Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(9):105-111
(http://derpharmachemica.com/archive.html)

Aqua mediated sodium acetate catalysed one-pot synthesis of pyrimidine derivatives as anti-inflammatory and antioxidant agent

Dipen K. Sureja[1*], Sandip P. Dholakia[2] and Kantilal R. Vadalia[3]

[1]*Department of Pharmacy, Sumandeep Vidyapeeth, Po - Piparia, Ta - Waghodia, Vadodara - 391760, Gujarat, India*
[2]*Shankersinh Vaghela Bapu Institute of Pharmacy, Po - Vasan, Gandhinagar - 382650, Gujarat, India*
[3]*Atmiya Institute of Pharmacy, Yogidham Gurukul, Kalawad road, Rajkot - 360005, Gujarat, India*

ABSTRACT

The present investigation is focused on one pot synthesis of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto) pyrimidine derivatives with the objective of discovering a novel and potent anti-inflammatory and antioxidant agent. The formation of compounds was recognized by preliminary laboratory techniques like melting point, R_f value and further confirmed by spectral analysis. Furthermore, they were screened in-vitro to study their anti-inflammatory and antioxidant activity, which shows moderate to good potency.

Keywords: One pot synthesis, Pyrimidine derivatives, Anti-inflammatory activity, Antioxidant activity.

INTRODUCTION

Pyrimidine and its derivatives are incessantly pulling attention of the medicinal chemists from the first day of their discovery as important constituents of nucleic acid and are known to have diverse pharmacological activities [1,2] like antimicrobial, antiviral, antifilarial, antimalarial, analgesic, anthelmintic, antihypertensive, anti-HIV and anticancer activity. Certain pyrimidine derivatives are also reported to possess anti-inflammatory [3,4] and antioxidant [4-6] activity. Recently it has been reviewed that pyrimidine and its fused derivatives are widely used as drugs to treat various diseases [7].

The various synthetic routes for pyrimidine derivatives have been extensively reviewed [8-10]. Various reports are also available on one pot synthesis [11-14] of pyrimidine by three component condensation reaction using aromatic aldehydes, ethyl cyanoacetate or malononitrile and urea or thiourea or guanidine using various catalysts. These single step methods are more convenient as compared with two step strategies as it is selective, less time consuming and produce higher yields.

Nowadays, in new drug development process, consideration of ecological point of view is important along with the economy point of view. In any synthesis, the solvents are important, as they are generally used in enormous quantities. Many organic solvents are ecologically harmful, and their use should therefore be minimized as far as possible or even avoided altogether [15]. In the current scenario, organic reactions in water as solvent have attracted much attention, because of its usefulness as a cheap, safe and environment friendly solvent [16]. The enhanced reactivity and selectivity observed in some reactions have been rationalized by various authors as being a consequence of hydrophobic effects and enforced hydrophobic interactions [17].

To the best of our knowledge, there are no reports available on single step synthesis of pyrimidine derivatives using aromatic aldehydes, malononitrile and urea or thiourea. So, herewith we would like to report one-pot and efficient synthesis of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidine derivatives by three-component

condensation of aromatic aldehydes, malononitrile and urea or thiourea using sodium acetate as catalyst and water as solvent in order to investigate its *in-vitro* anti-inflammatory and antioxidant activity.

MATERIALS AND METHODS

All reagents and solvents used were of LR grade, obtained from SD fine chemicals (Mumbai, India) and were used without further purification. The progress of the reaction and purity of the synthesized compounds were checked on the precoated silica gel F_{254} plates obtained from Merck (Mumbai, India) using chloroform and ethyl acetate (7:3) as mobile phase. Iodine chamber and UV lamp (λ = 254 nm) were used for visualization of the spots. Melting points were determined in an open capillary tube on Chemline CL726 melting point apparatus and are uncorrected. Double beam Shimandu 1800 UV spectrophotometer was used for the measurement of absorbance. IR spectra in KBr (ν_{max}, cm^{-1}) were recorded on Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR (δ, ppm) spectra were recorded in $CDCl_3$ or DMSO-d_6 with TMS as internal standard on Bruker advance III NMR spectrophotometer at 400 MHz. Mass spectra were determined using direct inlet probe on a Shimadzu GC-MS QP 2010 mass spectrometer.

General procedure for synthesis of pyrimidine derivatives (1a-1l and 2a-2l):
A mixture of substituted aldehyde (20 mM), malononitrile (20 mM), urea or thiourea (20 mM) and sodium acetate (20 mM) in water (50 mL) was refluxed with stirring for 3.5-6 h (the progress of the reaction being monitored by TLC). The product precipitated from the reaction mixture after cooling was filtered, washed with ice cold water and recrystallized from suitable solvent.

