ISSN 2394-2789(Print)
e-ISSN 2394-2797(Online)

Research Communication

ANTI-INFLAMMATORY, ANALGESIC AND ANTIMICROBIAL ACTIVITY STUDIES OF NOVEL 4, 6-DISUBSTITUTED-2-AMINO-3-CYANOPYRIDINES

*** Srinivasa Rao Atla[1], Neelakanta Rao Nagireddy[2], Rajendra Prasad Yejella[3]**
[1] Department of Pharmaceutical Chemistry, Shri Vishnu College of Pharmacy
(Affiliated to Andhra University), Bhimavaram-534202, Andhra Pradesh, India.
[2] Rite Aid Department of Pharmacy, State TN, Ountry USA-38109.
[3]Department of Pharmaceutical Chemistry, Andhra University, Visakhapatnam-530 003,
Andhra Pradesh, India.

***Corresponding author:**
E-mail: drasr@svcp.edu.in

Abstract: *A new series of 4-arylsubstituted-6-(p-hydroxyphenyl/ p-aminophenyl)-3-cyano-2-amino pyridines were synthesized by an efficient one-pot cyclocondensation reaction of 4-amino/ 4-hydroxyacetophenone, aromatic aldehydes, malononitrile and ammonium acetate. The structures of these compounds were confirmed by IR, NMR (^1H & ^{13}C) and Mass spectral analysis. All the synthesized compounds were subjected to evaluation for their anti-inflammatory, analgesic and antimicrobial properties. The electro negativity of the substituents and their displacement on the 4- or 6-aryl ring of the 4,6-diaryl-3-cyano-2-aminopyridine nucleus **(4a-p)** influenced the anti-inflammatory and analgesic activity which was higher in the presence of electron releasing groups. The introduction of the p-hydroxyphenyl substituent in the 6-position of the 3-cyano-2-aminopyridine nucleus **(4i-p)** increased the anti-inflammatory and analgesic power, but there was no evidence of the relationship among the electronic characteristic of the substituents, their displacement on the 4-phenyl ring and the activity. These results indicated that **4k** and **4p** are more promising molecules as anti-inflammatory and analgesic agents. All compounds were also evaluated for their antimicrobial activity against variety of bacterial and fungal strains. Compounds **4b, 4f, 4h, 4j** and **4p** showed maximum activity comparable to the reference standards.*

Keywords: *3-cyano-2-aminopyridine; Anti-inflammatory; Analgesic; Antimicrobial activity*

Introduction

Multi-component reactions (MCRs) are powerful tools in modern medicinal chemistry, enabling straightforward access to large libraries of structurally related, drug-like compounds and there by facilitating lead generation. Hence, combined with the use of combinatorial chemistry and high-through put parallel synthesis, such reactions have constituted an increasingly valuable approach to drug discovery efforts in recent years [1]. And also the most suitable protocol for the synthesis of functionalized organic compounds would be a one-pot reaction due to the fact that the synthesis can be performed without the isolation of the intermediates, without discharging any functional groups in short reaction time [2]. Many of the drug development directions aimed to influence multiple targets in a parallel fashion. One of the newly developed combination therapy is multi-target lead discovery which is a promising tool for the identification of unexpectedly novel effects of drug combinations [3]. Several efficient drugs, such as salicylates, non-steroidal anti-inflammatory drugs (NSAIDs), metformin, antidepressants, anti-neurodegenerative agents, multi-target kinase inhibitors affect many targets simultaneously. And also multi-target antibodies are increasingly used in cancer therapy to delay the development of resistance [4].

The pyridine derivatives have shown important biological activities as pharmaceuticals and potential agrochemicals which occurs naturally and synthetically [5]. Some new 2-amino-3-cyano-6-(3,5-dibromo-4-methoxyphenyl)-4-arylpyridines/pyrans were synthesized and screened for their antitubercular and antimicrobial activities and were found to be active [6]. Furthermore, 2-amino-3-cyano-4-tetrazoloquinolinylpyridines were synthesized by the one-pot cyclocondensation and tested for their antimicrobial activity against a panel of pathogenic strains of bacteria and fungi and found to be equipotent or more potent than commercial antibiotics [7].

In association with those, 2-amino-3-cyanopyridine derivatives have been identified as IKK-β inhibitors in conjunction with its importance and utility as intermediates in preparing variety of heterocyclic compounds [8, 9]. Consequently, the synthesis of 2-amino-3-cyanopyridine derivatives keeps on attracting much interest in organic chemistry. Various routes for the synthesis of 2-amino-3-cyanopyridine derivatives have been reported using two-component as well as three-component reactions [6, 10, and 11]. Manna and coworkers have reported the synthesis of 4, 6-disubstituted-3-cyano-2-aminopyridines and their anti-inflammatory, analgesic and antipyretic activity properties which are ten times less active than indomethacin [12]. Some researchers have reported a facile one-pot multi-component synthesis of 2-amino-3-cyanopyridine derivatives by the condensation of malononitrile with aromatic aldehydes, alkyl ketones in the presence of ammonium acetate because active methylene compounds give heterocyclic compounds containing one nitrogen atom by condensation with ketones in the presence of ammonium acetate [13,14].

In view of these references, we would like to report the synthesis of a new series of 2-amino-3-cyano-4-(substituted aryl)-6-(4-aminophenyl)pyridines **(4a-h)** and 2-amino-3-cyano-4-(substituted aryl)-6-(4-hydroxyphenyl)pyridines **(4i-p)** by an efficient one-pot multi-component reaction of 4-amino/hydroxyacetophenone, aromatic aldehydes, malononitrile and ammonium acetate (Figure 1) through Michael reaction, with the elimination of 1 mol each of water and hydrogen and all these synthesized cyanopyridines (Table 1) were screened for their anti-inflammatory, analgesic and antimicrobial activity.

Materials and Methods

General

Melting points were determined on a standard Boetius apparatus and are uncorrected. IR spectra were recorded in Bruker FT-IR Opus Spectroscopic Software Version 2.0 using KBr disc method. **^{1}H** and **^{13}C NMR** spectra were recorded in the indicated solvent on a Bruker Avance 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard (chemical shifts in δ ppm). LC-MS [API-ESI-MS (80 eV)] spectra were recorded on Agilent HPLC 1100 series. Elemental analyses (% C, H, N) of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within ± 0.4% of the theoretical values.

