June 2010 ISSN: 0974 - 7516 Volume 6 Issue 2

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 6(2), 2010 [160-165]

# Synthesis of 6-chloro-8-substuted-9[H]-purine derivatives and bioactivity studies

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*Received: 14th February, 2010 ; Accepted: 24th February, 2010*

## ABSTRACT

Selective preparation of 6-chloro-8-subtituted-9[H]-purines has been achieved, by treating 6-halo-4,5-diaminopyrimidine and substituted aromatic acid with pyridine reagent to get 75–90% yields by refluxing at short reaction time 1.5 to 2.0 hours. Their chemical structures were characterized using IR, $H^1$ NMR and Mass spectral studies. All the above compounds were screened for anti-microbial activity, anti-oxidant activity and their bioassay showed them to possess significant antimicrobial activity and anti-oxidant activity. © 2010 Trade Science Inc. - INDIA

## KEYWORDS

Purine derivatives;
6-chlorodiaminopyrimidine;
Aromatic acids;
Pyridine and acetonitrile;
Anti-microbial activity;
Anti-oxidant activity.

## INTRODUCTION

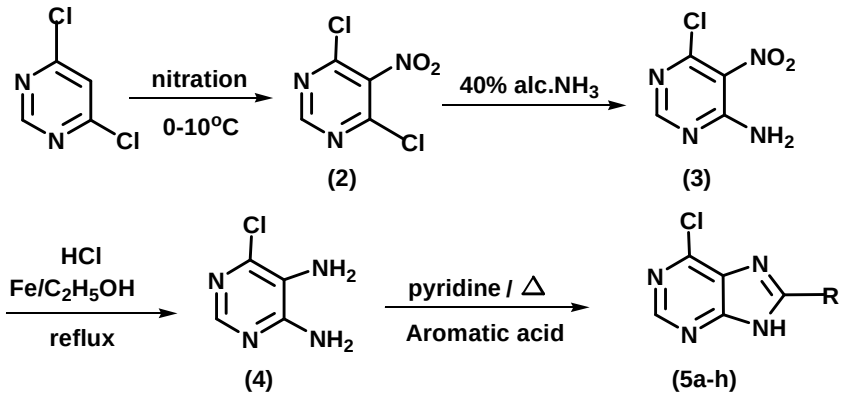
Purines derivatives have been the subject of considerable interest from many biological[1-5] point of interest and consequently the synthesis of these compounds have gained importance. The condensation of pyrimidines[6,7] and substituted aromatic acids in the presence of pyridine under reflux condition yields 6-chloro-8-subtituted-9[H]-purines derivatives **(5)** in good yield. The reaction is very fast and 90% conversation was observed in 2 hours time of reaction. We have been encouraged by these results and examined with several aromatic acids under the optimized conditions (TABLE 1). Here the acetonitrile is much better solvent than all other tested solvents such as MeOH, DCM, THF in small amount of pyridine to get high yields 75-90%.

4,6-dichloro-5-nitropyrimidine **(2)**[8-11] was prepared form the reaction of 4,6-dichloropyrimidine with nitration to obtained 90% yield due to the mild conditions in strong nitric acid was used. Compound **(2)** was further converted to compound **(3)**[12,13] by reaction with 40% ethanolic ammonia[14] in good yield. Further compound **(3)** under reduction[15-18] with Fe/HCl in ethanol to give 6-chloro-4,5-diaminopyrimidine **(4)**[19-22].

Here we have developed an efficient synthesis of several new 6-chloro-8-substituted-9[H]-purine derivatives through the condensation between 6-chloro-4,5-diaminopyrimidines and substituted benzoic acids and phenyl acetic acids using pyridine as the catalyst. The attractive features of this process are low cost reagents, good reaction yields and short reaction time. There fore it is an effective method for the synthesis of 8-subtituted-9[H]-purine derivatives TABLE 1.

## EXPERIMENTAL PROCEDURE

The $^1$H-NMR spectra were recorded at 500 MHz with a Bruker Avance DPX 300 instrument. Mass spectra were recorded under ESI-Mass with a LC-Trap-SL instrument and presented as m/z (% rel int.). Elemental analyses (C, N, H) results were found to




5a R= 4-Methoxy phenyl
5b R= P-Tolyl
5c R= Benzyl
5d R= 3,4 Dimethoxy phenyl
5e R = 2-Chloro phenyl
5f R = 4-Bromo benzyl
5g R = 2-Ethoxy phenyl
5h R = 4-Chloro-2-ethoxy phenyl

**Scheme 1**

be in good agreement with the calculated values. Melting points were determined with Capillaries Thomas Hoover melting point apparatus and are uncorrected. TLC monitored all reactions and purity of the synthesized compounds.

