against the bacteria and fungi. The newly synthesized compounds exhibited promising antibacterial activities against *E. coli*, *S. aureus,* & *P. aeruginosa* and exhibited excellent antifungal activity against *C. albicans*. These results make novel pyrimidines interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of antimicrobial agents.

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REFERENCES

[1] (a)Lafon L Ger Offen. 2010; 180, Chem Abstr 1970; 73: 120342. (b) Misra SS, Tewari RS. J Ind Chem Soc 1973; 1: 68.
[2] Li R, Kenyon GL, Cohen FE, Chen X, Gong B, Dominguez JN, Davidson E, Kurzban G, Miller RE, Nuzum EO, Rosenthal PJ, McKerrow JH. J Med Chem 1995; 38(26): 5031.
[3] Onyilagha JC, Malhotra B, Elder M, Christopher J, Towers GH. Can J Plant Pathol 1997; 19(2): 133; Chem Abstr 1997; 127(12): 157896.
[4] Ikeda T, Sakuta K. Jpn Kokai Tokkyo Koho JP 05,178,865; Chem Abstr 1993; 119: 188298.
[5] (a) Townsend LB, Drach JC. US 6,342,501; Chem Abstr 2002; 136(9): 134778. (b) Gangjee A, Zeng Y, McGuire JJ, Kisluik RL. J Med Chem 2000; 43(16): 3125.
[6] Huang J, Huang Z Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,298,743 Chem Abstr 2002; 136(18): 284403.
[7] Marquez VE, Russ PL, PCT Int. Appl. WO 02 08,204, Chem Abstr 2002; 136(8): 118704.
[8] Agarwal A, Srivastava K, Puri SK, Sinha S, Chauhan PMS, Bioorg Med Chem Lett 2005; 15: 4923.
[9] Grigoryan LA, Kaldrikyan MA, Melik-ogandzhanyan RG, Arsenyan FG, Stepanyan GM, Garibdzhanyan BG. Pharm Chem J 2005; 18: 468.
[10] Sayed HH, Shamroukh AH, Rashad AE. Acta Pharm 2006; 56: 231.
[11] Venkatesan J, Pandeya SN, Selvakumar D. Ind J Pharm Sci 2007; 69: 586.
[12] Munawar MA, Azad M, Siddiqui HL, Nasim FH. J Chin Chem Soc 2008; 55: 394.
[13] Moustafa AH, Saad HA, Shehab WS, El-Mobayed MM. Phosphorus Sulfur Silicon Rel Elem 2008; 183: 115.
[14] Grigoryan LA, Kaldrikyan MA, Melik-Ogandzhanyan RG, Arsenyan FG. Pharm Chem J 2008; 42: 115.
[15] Pandya SS, Chowdhary PVR. Ind J Pharm Sci 2008; 70: 208.
[16] Amir M, Akhtar S, Kumar JH. Acta Pharm 2008; 58: 467.

[17] Xie F, Zhao H, Zhao L, Lou L, Hu Y. Bioorg Med Chem Lett 2009; 19: 275.
[18] Rattan A, Antimicrobials in laboratory medicine, Churchill B I, Livingstone, New Delhi, 2000; 85.