Chemicals

All chemicals, reagents and solvents were obtained from Sigma-Aldrich and Merck chemical companies and were used without further purification. Analytical TLC was performed on Silica Gel F_{254} plates (Merck) with visualization by UV (254 nm) chamber. All the cyanopyridines have been purified by column chromatography performed on silica gel (100-200 mesh, Merck).

Experimental

General procedure for the synthesis of 6-(4-aminophenyl)-4-(substituted aryl)-3-cyano-2-aminopyridines (4a-h)

Substituted aryl aldehydes **1a-h** (0.005 mol), malononitrile **2** (0.005 mol), 4-aminoacetophenone **3a** (0.005 mol), ammonium acetate (0.02 mol) and absolute alcohol (15 ml) were charged in a 50 ml round bottom flask. Then, the reaction mixture was refluxed for 2 to 3 h. Progress of the reaction was monitored by the TLC using Silica gel-G. After completion of the reaction, poured the reaction mixture into crushed ice with constant stirring. The solid separated was filtered and dried. It was purified by column chromatography performed on Silica gel (100-200 mesh), using ethylacetate and hexane mixture as mobile phase. The pyridine derivatives **4a-h** on purification obtained as orange red to yellowish red fine powder with 30-70% yields.

2-amino-3-cyano-4-(2-chlorophenyl)-6-(4-aminophenyl)pyridine **(4a):** Orange yellow solid; Yield 54%; mp 176-178 °C; IR (KBr) cm^{-1}: 3415, 3346, 2205, 1617, 1568, 1367, 822; ^{1}H-NMR (DMSO-d_6) δ: 5.66 (2H, br s), 6.62 (2H, d, J = 8.6 Hz), 6.83 (2H, br s), 7.00 (1H, s), 7.64-7.48 (4H, m), 7.86 (2H, d, J = 8.6 Hz); ^{13}C-NMR (DMSO-d_6) δ: 85.92, 107.96, 117.41, 118.65, 125.23, 127.40, 128.61, 129.59, 130.58, 131.29, 136.61, 151.19, 152.13, 159.06, 159.99; LC-MS *m/z*: 321.32 [{M+H}$^+$]. *Anal.* Calcd for $C_{18}H_{13}N_4Cl$: C, 67.40; H, 4.04; N, 17.48. Found: C, 68.83; H, 4.16; N, 17.95.

2-amino-3-cyano-4-(4-chlorophenyl)-6-(4-aminophenyl)pyridine **(4b):** Orange yellow solid; Yield 62%; mp 188-189 °C; IR (KBr) cm^{-1}: 3402, 3338, 2200, 1618, 1574, 1367, 820; ^{1}H-NMR (DMSO-d_6) δ: 5.65 (2H, bs), 6.61 (2H, d, J = 8.4 Hz), 6.78 (2H, bs), 7.08 (1H, s), 7.60 (2H, d, J = 8.4 Hz), 7.66 (2H, d, J = 8.4 Hz), 7.87 (2H, d, J = 8.4 Hz); ^{13}C-NMR (DMSO-d_6) δ: 85.89, 107.90, 114.34, 117.43, 125.41, 127.40, 128.74, 130.58, 134.29, 136.61, 140.63, 154.19, 159.06, 162.99; LC-MS *m/z*: 321.32 [{M+H}$^+$]. *Anal.* Calcd for $C_{18}H_{13}N_4Cl$: C, 67.40; H, 4.04; N, 17.48. Found: C, 68.59; H, 4.18; N, 17.87.

2-amino-3-cyano-4-(2,4-dichlorophenyl)-6-(4-aminophenyl)pyridine **(4c):** Orange red solid; Yield 55%; mp 206-207 °C; IR (KBr) cm^{-1}: 3425, 3352, 2202, 1630, 1580, 1368, 829; ^{1}H-NMR (DMSO-d_6) δ: 5.67 (2H, br s), 6.60 (2H, d, J = 8.8 Hz), 6.85 (2H, br s), 7.00 (1H, s), 7.65-7.44 (3H, m), 7.84 (2H, d, J = 8.8 Hz); ^{13}C-NMR (DMSO-d_6) δ: 95.92, 111.43, 116.5, 117.55, 125.76, 128.52, 129.43, 131.63, 134.75, 135.86, 136.34, 138.54, 143.59, 151.19, 155.38, 160.54; LC-MS *m/z*: 356.08 [{M+H}$^+$]. *Anal.* Calcd for $C_{18}H_{12}N_4Cl_2$: C, 60.90; H, 3.41; N, 15.77. Found: C, 61.86; H, 3.53; N, 16.15.

2-amino-3-cyano-4-(4-fluorophenyl)-6-(4-aminophenyl)pyridine **(4d):** Yellow solid; Yield 70%; mp 192-194 °C; IR (KBr) cm^{-1}: 3466, 3425, 2200, 1615, 1574, 1371, 1238; ^{1}H-NMR (DMSO-d_6) δ: 5.63 (2H, br s), 6.61 (2H, d, J = 8.8 Hz), 6.76 (2H, br s), 7.08 (1H, s), 7.37 (2H, dd, J = 10.2 Hz, J = 8.8 Hz), 7.70 (2H, dd, J = 9.2 Hz, J = 8.6 Hz), 7.87 (2H, d, J = 8.4 Hz); ^{13}C-NMR (DMSO-d_6) δ: 90.82, 109.45, 114.37, 117.29, 118.71, 126.43, 128.21, 135.53, 136.23, 145.65, 153.76, 154.64, 163.97, 164.21; LC-MS *m/z*: 305.11 [{M+H}$^+$]. *Anal.* Calcd for $C_{18}H_{13}N_4F$: C, 71.11; H, 4.31; N, 18.41. Found: C, 72.35; H, 4.76; N, 19.12.

2-amino-3-cyano-4-(3-bromophenyl)-6-(4-aminophenyl)pyridine **(4e):** Orange red solid; Yield 50%; mp 205-207 °C; IR (KBr) cm^{-1}: 3424, 3348, 2203, 1629, 1571, 1367, 639; ^{1}H-NMR (DMSO-d_6) δ: 5.65 (2H, br s), 6.62 (2H, d, J = 8.4 Hz), 6.80 (2H, br s), 7.12 (1H, s), 7.52-7.48 (1H, t, J = 7.1 Hz), 7.64 (1H, d, J = 7.6 Hz), 7.72 (1H, d, J = 7.6 Hz), 7.83 (1H, s), 7.90 (2H, d, J = 8.4 Hz); ^{13}C-NMR (DMSO-d_6) δ: 83.96, 109.48, 117.28, 123.35, 125.31, 127.40, 128.71, 130.70, 132.05, 133.79, 139.66, 151.20, 155.29, 159.27, 160.65; LC-MS *m/z*: 367.13 [{M+H}$^+$]. Anal. Calcd for $C_{18}H_{13}N_4Br$: C, 59.23; H, 3.59; N, 15.34. Found: C, 60.17; H, 3.64; N, 16.21.