4-amino-2-hydroxy-6-phenylpyrimidine-5-carbonitrile **(1a)**
Reac. time: 6.0 h; Yield: 76% (Ethanol); R_f: 0.72; m.p. 179-180 ºC; IR (KBr, cm^{-1}): 3473, 3233, 2221, 1635, 1286, 1170; ^{1}H NMR ($CDCl_3$, δ): 5.44 (s, 2H, NH_2), 7.55-7.59 (t, 2H, aro. CH), 7.65-7.68 (t, 1H, aro. CH), 7.81 (s, 1H, OH), 7.93-7.95 (d, 2H, aro. CH); MF: $C_{11}H_8N_4O$ (212.21); MS: *m/z* 212 (M^+).

4-amino-2-hydroxy-6-styrylpyrimidine-5-carbonitrile **(1b)**
Reac. time: 5.0 h; Yield: 63% (Ethanol); R_f: 0.67; m.p. 151-153 ºC; IR (KBr, cm^{-1}): 3410, 3238, 3086, 2225, 1687, 1307; ^{1}H NMR ($CDCl_3$, δ): 5.34 (s, 2H, NH_2), 7.26-7.27 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.42-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH), 7.82 (s, 1H, OH); MF: $C_{13}H_{10}N_4O$ (238.24); MS: *m/z* 238 (M^+).

4-amino-2-hydroxy-6-(4-hydroxyphenyl)pyrimidine-5-carbonitrile **(1c)**
Reac. time: 5.0 h; Yield: 77% (EtOH:H_2O, 1:1); R_f: 0.51; m.p. 173-175 ºC; IR (KBr, cm^{-1}): 3493, 3307, 3233, 2231, 1627, 1288; ^{1}H NMR (DMSO-d_6, δ): 5.40 (s, 2H, NH_2), 6.86-6.87 (d, 2H, aro. CH), 7.05 (s, 1H, OH), 7.48-7.50 (d, 2H, aro. CH), 7.65 (s, 1H, OH); MF: $C_{11}H_8N_4O_2$ (228.21); MS: *m/z* 228 (M^+).

4-amino-6-(2-chlorophenyl)-2-hydroxypyrimidine-5-carbonitrile **(1d)**
Reac. time: 4.0 h; Yield: 78% (Ethanol); R_f: 0.65; m.p. 180-182 ºC; IR (KBr, cm^{-1}): 3463, 3225, 2226, 1578, 1290, 749; ^{1}H NMR ($CDCl_3$, δ): 5.18 (s, 2H, NH_2), 7.46-7.49 (m, 1H, aro. CH), 7.57-7.58 (d, 2H, aro. CH), 7.82 (s, 1H, OH), 8.19-8.21 (d, 1H, aro. CH); MF: $C_{11}H_7ClN_4O$ (246.65); MS: *m/z* 246 (M^+), 248 (M^{+2}).

4-amino-6-(4-chlorophenyl)-2-hydroxypyrimidine-5-carbonitrile **(1e)**
Reac. time: 3.5 h; Yield: 79% (Ethanol); R_f: 0.68; m.p. 162-164 ºC; IR (KBr, cm^{-1}): 3445, 3214, 2220, 1571, 1240, 759 ; ^{1}H NMR ($CDCl_3$, δ): 5.30 (s, 2H, NH_2), 7.53-7.56 (d, 2H, aro. CH), 7.76 (s, 1H, OH), 7.87-7.89 (d, 2H, aro. CH); MF: $C_{11}H_7ClN_4O$ (246.65); MS: *m/z* 246 (M^+), 248 (M^{+2}).

4-amino-6-(4-(dimethylamino)phenyl)-2-hydroxypyrimidine-5-carbonitrile **(1f)**
Reac. time: 3.5 h; Yield: 77% (Ethanol); R_f: 0.58; m.p. 191-192 ºC; IR (KBr, cm^{-1}): 3434, 3225, 2215, 1565, 1293, 1169; ^{1}H NMR ($CDCl_3$, δ): 3.17 (s, 6H, $N(CH_3)_2$), 5.33 (s, 2H, NH_2), 6.70-6.72 (d, 2H, aro. CH), 7.48 (s, 1H, OH), 7.83-7.84 (d, 2H, aro. CH); MF: $C_{13}H_{13}N_5O$ (255.28); MS: *m/z* 255 (M^+).