## General procedure

6-Chloro-8-subtituted-9[H]-purines: A mixture of 6-chloro-4,5 diaminopyrimi-dines (1mmol), substituted benzoic acids and phenyl acetic acids (1.1mmol), acetonitile (5ml) and catalytic amount of pyridine were stirred under reflux for about two hours. The resulting mixture was cooled and acidified and recrystallized with ethanol.

## 6-Chloro-8-(4-methoxy phenyl)-9[H]-purine (5a)

0.76g (85% yield), Solid, mp.: 242-244°C (ethanol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 3.8 (3H, s); 7.0 (2H, d); 8.25 (2H, d); 8.6 (1H, s). ESI-Mass spectrum, m/z (I, %): 261[M+H]$^+$ (100). Found, %: C 55.26; H 3.44; N 21.48; Cl 13.61; O 6.13. $C_{12}H_9ClN_4O$. Calculated, %: C 55.28; H 3.46; N 21.50; Cl 13.63.

## 6-Chloro-8-p-tolyl-9[H]-purine (5b)

0.71g (83.5% yield), Solid, mp.: 257-258°C (ethanol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 2.45 (3H, s); 7.30 (2H, d); 8.2 (2H, d); 8.6 (1H, s). ESI-Mass spectrum, m/z (I, %): 244.9[M+H]$^+$ (100). Found, %: C 58.87; H 3.71; N 22.89; Cl 14.50. $C_{12}H_9ClN_4$. Calculated, %: C 58.89; H 3.70; N 22.90; Cl 14.52.

## 6-Chloro-8-benzyl-9[H]-purine (5c)

0.7g (81% yield), Solid, mp.: 160-163°C (ethanol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 4.22 (2H, s); 7.33 (5H, m); 8.6 (1H, s); 13.4 (1H, s). ESI-Mass spectrum, m/z (I, %): 244.9[M+H]$^+$ (100). Found, %: C 58.87; H 3.66; N 22.88; Cl 14.50. $C_{12}H_9ClN_4$. Calculated, %: C 58.89; H 3.68; N 22.90; Cl 14.51.

## 6-Chloro-8-(3,4-dimethoxyphenyl)-9[H]-purine (5d)

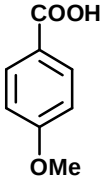
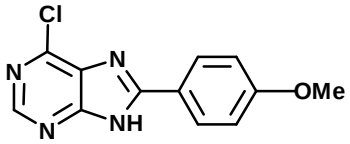
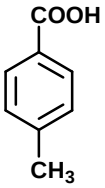
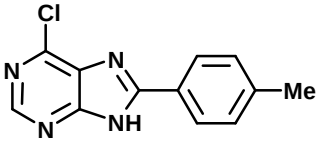
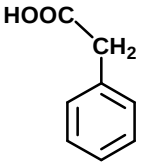
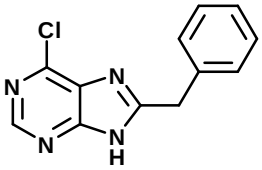
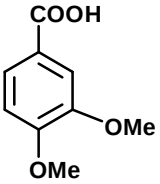
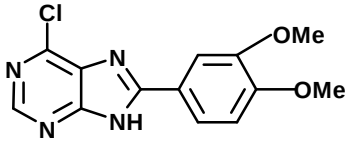
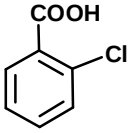
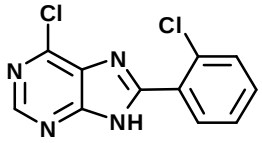
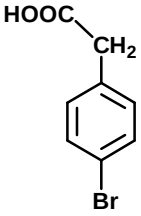
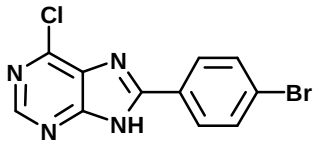
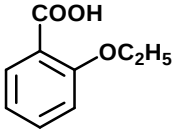
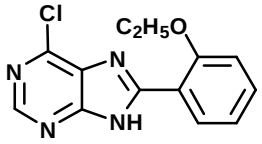
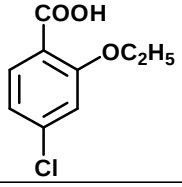
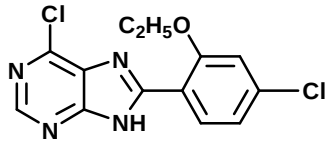
0.81g (90% yield), Solid, mp.: 250-252°C (ethanol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 3.90 (3H, s); 3.95 (3H, s); 7.08 (1H, d); 7.85 (2H, m); 8.6 (1H, s). ESI-Mass spectrum, m/z (I, %): 291[M+H]$^+$ (100). Found, %: C 53.69; H 3.74; N 19.25; Cl 12.23. $C_{13}H_{11}ClN_4O_2$. Calculated, %: C 53.70; H 3.78; N 19.27; Cl 12.22.