2-amino-3-cyano-4-(4-methoxyphenyl)-6-(4-aminophenyl) pyridine **(4f):** Orange yellow solid; Yield 19%; mp 178-179 °C; IR (KBr) cm^{-1}: 3468, 3358, 2200, 1610, 1577, 1373, 1251; ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s), 5.62 (2H, br s), 6.55 (2H, d, J = 8.4 Hz), 6.68 (2H, br s), 7.05 (1H, s), 7.09 (2H, d, J = 8.4 Hz), 7.61 (2H, d, J = 8.8 Hz), 7.86 (2H, d, J = 8.8 Hz); ^{13}C-NMR (DMSO-d_6) δ: 59.67, 92.45, 108.34, 115.69, 116.75, 118.42, 121.76, 128.65, 129.55, 138.24, 142.31, 151.82, 155.22, 158.21, 162.27; LC-MS *m/z*: 317.14 [{M+H}$^+$]. Anal. Calcd for $C_{19}H_{16}N_4O$: C, 72.11; H, 5.31; N, 17.71. Found: C, 73.05; H, 5.29; N, 17.90.

2-amino-3-cyano-4-(4-methylphenyl)-6-(4-aminophenyl) pyridine **(4g):** Yellow solid; Yield 40%; mp 172-174 °C; IR (KBr) cm^{-1}: 3462, 3363, 2202, 1622, 1583, 1371; ^{1}H-NMR (DMSO-d_6) δ: 2.39 (3H, s), 5.62 (2H, br s), 6.55 (2H, d, J = 8.8 Hz), 6.70 (2H, br s), 7.05 (1H, s), 7.17 (2H, d, J = 8.0 Hz), 7.53 (2H, d, J = 8.0 Hz), 7.68 (2H, d, J = 8.6 Hz); ^{13}C-NMR (DMSO-d_6) δ: 28.43, 102.72, 115.67, 118.68, 119.35, 127.03, 128.77, 130.24, 137.43, 140.58, 142.73, 146.34, 157.45, 162.33, 164.95; LC-MS *m/z*: 301.14 [{M+H}$^+$]. Anal. Calcd for $C_{19}H_{16}N_4$: C, 75.98; H, 5.37; N, 18.65. Found: C, 76.86; H, 5.39; N, 19.16.

2-amino-3-cyano-4-(4-dimethylaminophenyl)-6-(4-aminophenyl) pyridine **(4h):** Crimson red solid; Yield 44%; mp 208-209 °C; IR (KBr) cm^{-1}: 3444, 3328, 2212, 1614, 1561, 1360; ^{1}H-NMR (DMSO-d_6) δ: 3.10 (6H, s), 5.60 (2H, br s), 6.84 (2H, d, *J* = 10.2 Hz), 7.07 (1H, s), 7.26 (2H, d, *J* = 9.8 Hz), 7.83 (2H, d, *J* = 10.0 Hz), 8.02 (2H, br s), 8.13 (2H, d, *J* = 9.8 Hz); ^{13}C-NMR (DMSO-d_6) δ: 43.60, 93.33, 115.73, 117.92, 118.12, 126.75, 127.90, 129.47, 135.65, 136.85, 145.43, 145.83, 158.92, 161.65, 163.45; LC-MS *m/z*: 330.17 [{M+H}$^+$]. Anal. Calcd for $C_{20}H_{19}N_5$: C, 73.01; H, 5.81; N, 21.26. Found: C, 73.94; H, 5.86; N, 21.97.

General procedure for the synthesis of 6-(4-hydroxyphenyl)-4-(substituted aryl)-3-cyano-2-aminopyridines (4i-p)

Substituted aryl aldehydes **1a-h** (0.005 mol), malononitrile **2** (0.005 mol), 4-hydroxyacetophenone **3b** (0.005 mol), ammonium acetate (0.02 mol), piperidine (5 mol) and absolute alcohol (15 ml) were charged in a 50 ml round bottom flask. Then, the reaction mixture was refluxed for 2 to 3 h. Progress of the reaction was monitored by the TLC using Silica gel-G. After completion of the reaction, poured the reaction mixture into crushed ice with constant stirring. The solid separated was filtered and dried. It was purified by column chromatography performed on Silica gel (100-200 mesh, Merck), using ethylacetate and hexane mixture as mobile phase. The pyridine derivatives **4i-p** on purification obtained as orange to yellowish red fine powder with 35-75% yields.

2-amino-3-cyano-4-(2-chlorophenyl)-6-(4-hydroxyphenyl)pyridine **(4i):** Yellow solid; Yield 68%; mp 165-167 °C; IR (KBr) cm^{-1}: 3420, 3358, 2210, 1610, 1570, 1377, 824; ^{1}H-NMR (DMSO-d_6) δ: 6.45 (2H, d, *J* = 8.8 Hz), 6.76 (2H, br s), 7.08 (1H, s), 7.44-7.50 (4H, m), 7.76 (2H, d, *J* = 8.6 Hz), 13.42 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 85.42, 107.68, 117.35, 118.46, 125.64, 128.80, 129.32, 130.86, 131.64, 132.00, 137.42, 152.26, 154.43, 159.44, 161.64; LC-MS *m/z*: 322.04 [{M+H}$^+$]. Anal. Calcd for $C_{18}H_{12}N_3OCl$: C, 67.20; H, 3.76; N, 13.06. Found: C, 68.19; H, 3.89; N, 13.85.

2-amino-3-cyano-4-(4-chlorophenyl)-6-(4-hydroxyphenyl)pyridine **(4j):** Dark yellow solid; Yield 72%; mp 170-172 °C; IR (KBr) cm^{-1}: 3432, 3338, 2205, 1622, 1574, 1371, 826; ^{1}H-NMR (DMSO-d_6) δ: 6.64 (2H, d, *J* = 8.8 Hz), 6.84 (2H, br s), 7.18 (1H, s), 7.68 (2H, d, *J* = 8.6 Hz), 7.68 (2H, d, *J* = 8.8 Hz), 7.77 (2H, d, *J* = 8.6 Hz), 13.65 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 85.44, 112.48, 116.86, 121.26, 123.87, 124.61, 127.64, 130.18, 131.68, 134.64, 150.87, 158.88, 159.45, 163.65; LC-MS *m/z*: 322.04 [{M+H}$^+$]. Anal. Calcd for $C_{18}H_{12}N_3OCl$: C, 67.19; H, 3.76; N, 13.06. Found: C, 68.04; H, 3.90; N, 13.54.