4-amino-2-hydroxy-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile **(1g)**
Reac. time: 3.5 h; Yield: 79% (Ethanol); R_f: 0.60; m.p. 156-157 ºC; IR (KBr, cm^{-1}): 3450, 3237, 2224, 1570, 1263, 1177; ^{1}H NMR ($CDCl_3$, δ): 3.94 (s, 3H, OCH_3), 5.50 (s, 2H, NH_2), 7.03-7.05 (d, 2H, aro. CH), 7.68 (s, 1H, OH), 7.93-7.95 (d, 2H, aro. CH); MF: $C_{12}H_{10}N_4O_2$ (242.23); MS: *m/z* 242 (M^+).

***4-amino-2-hydroxy-6-(4-hydroxy-3-methoxyphenyl)pyrimidine-5-carbonitrile* (1h)**
Reac. time: 5.0 h; Yield: 65% (Ethanol); R_f: 0.52; m.p. 194-196 ºC; IR (KBr, cm^{-1}): 3486, 3322, 3227, 2221, 1630, 1165; ^{1}H NMR ($CDCl_3$, δ): 3.98 (s, 3H, OCH_3), 5.14 (s, 2H, NH_2), 7.01-7.03 (d, 1H, aro. CH), 7.26 (s, 1H, OH), 7.30-7.32 (dd, 1H, aro. CH), 7.63 (s, 1H, OH), 7.73 (d, 1H, aro. CH); MF: $C_{12}H_{10}N_4O_3$ (258.23); MS: *m/z* 258 (M^+).

***4-amino-2-hydroxy-6-(2-nitrophenyl)pyrimidine-5-carbonitrile* (1i)**
Reac. time: 3.5 h; Yield: 83% (Ethanol); R_f: 0.59; m.p. 199-200 ºC; IR (KBr, cm^{-1}): 3445, 3230, 2223, 1601, 1529, 1347; ^{1}H NMR ($CDCl_3$, δ): 5.18 (s, 2H, NH_2), 7.82-7.85 (m, 2H, aro. CH), 7.89-7.92 (m, 1H, aro. CH), 8.37-8.39 (dd, 1H, aro. CH), 7.87 (s, 1H, OH); MF: $C_{11}H_7N_5O_3$ (257.20); MS: *m/z* 257 (M^+).

***4-amino-2-hydroxy-6-(4-nitrophenyl)pyrimidine-5-carbonitrile* (1j)**
Reac. time: 3.5 h; Yield: 80% (Ethanol); R_f: 0.64; m.p. 221-223 ºC; IR (KBr, cm^{-1}): 3432, 3226, 2211, 1629, 1533, 1354; ^{1}H NMR ($CDCl_3$, δ): 5.13 (s, 2H, NH_2), 7.91 (s, 1H, OH), 8.09-8.11 (d, 2H, aro. CH), 8.40-8.43 (d, 2H, aro. CH); MF: $C_{11}H_7N_5O_3$ (257.20); MS: *m/z* 257 (M^+).

***4-amino-2-hydroxy-6-(3-methoxyphenyl)pyrimidine-5-carbonitrile* (1k)**
Reac. time: 4.0 h; Yield: 75% (Ethanol); R_f: 0.73; m.p. 167-169 ºC; IR (KBr, cm^{-1}): 3445, 3243, 2215, 1570, 1256, 1170; ^{1}H NMR ($CDCl_3$, δ): 3.93 (s, 3H, OCH_3), 5.33 (s, 2H, NH_2), 7.00-7.02 (d, 1H, aro. CH), 7.45-7.50 (m, 3H, aro. CH), 7.65 (s, 1H, OH); MF: $C_{12}H_{10}N_4O_2$ (242.23); MS: *m/z* 242 (M^+).

***4-amino-2-hydroxy-6-(3-nitrophenyl)pyrimidine-5-carbonitrile* (1l)**
Reac. time: 4.0 h; Yield: 76% (Ethanol); R_f: 0.69; m.p. 160-162 ºC; IR (KBr, cm^{-1}): 3420, 3221, 2223, 1632, 1529, 1351; ^{1}H NMR ($CDCl_3$, δ): 5.24 (s, 2H, NH_2), 7.77-7.79 (dd, 1H, aro. CH), 7.84 (s, 1H, OH), 8.11-8.13 (dd, 1H, aro. CH), 8.52 (m, 1H, aro. CH), 8.62 (s, 1H, aro. CH); MF: $C_{11}H_7N_5O_3$ (257.20); MS: *m/z* 257 (M^+).

***4-amino-2-mercapto-6-phenylpyrimidine-5-carbonitrile* (2a)**
Reac. time: 6.0 h; Yield: 76% (Ethanol); R_f: 0.76; m.p. 152-153 ºC; IR (KBr, cm^{-1}): 3464, 3219, 2227, 1623, 1347, 1017; ^{1}H NMR ($CDCl_3$, δ): 4.81 (s, 1H, SH), 5.48 (s, 2H, NH_2), 7.56-7.59 (t, 2H, aro. CH), 7.64-7.68 (t, 1H, aro. CH), 7.93-7.95 (d, 2H, aro. CH); MF: $C_{11}H_8N_4S$ (228.27); MS: *m/z* 228 (M^+).