## 6-Chloro-8-(2-chlorophenyl)-9[H]-purine (5e)

0.64g (80% yield), Solid, mp.: 204-205°C (ethanol). $^1$H NMR (DMSO-$d_6$, 200 MHz): δ 7.4 (3H, m); 7.95 (1H, d); 8.7 (1H, s); 13.9 (1H, s/m). ESI-Mass spectrum, m/z (I, %): 291[M+H]$^+$ (100). Found, %: C 49.80; H 2.24; N 21.11; Cl 26.80. $C_{11}H_6Cl_2N_4$. Calculated, %: C 49.81; H 2.26; N 21.13; Cl 26.79.

Full Paper

**TABLE 1 : Synthesis of 6-chloro 8-subtituted-9[H]-purines**

| Entry | R | Product | Time (h) | Yield (%) |
|---|---|---|---|---|
| 5a |  |  | 2.0 | 85% |
| 5b |  |  | 1.5 | 85% |
| 5c |  |  | 1.5 | 85% |
| 5d |  |  | 1.5 | 90% |
| 5e |  |  | 2.0 | 80% |
| 5f |  |  | 2.0 | 75% |
| 5g |  |  | 1.5 | 85% |
| 5h |  |  | 2.0 | 83% |

### 6-Chloro-8-(4-bromobenzyl)-9[H]-purine (5f)

0.53g (75% yield), Solid, mp.: 181-182°C (ethanol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 4.22 (2H, s); 7.24 (2H, d); 7.42 (2H, d); 8.6 (1H, s); 13.6 (1H, s/m). ESI-Mass spectrum, m/z (I, %): 322.8[M+H]$^+$ (100) & 324.9[(M+2)+H]$^+$ (100). Found, %: C 45.95; H 2.53; N 17.84; Cl 11.30; Br 22.30. $C_{12}H_8BrClN_4$ Calculated, %: C 45.93; H 2.55; N 17.86; Cl 11.32; Br 22.32.

### 6-Chloro-8-(2-ethoxyphenyl)-9[H]-purine (5g)

0.56g (78% yield), Solid, mp.: 170-172°C (etha-

TABLE 2 : Antibacterial activity* of the target compounds (5a-h)

| Compound | Concentration (µg) | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Gram-positive bacteria | | Gram-negative bacteria | |
| | | *S.aureus* | *B.subtilis* | *P.vulgaris* | *K.pneumoniae* |
| 5a | 100 | 30 | 31 | 31 | 35 |
| | 200 | 35 | 38 | 40 | 39 |
| 5b | 100 | 20 | 22 | 17 | 19 |
| | 200 | 26 | 25 | 22 | 21 |
| 5c | 100 | 25 | 28 | 20 | 25 |
| | 200 | 30 | 32 | 23 | 28 |
| 5d | 100 | 32 | 30 | 26 | 26 |
| | 200 | 35 | 35 | 28 | 30 |
| 5e | 100 | 12 | 11 | 14 | 13 |
| | 200 | 15 | 13 | 17 | 16 |
| 5f | 100 | 26 | 26 | 20 | 21 |
| | 200 | 32 | 30 | 24 | 24 |
| 5g | 100 | 27 | 26 | 31 | 34 |
| | 200 | 32 | 30 | 36 | 40 |
| 5h | 100 | 11 | 12 | 11 | 11 |
| | 200 | 14 | 16 | 14 | 13 |
| Chloramphenicol | 100 | 35 | 38 | 40 | 42 |
| | 200 | 39 | 41 | 44 | 45 |

***c = 100µg / ml. * c = 200µg / ml**

nol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 4.22 (2H, q); 1.48 (3H, t); 7.18 (2H, m); 7.45 (1H, m); 8.25 (1H, d); 8.48 (1H, s); 12.8 broad (1H, s). ESI-Mass spectrum, m/z (I, %): 275[M+H]$^+$ (100). Found, %: C 56.819; H 4.02; N 20.38; Cl 12.91. $C_{13}H_{11}ClN_4O$. Calculated, %: C 56.83; H 4.00; N 20.40; Cl 12.93.