2-amino-3-cyano-4-(2,4-dichlorophenyl)-6-(4-hydroxyphenyl)pyridine **(4k):** Orange red solid; Yield 55%; mp 206-207 °C; IR (KBr) cm^{-1}: 3423, 3348, 2210, 1628, 1575, 1347, 832; ^{1}H-NMR (DMSO-d_6) δ: 6.68 (2H, d, *J* = 8.8 Hz), 6.76 (2H, br s), 7.08 (1H, s), 7.57-7.48 (3H, m), 7.68 (2H, d, *J* = 8.8 Hz), 13.58 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 95.80, 115.8, 117.17, 123.67, 127.74, 128.62, 129.68, 130.83, 134.34, 136.84, 138.24, 150.64, 155.07, 154.58, 158.64, 162.58; LC-MS *m/z*: 357.21 [{M+H}$^+$]. Anal. Calcd for $C_{18}H_{11}N_3OCl_2$: C, 60.69; H, 3.11; N, 11.80. Found: C, 61.58; H, 3.26; N, 12.19.

2-amino-3-cyano-4-(4-fluorophenyl)-6-(4-hydroxyphenyl)pyridine **(4l):** Yellow solid; Yield 75%; mp 188-189 °C; IR (KBr) cm^{-1}: 3413, 3326, 2202, 1620, 1553, 1358, 1274; ^{1}H-NMR (DMSO-d_6) δ: 6.58 (2H, d, *J* = 8.6 Hz), 6.82 (2H, br s), 7.16 (1H, s), 7.31 (2H, dd, *J* = 10.0 Hz, *J* = 8.0 Hz), 7.67 (2H, dd, *J* = 9.4 Hz, *J* = 8.6 Hz), 7.80 (2H, d, *J* = 8.4 Hz), 13.61 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 90.68, 107.34, 114.31, 117.22, 118.75, 126.34, 127.28, 135.49, 136.42, 155.47, 156.53, 162.13, 164.52, 165.64; LC-MS *m/z*: 306.2 [{M+H}$^+$]. Anal. Calcd for $C_{18}H_{12}N_3OF$: C, 70.82; H, 3.96; N, 13.76. Found: C, 71.41; H, 4.06; N, 14.17.

2-amino-3-cyano-4-(3-bromophenyl)-6-(4-hydroxyphenyl)pyridine **(4m):** Orange red solid; Yield 43%; mp 200-201 °C; IR (KBr) cm^{-1}: 3447, 3378, 2210, 1643, 1564, 1364, 631; ^{1}H-NMR (DMSO-d_6) δ: 6.64 (2H, d, *J* = 8.8 Hz), 6.86 (2H, br s), 7.12 (1H, s), 7.52-7.42 (1H, t, *J* = 7.0 Hz), 7.60 (1H, d, *J* = 7.8 Hz), 7.75 (1H, d, *J* = 8.0 Hz), 7.80 (1H, s), 7.94 (2H, d, *J* = 8.0 Hz), 13.57 (1H, br

s); ^{13}C-NMR (DMSO-d_6) δ: 84.76, 106.16, 117.0, 122.55, 123.64, 125.67, 127.13, 128.68, 131.63, 132.62, 133.82, 139.88, 151.43, 152.20, 159.32, 162.58; LC-MS *m/z*: 366.02 [{M+H}$^+$]. Anal. Calcd for $C_{18}H_{12}N_3OBr$: C, 59.03; H, 3.30; N, 11.45. Found: C, 60.05; H, 3.42; N, 11.84.

2-amino-3-cyano-4-(4-methoxyphenyl)-6-(4-hydroxyphenyl)pyridine **(4n):** Orange yellow solid; Yield 50%; mp 172-173°C; IR (KBr) cm^{-1}: 3456, 3354, 2206, 1614, 1589, 1370, 1258; ^{1}H-NMR (DMSO-d_6) δ: 3.82 (3H, s), 6.51 (2H, d, *J* = 8.4 Hz), 6.73 (2H, br s), 7.07 (1H, s), 7.19 (2H, d, *J* = 8.6 Hz), 7.41 (2H, d, *J* = 8.6 Hz), 7.74 (2H, d, *J* = 8.8 Hz), 13.62 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 58.58, 91.86, 107.58, 115.86, 117.89, 126.53, 128.19, 129.32, 134.64, 137.85, 145.82, 150.65, 154.47, 157.53, 161.43; LC-MS *m/z*: 318.12 [{M+H}$^+$]. Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 72.56; H, 4.82; N, 13.24.

2-amino-3-cyano-4-(4-methylphenyl)-6-(4-hydroxyphenyl)pyridine **(4o):** Yellow solid; Yield 57%; mp 168-170 °C; IR (KBr) cm^{-1}: 3444, 3346, 2207, 1622, 1580, 1376; ^{1}H-NMR (DMSO-d_6) δ: 2.37 (3H, s), 6.57 (2H, d, *J* = 8.8 Hz), 6.68 (2H, br s), 7.02 (1H, s), 7.13 (2H, d, *J* = 8.2 Hz), 7.50 (2H, d, *J* = 8.4 Hz), 7.64 (2H, d, *J* = 8.4 Hz), 13.62 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 30.56, 92.54, 109.13, 117.75, 119.58, 126.43, 128.25, 132.47, 137.00, 140.23, 142.47, 146.65, 157.08, 162.54, 164.42; LC-MS *m/z*: 302.34 [{M+H}$^+$]. Anal. Calcd for $C_{19}H_{15}N_3O$: C, 75.73; H, 5.02; N, 13.94. Found: C, 76.54; H, 5.09; N, 14.13.