***4-amino-2-mercapto-6-styrylpyrimidine-5-carbonitrile* (2b)**
Reac. time: 5.0 h; Yield: 60% (Ethanol); R_f: 0.73; m.p. 118-120 ºC; IR (KBr, cm^{-1}): 3450, 3239, 3079, 2228, 1681, 1315; ^{1}H NMR ($CDCl_3$, δ): 4.82 (s, 1H, SH), 5.23 (s,2H, NH_2), 7.25-7.26 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.43-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH); MF: $C_{13}H_{10}N_4S$ (254.31); MS: *m/z* 254 (M^+).

***4-amino-6-(4-hydroxyphenyl)-2-mercaptopyrimidine-5-carbonitrile* (2c)**
Reac. time: 5.0 h; Yield: 75% (EtOH:H_2O, 1:1); R_f: 0.48; m.p. 141-142 ºC; IR (KBr, cm^{-1}): 3499, 3323, 3219, 2220, 1634, 1294; ^{1}H NMR (DMSO-d_6, δ): 4.65 (s, 1H, SH), 5.42 (s, 2H, NH_2), 6.85-6.86 (d, 2H, aro. CH), 7.12 (s, 1H, OH), 7.49-7.50 (d, 2H, aro. CH); MF: $C_{11}H_8N_4OS$ (244.27); MS: *m/z* 244 (M^+).

***4-amino-6-(2-chlorophenyl)-2-mercaptopyrimidine-5-carbonitrile* (2d)**
Reac. time: 4.0 h; Yield: 80% (Ethanol); R_f: 0.71; m.p. 148-149 ºC; IR (KBr, cm^{-1}): 3421, 3234, 2221, 1596, 1260, 760; ^{1}H NMR ($CDCl_3$, δ): 4.62 (s, 1H, SH), 5.26 (s, 2H, NH_2), 7.46-7.48 (m, 1H, aro. CH), 7.57-7.58 (d, 2H, aro. CH), 8.19-8.20 (d, 1H, aro. CH); MF: $C_{11}H_7ClN_4S$ (262.72); MS: *m/z* 262 (M^+), 264 (M^{+2}).

***4-amino-6-(4-chlorophenyl)-2-mercaptopyrimidine-5-carbonitrile* (2e)**
Reac. time: 3.5 h; Yield: 76% (Ethanol); R_f: 0.75; m.p. 123-124 ºC; IR (KBr, cm^{-1}): 3421, 3228, 2232, 1585, 1215, 617; ^{1}H NMR ($CDCl_3$, δ): 4.74 (s, 1H, SH), 5.46 (s, 2H, NH_2), 7.52-7.54 (d, 2H, aro. CH), 7.85-7.87 (d, 2H, aro. CH); MF: $C_{11}H_7ClN_4S$ (262.72); MS: *m/z* 262 (M^+), 264 (M^{+2}).

***4-amino-6-(4-(dimethylamino)phenyl)-2-mercaptopyrimidine-5-carbonitrile* (2f)**
Reac. time: 3.5 h; Yield: 79% (Ethanol); R_f: 0.63; m.p. 172-173 ºC; IR (KBr, cm^{-1}): 3419, 3242, 2223, 1570, 1299, 1173; ^{1}H NMR ($CDCl_3$, δ): 3.16 (s, 6H, $N(CH_3)_2$), 4.67 (s, 1H, SH), 5.26 (s, 2H, NH_2), 6.71-6.72 (d, 2H, aro. CH), 7.82-7.84 (d, 2H, aro. CH); MF: $C_{13}H_{13}N_5S$ (271.34); MS: *m/z* 271 (M^+).

***4-amino-2-mercapto-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile* (2g)**
Reac. time: 3.5 h; Yield: 74% (Ethanol); R_f: 0.55; m.p. 120-121 ºC; IR (KBr, cm^{-1}): 3446, 3249, 2229, 1563, 1278, 1183; ^{1}H NMR ($CDCl_3$, δ): 3.92 (s, 3H, OCH_3), 4.68 (s, 1H, SH), 5.09 (s, 2H, NH_2), 7.03-7.05 (d, 2H, aro. CH), 7.93-7.94 (d, 2H, aro. CH); MF: $C_{12}H_{10}N_4OS$ (258.30); MS: *m/z* 258 (M^+).