### 6-Chloro-8-(4-chloro-2-ethoxyphenyl)-9[H]-purine (5h)

0.60g (83% yield), Solid, mp.: 157-158°C (ethanol). $^1$H NMR spectrum (DMSO-$d_6$, 200 MHz): δ 4.22 (2H, q); 1.48 (3H, t); 7.18 (2H, d); 7.45 (1H, s); 8.35 (1H, s); 12.8 broad (1H, s). ESI-Mass spectrum, m/z (I, %): 309[M+H]$^+$ (100). Found, %: C 50.48; H 3.24; N 18.17; Cl 22.90. $C_{13}H_{11}ClN_4O$. Calculated, %: C 50.51; H 3.26; N 18.12; Cl 22.94.

### Antimicrobial testing

The compound **(5a-h)** was tested for in vitro antimicrobial activity at two different concentrations 100 and 200µg per disc. The antibacterial activity was screened against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Proteus vulgaris*, *Klebsiella pneumoniae* (Gram-nagative bacteria) on nutrient agar plates at 37°C for 24 hrs using chloramphenicol as reference during. The compounds were also evaluated for their antifungal activity against *Aspergillus niger* and *Pencillium chrysogenium* using fluconazole as standard drug. Fungi cultures were grown on potato dextrose agar (PDA) medium at 25°C. The spore suspension was adjusted to $10^6$ pores ml$^{-1}$ at an mg ml$^{-1}$ concentration by the Vincent and Vincent method.

### Antioxidant testing

The compounds **(5a-h)** is tested for antioxidant property by nitric oxide and DPPH methods.

Assay for Nitric Oxide (NO) Scavenging Activity Sodium nitroprusside (5µM) in phosphate buffer pH 7.4 was incubated with 100µM concentration of test compounds dissolved in a suitable solvent (methanol) and tubes were incubated at 25°C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals 0.5ml of incubation solution was taken and diluted with 0.5ml

**TABLE 3 : Antifungal activity* of the target compounds (5a-h)**

| Compound | Concentration | Zone of Inhibition (mm) | |
|---|---|---|---|
| | (µg/ml) | *A.niger* | *P.chrysogenium* |
| 5a | 100 | 24 | 20 |
| | 200 | 27 | 26 |
| 5b | 100 | 14 | 14 |
| | 200 | 19 | 17 |
| 5c | 100 | 26 | 26 |
| | 200 | 30 | 29 |
| 5d | 100 | 31 | 18 |
| | 200 | 38 | 19 |
| 5e | 100 | 15 | 25 |
| | 200 | 18 | 28 |
| 5f | 100 | 17 | 30 |
| | 200 | 22 | 32 |
| 5g | 100 | 30 | 26 |
| | 200 | 33 | 28 |
| 5h | 100 | 28 | 16 |
| | 200 | 32 | 18 |
| Fluconazole | 100 | 38 | 41 |
| | 200 | 42 | 44 |

***c = 100µg /ml, *c = 200µg / ml**

of griess reagent (1% sulfanilamide, 0.1% N-naphthyle-thylenediamine dihydrochloride and 2% o-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent N-naphthyle-thylenediamine dihydrochloride was read at 546nm.

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical (DPPH Method): The nitrogen centered stable free radical DPPH has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at 517nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of test compounds (100µM) were added to DPPH (100µM) in ethanol. The tubes were kept at an ambient temperature for 25 minutes and the absorbance was measured at 517nm. The difference between the test and the control experiments was taken and expressed as the percentage scavenging of the DPPH radical.