2-amino-3-cyano-4-(4-dimethylaminophenyl)-6-(4-hydroxyphenyl)pyridine **(4p):** Crimson red solid; Yield 40%; mp 202-203 °C; IR (KBr) cm^{-1}: 3456, 3350, 2210, 1615, 1567, 1354; ^{1}H-NMR (DMSO-d_6) δ: 3.10 (6H, s), 6.76 (2H, d, *J* = 9.2 Hz), 7.00 (1H, s), 7.22 (2H, d, *J* = 8.8 Hz), 7.80 (2H, d, *J* = 9.0 Hz), 7.94 (2H, br s), 8.24 (2H, d, *J* = 9.8 Hz), 13.53 (1H, br s); ^{13}C-NMR (DMSO-d_6) δ: 45.45, 106.74, 112.68, 114.45, 117.43, 118.53, 127.78, 127.96, 128.42, 132.36, 144.19, 155.24, 157.65, 160.78, 163.69; LC-MS *m/z*: 331.28 [{M+H}$^+$]. Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 73.50; H, 5.63; N, 17.24.

Pharmacology

Animals

Wistar albino rats (150-200 g) and albino mice (20-25 g) of either sex (M/S Ghosh Enterprises, Calcutta, West Bengal, India) were selected for the experiments. Animals were allowed to be acclimatizing for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (6 animals per cage), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark sequence; at an ambient temperature of 25±2°C; 35-60% relative humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd., Mumbai) and water *ad libitum.* Permission has been obtained from our institutional animal ethical committee (439/PO/A/01/CPCSEA) for conducting the experiments.

Anti-inflammatory activity

The compounds were tested for anti-inflammatory activity by carrageenan-induced rat paw edema method in albino rats [15,16]. Sodium CMC (1% W/V) suspension was prepared as vehicle to suspend the test compounds and standard drug. 1% W/V suspension of carrageenan sodium salt was prepared to induce inflammation in rats. Albino rats (150-200 g) were divided into nineteen groups of six animals each and they were numbered individually. All groups were fasted for overnight and allowed water *ad libitum*. Inflammation was induced by injecting 0.05 ml of 1% carrageenan suspension subcutaneously into the sub-plantar region of the right hind paw and 0.05 ml of saline was injected into the sub-plantar region of the left hind paw for all groups. Group-1 considered as sham control animals. One hour prior to carrageenan injection, group-2 was administered with 1% sodium CMC gel (1 ml/kg, p.o.) used as carrageenan treated control animals. Group-3 treated with standard drug ibuprofen (10 mg/kg, p.o.) and the group-

4 to 19 treated with synthesized cyanopyridines (10 mg/kg, p.o.) respectively. The thickness of the both paws of each rat was measured before carrageenan injection and after carrageenan injection at time intervals 0, 0.5, 1, 2 and 4 h using digital plethysmometer apparatus by mercury displacement due to dipping of the paw can be directly read from graduated micro scale attached to the mercury column to magnify the small changes in paw thickness during the course of the experiment. The percent inhibition of paw edema thickness of control, reference drug and compound treated animals were calculated. The results and statistical analysis of anti-inflammatory activity of control, reference drug and the compounds tested are shown in Table 2.

Analgesic activity

Tail flick (tail-withdrawal from the radiant heat) method was conducted according to D'Amour et al. [17] and Kulkarni [18] using an analgesiometer was adopted for evaluation of analgesic activity of the test compounds and standard. Basal reaction time has been taken to radiant heat by placing the tip (last 1-2 cm) of the tail of the animals (control, standard and test groups) individually. The tail-withdrawal from the heat is taken as the end point. Ibuprofen and test compounds were suspended in sodium CMC (1% W/V) aqueous suspension. Albino mice of either sex (20-25 g) were divided into eighteen groups of six animals each and they were numbered individually. All groups were fasted for 24 h before administering the drug with water *ad libitum.* Group-1 was administered with only 1% w/v sodium CMC suspension (1 ml/kg, p.o.) which served as control. Group-2 was administered with ibuprofen (10 mg/kg, p.o.) which served as a standard. Group-3 to 18 (10 mg/kg, p.o.) were administered with test compounds respectively. All the animals were held in position by a suitable restrained with the tail extending out. The time in seconds taken to withdraw the tail as the reaction time and was recorded at 0, 0.5, 1, 2 and 4 h after administration of compounds. A cut off point of 10 sec was observed to prevent the tail damage. The percentage of protection in the control, standard drug and compound treated animals were calculated. The results and statistical analysis of analgesic activity of control, ibuprofen and the compounds tested are shown in Table 3.

Antimicrobial activity

The *in vitro* antimicrobial activity of all the synthesized compounds was carried out by broth micro dilution method [19, 20]. Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth used for fungal nutrition. Inoculums' size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strains employed for the activity were procured from [MTCC-Micro Type Culture Collection] Institute of Microbial Technology, Chandigarh, India. The compounds **4a-p** were screened for their antibacterial activity against *Bacillus subtilis* (MTCC 441), *Clostridium tetani* (MTCC 449), *Streptococcus pneumonia* (MTCC 3906), *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 98) as well as antifungal activity against *Aspergillus fumigates* (MTCC 3008) and *Candida albicans* (MTCC 227). DMSO (dimethyl sulphoxide) was used as vehicle to get desired concentration of compounds to test upon microbial strain. The lowest concentration, which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for comparison in the present study were ampicillin for evaluating antibacterial activity as well as griseofulvin and nystatin for antifungal activity. The results are summarized in Table 4.

Results and Discussion

Chemistry

Target compounds, **4a-p** were synthesized by the reaction proceed via initial formation of the arylidenemalononitrile (Michael reaction) from aromatic aldehydes and malononitrile, which then reacts with the aryl ketones **3a-b** and ammonium acetate to give the 2-amino-3-cyanopyridines. The structure of the products, **4a-p** was established by spectroscopic analysis. The IR spectra of **4a-p** showed bands at 3400-3200 cm^{-1} (NH_2), 3450-3425 (OH) cm^{-1}, 2220-2210

cm^{-1} (C≡N) and 1640-1600 cm^{-1} (C=N). The ^{1}H-NMR spectra of these compounds gave further support for the cyanopyridines structure, since they showed a broad singlet at ppm 13.70-13.40 and 5.60-5.70 attributed to the hydroxylic proton and amino protons respectively and an another broad singlet at ppm 6.85-6.70 attributed to the amino protons of cyanopyridine nucleus which were disappeared when the deuteriodimethylsulphoxide solution was shaken with deuterium oxide. And also the characteristic singlet peak observed at ppm 7.20-7.10 indicates the presence of single proton at C-5 position of pyridine ring further confirms the formation of 2-amino-3-cyano-4, 6-disubstituted pyridine nucleus. Other aromatic proton signals were appeared at ppm 6.0-8.0. In the ^{13}C-NMR spectrum exhibited characteristic peaks between ppm 180-160 for ring carbons adjacent to nitrogen atom in pyridine nucleus, ppm 150-120 for other ring carbons, ppm 119-116 for cyanide carbon and ppm 60-30 outside the ring carbons confirming the pyridine structure. The mass spectra showed the corresponding molecular ion peak $[M+H]^{+}$ as the base peak and the fragmentation patterns was characteristic of respective pyridines. The elemental analyses of all the newly synthesized compounds confirmed their structures.