***4-amino-6-(4-hydroxy-3-methoxyphenyl)-2-mercaptopyrimidine-5-carbonitrile* (2h)**
Reac. time: 5.0 h; Yield: 66% (EtOH:H_2O, 1:1); R_f: 0.67; m.p. 167-168 ºC; IR (KBr, cm^{-1}): 3491, 3330, 3211, 2222, 1636, 1157; ^1H NMR ($CDCl_3$, δ): 3.97 (s, 3H, OCH_3), 4.63 (s, 1H, SH), 5.23 (s, 2H, NH_2), 7.02-7.03 (d, 1H, aro. CH), 7.26 (s, 1H, OH), 7.30-7.32 (dd, 1H, aro. CH), 7.73 (d, 1H, aro. CH); MF: $C_{12}H_{10}N_4O_2S$ (274.30); MS: *m/z* 274 (M^+).

***4-amino-2-mercapto-6-(2-nitrophenyl)pyrimidine-5-carbonitrile* (2i)**
Reac. time: 3.5 h; Yield: 81% (Ethanol); R_f: 0.68; m.p. 172-173 ºC; IR (KBr, cm^{-1}): 3441, 3223, 2221, 1596, 1523, 1340; ^1H NMR ($CDCl_3$, δ): 4.67 (s, 1H, SH), 5.19 (s, 2H, NH_2), 7.82-7.85 (m, 2H, aro. CH), 7.89-7.93 (m, 1H, aro. CH), 8.37-8.39 (dd, 1H, aro. CH); MF: $C_{11}H_7N_5O_2S$ (273.27); MS: *m/z* 273 (M^+).

***4-amino-2-mercapto-6-(4-nitrophenyl)pyrimidine-5-carbonitrile* (2j)**
Reac. time: 3.5 h; Yield: 78% (Ethanol); R_f: 0.73; m.p. 190-191 ºC; IR (KBr, cm^{-1}): 3412, 3220, 2213, 1629, 1533, 1354; ^1H NMR ($CDCl_3$, δ): 4.80 (s, 1H, SH), 5.28 (s, 2H, NH_2), 8.08-8.11 (d, 2H, aro. CH), 8.40-8.42 (d, 2H, aro. CH); MF: $C_{11}H_7N_5O_2S$ (273.27); MS: *m/z* 273 (M^+).

***4-amino-2-mercapto-6-(3-methoxyphenyl)pyrimidine-5-carbonitrile* (2k)**
Reac. time: 4.0 h; Yield: 73% (Ethanol); R_f: 0.71; m.p. 129-130 ºC; IR (KBr, cm^{-1}): 3434, 3244, 2219, 1556, 1278, 1191; ^1H NMR ($CDCl_3$, δ): 3.94 (s, 3H, OCH_3), 4.66 (s, 1H, SH), 5.27 (s, 2H, NH_2), 7.01-7.03 (d, 1H, aro. CH), 7.44-7.50 (m, 3H, aro. CH); MF: $C_{12}H_{10}N_4OS$ (258.30); MS: *m/z* 258 (M^+).

***4-amino-2-mercapto-6-(3-nitrophenyl)pyrimidine-5-carbonitrile* (2l)**
Reac. time: 4.0 h; Yield: 76% (Ethanol); R_f: 0.69; m.p. 131-132 ºC; IR (KBr, cm^{-1}): 3411, 3234, 2208, 1630, 1529, 1350; ^1H NMR ($CDCl_3$, δ): 4.84 (s, 1H, SH), 5.30 (s, 2H, NH_2), 7.76-7.78 (dd, 1H, aro. CH), 8.11-8.13 (dd, 1H, aro. CH), 8.53 (m, 1H, aro. CH), 8.61 (s, 1H, aro. CH); MF: $C_{11}H_7N_5O_2S$ (273.27); MS: *m/z* 273 (M^+).

Anti-inflammatory activity
The synthesized compounds were screened for *in-vitro* anti-inflammatory activity using inhibition of albumin denaturation technique according to reported method [18]. The standard drug and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that concentration of DMF in all solutions remains less than 2.5%. Test solution (1 mL, 100 mg/mL) was mixed with 1 mL of 1% albumin solution in phosphate saline buffer and incubated at 27˚C for 15 min. Denaturation was induced by keeping the reaction mixture at 60˚C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with UV-Vis spectrophotometer. The % inhibition of denaturation was calculated from control where no drug was added. Diclofenac sodium was used as a reference standard. The % inhibition was calculated using the following formula.

% Inhibition of denaturation = [(At/Ac) - 1] * 100

Where, At = Mean absorbance of test compound
Ac = Mean absorbance of control

Antioxidant activity
Hydrogen peroxide scavenging activity
A solution of hydrogen peroxide (20 mM) was prepared in phosphate saline buffer (pH 7.4). 1 mL of various dilutions (12.5, 25, 50, 100 mg/mL) of the test samples or standard, ascorbic acid in methanol were added to 2 mL of hydrogen peroxide solution in the phosphate saline buffer. The absorbance was measured at 230 nm after 10 min [19].