**TABLE 4 : Antioxidant activity* of the target compounds (5a-h)**

| Compound | % Inhibition at 100 µM | |
|---|---|---|
| | Nitric oxide method | DPPH method |
| 5a | 82.25 | 84.74 |
| 5b | 34.33 | 38.12 |
| 5c | 91.18 | 93.65 |
| 5d | 29.21 | 27.75 |
| 5e | 70.23 | 72.25 |
| 5f | 24.35 | 22.25 |
| 5g | 75.10 | 74.25 |
| 5h | 78.24 | 68.25 |

***c = 100µM**

## RESULTS AND DISCUSSION

The results of the compounds of preliminary antimicrobial testing are shown in TABLE 1 and 2. The results revealed that the inhibitory activity against Gram-positive bacteria was higher than Gram-negative bacteria. The imidazole derivatives **(5b)**, **(5e)**, and **(5h)** were displayed least activity. The compounds **(5a)**, **(5c)**, **(5d)**, **(5f)**, and **(5g)** showed excellent activity against Gram-positive bacteria (inhibitory zone>25mm) and good activity against Gram negative bacteria (inhibitory zone>20mm). All the test compounds **(5a)**, **(5c)**, **(5d)**, **(5g)**, and **(5h)** excellent activity and compounds **(5b)**, **(5e)** and **(5f)** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Aspergillus niger*. Compounds **(5a)**, **(5c)**, **(5e)**, **(5f)** and **(5g)** excellent activity and compounds **(5b)**, **(5d)** and **(5h)** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Pencillium chrysogenium* (TABLE 1 & 2). The compounds **(5a)**, **(5c)**, **(5e)**, **(5g)** and **(5h)** exhibited high antioxidant property in both Nitric Oxide and DPPH methods at 100 µM concentrations (TABLE 3).

## REFERENCES


**[1]** H.J.Schaeffer, R.N.Johnson, E.Odin, C.Hansch; J.Med.Chem., **13**, 452 **(1970)**.

**[2]** H.J.Schaeffer, E.Odin; J.Med.Chem., **10**, 181 **(1966)**.

**[3]** H.J.Kelly, E.F.Soroko; J.Med.Chem., **29**, 1133 **(1986)**.

**[4]** J.L.Kelley, M.P.Krochmal, J.A.Linn, E.W.Mc Lean, E.F.Soroko; J.Med.Chem., **31**, 606-612 **(1988)**.

**[5]** S.A.Lanfer, D.M.Domeyer, T.R.F.Scior, W.Albrecht, D.R.J.Hauser; J.Med.Chem., **48**, 710-722 **(2005)**.

**[6]** S.Banerjee, S.Dutta, K.S.Chakraborti; J.Indian Chem.Soc., **59(3)**, 417-418 **(1982)**.

**[7]** J.L.Kelley, E.W.Mc.Lean, R.M.Ferris, J.L.Howerd; J.Med.Chem., **33**, 1910-1914 **(1990)**.

**[8]** J.A.Montgomery, Jr., C.Temple; J.Am.Chem.Soc., **80**, 409 **(1958)**.

**[9]** S.M.Greeberg, L.O.Ross, R.K.Robins; J.Org. Chem., **24**, 1314 **(1959)**.

**[10]** D.J.Brown, N.W.Jacobsen; J.Chem.Soc., 3776 **(1965)**.

**[11]** A.Hari, B.L.Miller; Tetrahedron Lett., **40**, 245-248 **(1999)**.

**[12]** L.G.J.Hammarstrom, M.E.Meyer, D.B.Smith, F.X.Talamas; Tetrahedron Lett., **44**, 8361-8363 **(2003)**.

**[13]** W.R.Boon, W.G.M.Jones, G.R.Ramage; J.Chem. Soc., 96 **(1951)**.

**[14]** D.J.Brown; J.Applied Chem., **72**, 4 **(1954)**.

**[15]** C.L.Lee, K.P.Chan, Y.Lam, S.Y.Lee; Tetrahedron Lett., **42**, 1167 **(2001)**.

**[16]** R.A.Scheuerman, D.Tumelty; Tetrahedron Lett., **41**, 6531 **(2000)**.

**[17]** M.K.Schwarz, D.Tumelty, M.A.Gallop; Tetrahedron Lett., **39**, 8397 **(1998)**.

**[18]** R.K.Robins, K.L.Dille, B.E.Christensen; J.Org. Chem., **19**, 930 **(1954)**.

**[19]** R.K.Robins, K.L.Dille, C.H.Willets, B.E.Christensen; J.Am.Chem.Soc., **75**, 263 **(1953)**.

**[20]** N.J.Leonard, T.R.Henderson; J.Am.Chem.Soc., **97(17)**, 4990-4999 **(1997)**.

**[21]** B.K.Chun, S.Olgen, J.H.Hang, M.G.Newton, C.K.Chu; J.Org.Chem., **65**, 685-693 **(2000)**.

**[22]** A.Albert, D.J.Brown, H.C.S.Wood; J.Chem.Soc., 3832 **(1954)**.