Anti-inflammatory activity

All the synthesized compounds **(4a-p)** were screened for their anti-inflammatory activity by carrageenan induced rat paw edema model. The effect of the test compounds and ibuprofen, as a reference, was measured before and 0.5, 1, 2 and 4 h after carrageenan injection. Percent edema inhibition was calculated as regard to saline control group, as depicted in Table 2. When compared with ibuprofen, most of the compounds exhibited remarkable inhibition of edema size ($^{*}p < 0.01$). As revealed in Table 2, compounds **4c, 4e, 4k** and **4p** were found to be the most potent anti-inflammatory compounds, whereas compounds **4f** and **4h** in 4-aminophenyl series carrying 4-methoxyphenyl and 4-dimethylaminophenyl substituent and **4m** and **4n** in 4-hydroxyphenyl series carrying 3-bromophenyl and 4-methoxyphenyl substituent at C-4 position of pyridine nucleus respectively showed remarkable activity. And also it was found that compound **4k** identified as lead structure among the all. Analyzing the anti-inflammatory activity of the synthesized compounds **4a-p**, the following structure-activity relationship (SAR) was gained. Among five halogen substituted pyridine derivatives **4a-e** and **4i-m**, the potency order was found to be 2,4-Cl_2 > 3-Br > 4-Cl > 4-F > 2-Cl. Between three electron-donor substituted pyridine derivatives **4f-h** and **4n-p**, the potency order was 4-$N(CH_3)_2$ > 4-OCH_3 > 4-CH_3.

Analgesic activity

The analgesic activity of the synthesized compounds **(4a-p)** was evaluated by tail flick method, in which heat is used as a source to induce pain in mice. The increase in the reaction time (time interval) compared to basal is proportional to analgesic activity of the test compounds. The results are summarized in Table 3. Compounds **4c** and **4p** showed dose dependent activity with higher protection at 120 min which is comparable to the reference standard and exerted their activity in a manner similar to that of the well established drug ibuprofen because they carries 2,4-dichlorophenyl at C-4 in 4-aminophenyl series and 4-dimethylaminophenyl **(4p)** at C-4 in 4-hydroxyphenyl series on 2-amino-3-cyanopyridine nucleus. In addition it was found that the compounds having 3-bromophenyl **(4e)** and 4-dimethylaminophenyl **(4h)** substituents in 4-amino series and 2, 4-dichlorophenyl **(4k)** and 3-bromophenyl **(4m)** moieties in 4-hydroxy series at C-4 position in cyanopyridine nucleus exhibited moderate analgesic activity and the activity has been increased at 60 min and reached to the maximum at 120 min.

Analyzing the analgesic activity of all the compounds **4a-p**, the following SAR was gained. Among five halogen substituted cyanopyridines **4a-e** and **4i-m**, the potency order was 2,4-Cl_2 ≅ 3-Br > 4-F > 4-Cl > 2-Cl in both the 4-amino and 4-hydroxy series. Between three electron releasing substituted cyanopyridines **4f-h** and **4n-p**, the potency order was 4-$N(CH_3)_2$ > 4-OCH_3 > 4-CH_3 in both the series. Among all, compound **4p** was found to exhibit significant analgesic activity at 120 min. These results indicated that **4k** and **4p** are more promising molecules as

anti-inflammatory and analgesic agents respectively and further studies are required to elucidation of exact mechanism of action for their therapeutic potential.

Antimicrobial activity

The *in vitro* antimicrobial activity of all the synthesized compounds was carried out by broth microdilution method. An examination of the data (Table 4) reveals that amongst all the synthesized compounds **4a-p**, compound **4b** and **4j** exhibited excellent activity against Gram positive bacteria *Streptococcus pneumoniae* and Gram negative bacteria *Escherichia coli* while compounds **4h** and **4p** found to be highly active against Gram negative bacteria *Escherichia coli* only as compared to standard antibiotic ampicillin. Compounds **4b, 4d, 4f, 4h, 4j, 4l, 4n,** and **4p** are found to be more potent as compared to standard antibiotic ampicillin against Gram positive bacteria *Bacillus subtilis*. In case of Gram positive bacteria *Clostridium tetani*, compounds **4b, 4e, 4h, 4j, 4l** and **4p** are found to be more potent than ampicillin. Most of the compounds were not exhibited sufficiently potent activity to inhibit *Salmonella typhi*. Antifungal study revealed that compounds **4b, 4f, 4h, 4j** and **4p** are more potent as compared to standard fungicidal griseofulvin against *Candida albicans*. Most of the compounds were not found sufficiently potent to inhibit *Aspergillus fumigatus*.

Conclusion

A series of some new 2-amino-3-cyano-4,6-disubstituted pyridine derivatives have been synthesized through a facile one-pot multi-component reaction. This synthetic strategy allows the construction of relatively complicated nitrogen containing heterocyclic system as well as the introduction of various aromatic and heteroaromatic substitutions into 4- and 6- positions of pyridine. These new agents may be act by one of the mechanism discussed in introduction and can be further utilized for lead optimization purposes and also can be new leads for non-steroidal anti-inflammatory drugs (NSAIDs) and antimicrobial agents will be used in multi-target drug therapy which is one of the newly developed combination therapy as multi-target lead discovery. The electro negativity of the substituents and their displacement on the 4-aryl ring of the 4,6-diaryl-3-cyano-2-aminopyridines **4a-p** influenced the anti-inflammatory, analgesic and antimicrobial properties which was higher in the presence of electron-releasing groups. Furthermore, an increased potency was observed when the 4-aminophenyl group **(4a-h)** replaced with 4-hydroxyphenyl group **(4i-p)** at C-6 position of 3-cyano-2-aminopyridine nucleus.

Acknowledgements

The authors are thankful to Prof. A. Annapurna, Department of Pharmacology, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India, for her cooperation in the screening of pharmacological activities.