Nitric oxide scavenging activity
The reaction mixture containing 4 mL sodium nitroprusside (10 mM), 1 mL phosphate saline buffer (pH 7.4) and 1 mL test samples or ascorbic acid solution in DMSO at various concentrations (12.5, 25, 50, 100 mg/mL) was incubated at 25˚C for 150 min. After incubation, to 0.5 mL of reaction mixture, 1 mL of 2% w/v sulphanilic acid reagent added, mixed well and allowed to stand for 5 min. Then, 1 mL of 0.2% w/v 2-(1-naphthylamino)ethylamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 640 nm [20].

In above antioxidant activity determination methods, % inhibition was calculated by the following formula and IC_{50} value was derived from the % inhibition at different concentration.

% Inhibition = [1 - (At/Ac)] * 100

Where, At = Mean absorbance of test compound
Ac = Mean absorbance of control

Reducing power determination

The reducing power of test samples was determined according to reported method [21]. The test compounds and standard drug, ascorbic acid were dissolved in DMF to get different dilutions (12.5, 25, 50, 100 mg/mL). 1 mL of these dilutions then mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% w/v potassium ferricyanide solution. The mixture was incubated at 50˚C for 20 min. 2.5 mL of 10% w/v trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The upper layer of solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% w/v ferric chloride. The absorbance was measured at 700 nm, against reagent blank solution. The IC_{50} value was derived from the % inhibition at different concentration.

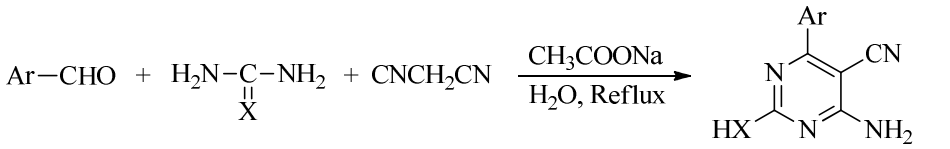
% Inhibition = [(At/Ac) - 1] * 100

Where, At = Mean absorbance of test compound
Ac = Mean absorbance of control.

RESULTS AND DISCUSSION

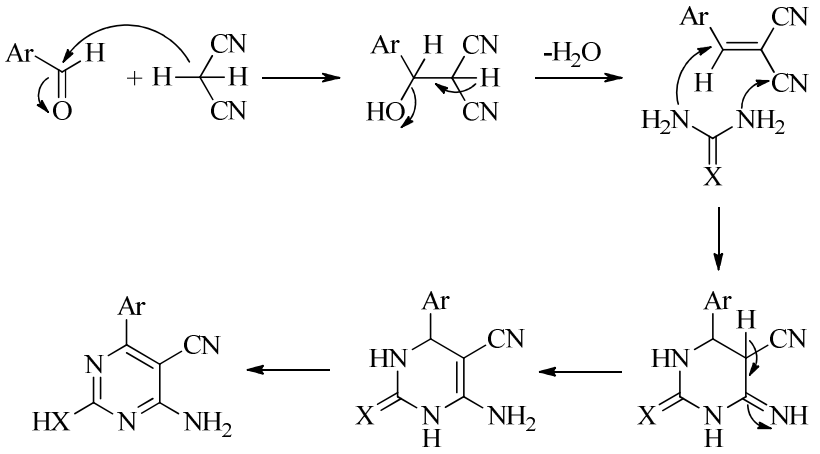
Synthetic approach

An aqua mediated novel multi-component reaction in the presence of equivalent amount of sodium acetate at reflux condition allows one-pot formation of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidines in good yields. These reactions are summarized in Scheme 1.



Scheme 1 Synthetic route of compounds 1a-1l and 2a-2l

The complete process represents an example of a one-pot synthesis. The reaction mechanism for synthesis of desired compounds is illustrated in scheme 2.



Scheme 2 Reaction mechanismfor synthesis of pyrimidine derivatives

In first step, benzylidene malononitrile containing an electron-poor C=C double bond was produced by rapid Knoevenagel condensation of malononitrile with the aromatic aldehyde. The second step was followed by Michael addition, cycloaddition, isomerization and aromatization to afford the 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidines. We believed that the driving force for such a transformation is the aromaticity of the final products.