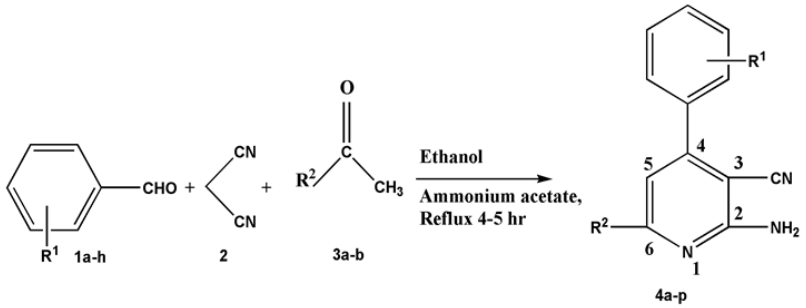

Figure 1. Multi-component synthesis of 4, 6-disubstituted-3-cyano-2-aminopyridines **4a-p**.

Table 1:

4, 6-disubstituted-3-cyano-2-aminopyridines (**4a-p)**

Compound	R^1	R^2	Compound	R^1	R^2
4a	2-Cl	4-$NH_2C_6H_4$	4i	2-Cl	4-OHC_6H_4
4b	4-Cl	4-$NH_2C_6H_4$	4j	4-Cl	4-OHC_6H_4
4c	2,4-Cl_2	4-$NH_2C_6H_4$	4k	2,4-Cl_2	4-OHC_6H_4
4d	4-F	4-$NH_2C_6H_4$	4l	4-F	4-OHC_6H_4
4e	3-Br	4-$NH_2C_6H_4$	4m	3-Br	4-OHC_6H_4
4f	4-OCH_3	4-$NH_2C_6H_4$	4n	4-OCH_3	4-OHC_6H_4
4g	4-CH_3	4-$NH_2C_6H_4$	4o	4-CH_3	4-OHC_6H_4
4h	4-$N(CH_3)_2$	4-$NH_2C_6H_4$	4p	4-$N(CH_3)_2$	4-OHC_6H_4

Table 2.

Anti-inflammatory activity of 4, 6-disubstituted-3-cyano-2-aminopyridines (**4a-p)**

Compound	Percent inhibition ± S.E.M. at various time intervals			
	0.5 h	1.0 h	2.0 h	4.0 h
4a	19.87±0.82	43.09±1.21	51.26±2.35*	49.85±1.92
4b	15.23±0.90	41.33±1.04*	74.54±2.62	53.54±1.75
4c	15.22±0.68*	50.45±1.23*	87.23±2.61*	59.94±1.79
4d	20.01±0.89	40.56±1.21	73.46±2.54	52.22±1.79
4e	18.26±0.68*	49.35±1.41*	86.99±2.62*	53.32±1.71
4f	17.32±0.62*	51.32±1.35	83.47±2.45*	54.57±1.68
4g	20.14±0.92	60.57±1.47	82.82±2.69	57.24±1.92
4h	20.06±0.92*	53.05±1.49	83.50±2.51*	55.42±1.80*
4i	21.53±0.76	48.17±1.04	55.74±2.02*	57.64±1.68
4j	18.95±0.63	46.56±1.28*	78.73±2.43	59.31±1.89
4k	20.47±0.57*	56.73±1.21*	89.53±2.44*	62.47±1.93
4l	22.74±0.79	41.37±1.39	74.37±2.61	54.58±1.68
4m	20.35±0.74*	52.62±1.30	86.37±2.51*	55.47±1.53
4n	19.64±0.81*	53.63±1.41	84.25±2.32*	53.46±1.72
4o	20.47±1.74	62.37±1.86	80.25±2.83*	49.78±1.79
4p	20.36±0.86*	52.94±1.51*	88.85±2.23*	59.61±1.47*
Ibuprofen	20.26±0.90*	53.95±0.97*	97.09±2.86*	68.02±1.27*

All values are represented as mean ± S.E.M. (n = 6).

*P < 0.01 compared to control group. One-way ANOVA, Dennett's t-test.

Dosage: Ibuprofen-10 mg/kg and test compounds-10 mg/kg body weight by orally.

Table 3.

Analgesic activity of 4, 6-disubstituted-3-cyano-2-aminopyridines (**4a-p)**

Compound	Percent inhibition ± S.E.M. at various time intervals			
	0.5 h	1.0 h	2.0 h	4.0 h
4a	28.35±1.34	47.21±1.68	69.39±2.71	34.28±1.41
4b	40.64±1.38	73.76±1.68	80.84±1.42	30.25±1.48
4c	50.56±0.59*	83.59±1.73*	90.04±1.39*	57.69±0.59
4d	42.67±2.86	77.81±1.97*	83.35±1.86	34.34±1.81
4e	24.75±0.86	80.73±1.29*	87.47±1.47*	30.73±1.09
4f	40.22±1.75	70.37±2.73*	82.46±1.82	42.34±2.12
4g	23.49±0.93	50.47±1.46	75.07±1.87	32.65±1.35

4h	39.88±0.81	82.35±1.31*	88.63±1.59*	30.35±1.06
4i	30.82±1.21	56.04±1.51	78.46±2.13	38.87±1.33
4j	46.14±1.57	77.54±1.51	82.24±1.33	32.64±1.59
4k	50.17±0.62*	83.22±1.69*	89.83±1.37*	56.14±0.55
4l	40.13±2.03	78.13±1.83*	85.23±1.77	30.53±1.73
4m	50.46±1.41*	83.41±1.74*	89.04±1.58*	56.62±1.40
4n	43.99±1.86*	71.89±2.81*	88.31±1.87	43.03±2.02
4o	23.13±0.97	52.03±1.31	75.37±1.97	33.17±1.21
4p	53.73±1.73*	88.27±1.83*	92.34±1.32*	58.31±1.52
Ibuprofen	55.26±0.90*	89.95±0.97*	99.87±1.86*	58.02±2.22*

All values are represented as mean ± S.E.M. (n = 6).
*p<0.01 compared to control group. One-way ANOVA, Dennett's t-test.
Dosage: Ibuprofen-10 mg/kg and test compounds-10 mg/kg body weight by orally.