Characterization

Structures of all the synthesized compounds 1a-1l and 2a-2l were established on the basis of spectral data. The IR, ^{1}H NMR and mass spectra supported the structure of various synthesized pyrimidine derivatives. For example, IR spectra of all compounds showed symmetric and asymmetric stretching of primary NH_2 group (3492-3420 and 3310-3220 cm^{-1}) and presence of cyano group (2232-2211 cm^{-1}). In the ^{1}H NMR spectrum, the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts and multiplicities. ^{1}H NMR spectrum of 1g showed a singlet at δ 3.94 ppm corresponding to methoxy group; a broad singlet at δ 5.50 ppm due to two proton of primary NH_2 group. The two doublets at δ 7.03-7.05 and 7.93-7.95 ppm are due to four aromatic protons. The phenolic proton appears at δ 7.68 ppm. In mass spectrum of 1g, the molecular ion peak (M^+) appeared at *m/z* 242, which was identical with its molecular formula.

Biological Screening

All the newly synthesized compounds were screened *in-vitro* for their preliminary anti-inflammatory and antioxidant activity. The results of preliminary *in-vitro* anti-inflammatory and antioxidant screening of compounds 1a-1l and 2a-2l are shown in Table 1.

Table 1 Anti-inflammatory and antioxidant activity of synthesized compounds

Compds.	Anti-inflammatory activity		Antioxidant activity		
	*Absorbance Mean ± #S.D.	% Inhibition of denaturation	*IC_{50} (µg/mL) ± #S.D.		
			Scavenging of NO radical	Scavenging of H_2O_2	Reducing Power
Control	0.1980 ± 0.024	---	---	---	----
1a	0.3038 ± 0.010	53.43	73 ± 0.087	58 ± 0.453	59 ± 0.066
1b	0.2885 ± 0.024	45.71	68 ± 0.183	44 ± 0.066	45 ± 0.183
1c	0.3021 ± 0.046	52.58	51 ± 0.050	39 ± 0.121	41 ± 0.087
1d	0.3429 ± 0.016	73.18	75 ± 0.333	56 ± 0.030	67 ± 0.279
1e	0.3584 ± 0.040	81.01	62 ± 0.066	50 ± 0.333	53 ± 0.414
1f	0.2399 ± 0.021	21.16	83 ± 0.279	74 ± 0.050	67 ± 0.268
1g	0.2464 ± 0.001	24.44	67 ± 0.318	40 ± 0.279	46 ± 0.118
1h	0.2825 ± 0.020	42.68	53 ± 0.453	36 ± 0.134	44 ± 0.087
1i	0.3311 ± 0.004	67.22	68 ± 0.024	55 ± 0.248	58 ± 0.040
1j	0.3405 ± 0.003	71.97	62 ± 0.016	49 ± 0.107	53 ± 0.196
1k	0.2680 ± 0.010	35.35	58 ± 0.024	39 ± 0.142	45 ± 0.052
1l	0.3239 ± 0.024	63.59	63 ± 0.118	52 ± 0.032	57 ± 0.150
2a	0.3012 ± 0.046	52.12	81 ± 0.066	67 ± 0.082	66 ± 0.453
2b	02853 ± 0.016	44.09	72 ± 0.453	53 ± 0.318	55 ± 0.121
2c	0.2961 ± 0.025	49.55	60 ± 0.121	41 ± 0.024	50 ± 0.333
2d	0.3409 ± 0.042	72.17	81 ± 0.162	67 ± 0.453	76 ± 0.121
2e	0.3547 ± 0.004	79.14	71 ± 0.082	56 ± 0.333	64 ± 0.024
2f	0.2363 ± 0.025	19.34	93 ± 0.780	84 ± 0.279	78 ± 0.066
2g	0.2401 ± 0.001	21.26	73 ± 0.087	48 ± 0.121	58 ± 0.333
2h	0.2785 ± 0.010	40.66	62 ± 0.279	40 ± 0.087	52 ± 0.162
2i	0.3256 ± 0.020	64.44	78 ± 0.050	64 ± 0.050	63 ± 0.453
2j	0.3374 ± 0.024	70.40	71 ± 0.162	58 ± 0.007	59 ± 0.318
2k	0.2602 ± 0.036	31.41	68 ± 0.082	42 ± 0.183	53 ± 0.024
2l	0.3187 ± 0.003	60.96	73 ± 0.318	60 ± 0.066	62 ± 0.050
Diclofenac Na	0.3630 ± 0.003	83.33	---	---	----
Ascorbic Acid	---	---	47 ± 0.087	33 ± 0.121	31 ± 0.183

**Average of triplicate reading; #S.D. = Standard Deviation*

Anti-inflammatory activity

Compared to the standard, diclofenac sodium, all the tested compounds have shown acceptable anti-inflammatory activity. The results revealed that the compounds 1d, 2d, 1e, 2e, 1j and 2j exhibited very good anti-inflammatory activities. Amongst all the tested compounds 1e found to be more potent. The compounds 1i, 2i, 1j and 2j have showed good activity, while other compounds showed weak to moderate activities. The results also showed that different substitution on aromatic ring system affect the anti-inflammatory activity. Electron withdrawing group like chlorine and nitro at any position shows good anti-inflammatory activity while electron releasing group shows weak to moderate activity. Furthermore, it can also be concluded that hydroxyl group is slightly more active compared to mercapto group at 2nd position of pyrimidine ring system.