Table 4:
Antimicrobial activity of 4, 6-disubstituted-3-cyano-2-aminopyridines (**4a-p)**

Compd.	Minimum inhibitory concentration (µg/mL)						
	Gram positive bacteria			Gram negative bacteria		Fungi	
	Bacillus subtilize MTCC 441	*Clostridium tetany* MTCC 449	*Streptococcus pneumonia* MTCC 1936	*Escherichia coli* MTCC 443	*Salmonella tophi* MTCC 98	*Aspergillums fumigates* MTCC 3008	*Candida albinos* MTCC 227
4a	1000	500	500	250	500	1000	1000
4b	**150**	**100**	**55**	**55**	200	**150**	**125**
4c	1000	500	500	250	250	>1000	1000
4d	**250**	500	125	**100**	250	1000	**500**
4e	500	**200**	500	250	500	>1000	>1000
4f	**250**	500	250	150	250	**200**	**250**
4g	500	500	500	250	500	1000	1000
4h	**250**	**125**	**100**	**55**	200	**250**	**125**
4i	1000	500	500	250	500	1000	1000
4j	**125**	**100**	**55**	**55**	150	**150**	**125**
4k	1000	500	250	200	1000	1000	1000
4l	**200**	**150**	**100**	**100**	500	1000	**500**
4m	500	500	200	200	500	>1000	1000
4n	**150**	500	**100**	**100**	200	**400**	**200**
4o	500	500	250	200	250	1000	1000
4p	**150**	**100**	**100**	**50**	150	**200**	**150**
Amp.	**250**	**250**	**110**	**100**	**100**	-	-
Grilse.	-	-	-	-	-	**100**	**500**
Nest.	-	-	-	-	-	**100**	**100**

Amp. : Ampicillin, Grilse. : Griseofulvin, Nyst. : Nystatin

References

1. Hulme C, Gore V: **Multi-component reactions: emerging chemistry in drug discovery from 'xylocain to crixivan'.** *Curr Med Chem* 2003, **10:**51-80.
2. Boulard L, BouzBouz S, Cossy J, Franck X, Figadere B: **Two successive one-pot reactions leading to the expeditious synthesis of (−)-centrolobine.** *Tetrahedron Lett* 2004, **45:**6603-6605.
3. Zimmermann GR, Lehar J, Keith CT: **Multi-target therapeutics: when the whole is greater than the sum of the parts.** *Drug Discovery Today* 2007, **12:**34-42.
4. Frantz S: **Drug discovery: Playing dirty.** *Nature* 2005, **437:**942-943.
5. Higashio Y, Shoji T: **Heterocyclic compounds such as pyrrole, pyridines, pyrrolidine, piperidine, indole, imidazol and pyrazines.** *Applied Catalysis A: General* 2004, **260:**251-259.

6. Vyas DH, Tala SD, Akbari JD, Dhaduk MF, Joshi KA: **Synthesis and antimicrobial activity of some new cyanopyridine and cyanopyrans towards *Mycobacterium tuberculosis* and other microorganisms.** *Indian J Chem* 2009, **48B:**833-839.
7. Divyesh MC, Manish Patel P, Ranjan Patel G: **An efficient one-pot synthesis and *in vitro* antimicrobial activity of new pyridine derivatives bearing the tetrazoloquinoline nucleus.** *Arkivoc* 2009, **xiv:**64-74.
8. Murata T, Shimada M, Sakakibara S, Yoshino T, Kadono H, Masuda T, Shimazaki M, Shintani T, Fuchikami K, Sakai K, Inbe H, Takeshita K, Niki T, Umeda M, Bacon KB, Ziegelbauer KB, Lowinger TB: **Discovery of novel and selective IKK-β serine-threonine protein kinase inhibitors. Part 1.** *Bioorg Med Chem Lett* 2003, **13:**913-918.
9. Abdel-Aziz AAM, El-Subbagh HI, Kunieda T: **Lewis acid-promoted transformation of 2-alkoxypyridines into 2-aminopyridines and their antibacterial activity. Part 2: Remarkably facile C–N bond formation.** *Bioorg Med Chem* 2005, **13:**4929-4935.
10. Feng S, Shujiang T, Fang F, Tuanjie L: **One-pot synthesis of 2-amino-3-cyanopyridine derivatives under microwave irradiation without solvent.** *Arkivoc* 2005, **i:**137-142.
11. Zhou JF, Gong GX, Zhu FX, Zhi SJ: **Microwave promoted one-pot synthesis of 3-(2′-amino-3′-cyano-4′-arylpyrid-6′-yl)coumarins.** *Chin Chem Lett* 2009, **20:**37-39.
12. Manna R, Chimenti F, Bolasco A, Bizzarri B, Filippelli W, Filippelli A, Ganliardi L: **Anti-inflammatory, analgesic and antipyretic 4,6-disubstituted 3-cyano-2-aminopyridines.** *Eur J Med Chem* 1999, **34:**245-254.
13. Akbarzadeh T, Rafinejad A, Mollaghasem J M, Safavi M, Fallah-Tafti A, Pordeli M, Ardestani SK, Shafiee A, Foroumadi A: **2-Amino-3-cyano-4-(5-arylisoxazol-3-yl)-4H-chromenes: Synthesis and In Vitro Cytotoxic Activity.** *Arch Pharm Chem Life Sci* 2012, **345:**386-392.
14. Murata T, Shimada M, Sakakibara S, Yoshino T, Masuda T, Shintani T, Sato H, Koriyama Y, Fukushima K, Nunami N, Yamauchi M, Fuchikami K, Komura H, Watanabe A, Ziegelbauer BK, Bacon BK, Lowinger BT: **Synthesis and structure-activity relationships of novel IKK-beta inhibitors. Part 3: Orally active anti-inflammatory agents.** *Bioorg Med Chem Lett* 2004, **14:**4019-4022.
15. Winter CA, Risley EA, Nus GN: **Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs.** *Proc Soc Exp Biol* 1962, **111:**544-547.
16. Kulkarni SK, Mehta AK, Kunchandy J: **Anti-inflammatory actions of clonidine, guanfacine and B-HT 920 against various inflammagen-induced acute paw oedema in rats.** *Arch Int Pharmacodyn Ther* 1986, **279:**324-334.
17. D'Amour FE, Smith DL: **A method for determining loss of pain sensation.** *J Pharmacol Exp Ther* 1941, **72:**74-79.
18. Kulkarni SK: **Heat and other physiological stress-induced analgesia: Catecholamine mediated and naloxone reversible response.** *Life Sci* 1980, **27:**185-188.
19. National Committee for Clinical Laboratory Standards (NCCLS), 940, West Valley Road, Suite 1400, Wayne, Pennsylvania, 19087-1898, USA. *Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement* (ISBN 1- 56238-454-6), 2002, M 100-S12 (M7).
20. Nishina C, Enoki N, Tawata S, Mori A, Kobayashi K, Fukushima M: **Antibacterial activity of flavonoids against *Staphylococcus epidermidis*, a skin bacterium.** *Agric Biol Chem* 1987, **51:**139-143.