Antioxidant activity

The antioxidant screening was carried out by various methods such as scavenging of hydrogen peroxide, scavenging of nitric oxide radical and reducing power determination. The results revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, hydroxyl derivatives showed good promising antioxidant activity as compared to that of standard, ascorbic acid due the availability of free hydroxyl group. The

compound 1c and 1h are more potent among the tested series. This may be due to additional hydroxyl group present on phenyl ring in the structure. Compounds 1k, 1g, 2c and 2h also showed moderate to good antioxidant activity.

CONCLUSION

A new, simple and efficient protocol towards one pot synthesis of biologically active 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidines has been developed using water as solvent at reflux temperature. Among the newly synthesized compounds, highest antioxidant activity for compounds 1c and anti-inflammatory activity for compound 1e was observed. Accordingly, these novel classes of pyrimidine derivatives emerged as a valuable lead series that might be useful as antioxidant and anti-inflammatory agents and hence promising candidates for further efficacy evaluation.

Acknowledgements
We are thankful to SAIF, IIT, Madras and Saurashtra University, Rajkot for providing spectral data of newly synthesized compounds.

REFERENCES

[1] I. M. Lagoja, *Chem Biodivers*, **2005**, 2(1), 1-50.
[2] H. J. Dhongade, H. Dansena, K. K. Chandrakar, *Asian J Pharm Clin Res*, **2015**, 8(4), 171-7.
[3] M. Amir, S. Javed, H. Kumar, *Indian J Pharm Sci*, **2007**, 69(3), 337-43.
[4] S. Panda, P. Chowdary, *Indian J Pharm Sci*, **2008**, 70(2), 208-15.
[5] G. Vanessa, M. Sidnei, F. C. F. Alex, C. F. Darlene, C. Pio, P. Ernani, *J Braz Chem Soc*, **2010**, 21(8), 1477-83.
[6] A. Padmaja, T. Payani, G. D. Reddy, V. Padmavathi, *Eur J Med Chem*, **2009**, 44(11), 4557-66.
[7] T. P. Selvam, C. R. James, P. V. Dniandev, S. K. Valzita, *Research in Pharmacy*, **2015**, 2(4), 1-9.
[8] C. O. Kappe, *Tetrahedron*, **1993**, 49(32), 6937-63.
[9] C. O. Kappe, *Acc Chem Res* **2000**, 33, 879-88.
[10] R. P. Gore, A. P. Rajput, *Drug Invent Today Drug*, **2013**, 5(2), 148-52.
[11] M. B. Deshmukh, P. V. Anbhule, S. D. Jadhav, A. R. Mali, S. S. Jagtap, S. A. Deshmukh, *Indian J Chem*, **2007**, 46B(9), 1445-8.
[12] M. B. Deshmukh, P. V. Anbhule, S. D. Jadhav, S. S. Jagtap, D. R. Patil, S. M. Salunkhe et al., *Indian J Chem*, **2008**, 47B(5), 792-5.
[13] B. Gangadasu, P. Narender, B. C. Raju, V. J. Rao, *Indian J Chem*, **2006**, 45B(5), 1259-63.
[14] A. Nimkar, M. M. V. Ramana, R. Betkar, P. Ranade, B. Mundhe, *New J Chem*, **2016**, 40(3), 2541-6.
[15] K. Tanaka, F. Toda, *Chem Rev*, **2000**, 100(3), 1025-74.
[16] C. J. Li, T. H. Chan. Comprehensive organic reactions in aqueous media, John Wiley and sons, New Jersy, **2007**, 2, 1-14.
[17] U. M. Lindstrom, *Chem Rev*, **2002**, 102(8), 2751-72.
[18] Y. Mizushima, M. Kobayashi, *J Pharm Pharmacol*, **1968**, 20(3), 169-73.
[19] G. K. Jayaprakasha, L. Jaganmohan Rao, K. K. Sakariah, *Bioorg Med Chem*, **2004**, 12(19), 5141-6.
[20] L. Marcocci, L. Packer, M.-T. Droy-Lefaix, A. Sekaki, M. Gardes-Albert, *Methods Enzymol*, **1994**, 234, 462-75.
[21] M. Oyaizu, *Jpn J Nutr*, **1986**, 44(6), 307-15.