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

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Introduction

In contemporary medicinal chemistry, multi-component reactions (MCRs) are potent instruments that facilitate lead generation by providing easy access to vast libraries of structurally related, drug-like molecules. Therefore, in recent years, such reactions have become an increasingly valuable approach to drug discovery efforts when paired with the use of combinatorial chemistry and high-throughput parallel synthesis [1]. Additionally, because a one-pot reaction can be carried out without the intermediates being isolated and without releasing any functional groups in a brief reaction time, it is the most appropriate technique for the synthesis of functionalized organic molecules [2]. The goal of many medication development strategies was to affect several targets simultaneously. Multi-target lead discovery is a recently developed combination therapy that holds promise for identifying unanticipated unique effects of medication combinations [3]. Many effective medications, including metformin, salicylates, non-steroidal anti-inflammatory medicines (NSAIDs), antidepressants, anti-neurodegenerative therapies, and multi-target kinase inhibitors, impact multiple targets at once. Additionally, multi-target antibodies are being utilized more often in cancer treatment to postpone the emergence of resistance [4]. Both naturally occurring and synthetically produced, pyridine derivatives have demonstrated significant biological activity as medicines and possible agrochemicals [5]. After being produced and tested for antitubercular and antibacterial properties, a few novel 2-amino-3-cyano-6-(3,5-dibromo-4-methoxyphenyl)-4-arylpyridines/pyrans were discovered to be effective [6]. Additionally, the one-pot cyclocondensation method was used to create 2-amino-3-cyano-4-tetrazoloquinolinylpyridines, which were then tested for antibacterial efficacy against a panel of pathogenic bacterial and fungal species and shown to be either equipotent or more potent than commercial antibiotics [7].

In collaboration with those, 2-amino-3-cyanopyridine derivatives have been found as IKK-β inhibitors in conjunction with their importance and usability as intermediates in manufacturing variety of heterocyclic compounds [8, 9]. As a result, organic chemistry continues to be very interested in the synthesis of 2-amino-3-cyanopyridine derivatives. Various approaches for the synthesis of 2-amino-3-cyanopyridine derivatives have been reported employing two-component as well as three-component processes [6, 10, and 11]. Manna and coworkers have described the synthesis of 4, 6-disubstituted-3-cyano-2-aminopyridines and their anti-inflammatory, analgesic and antipyretic activity qualities 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 Procedures

General Melting points were determined on a typical Boetius apparatus and are uncorrected. Using the KBr disc method, IR spectra were captured in Bruker FT-IR Opus Spectroscopic Software Version 2.0. 1H and 13C NMR spectra were acquired in the appropriate 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 obtained 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 bought from Sigma-Aldrich and Merck chemical companies and were utilized without additional processing. Analytical TLC was done on Silica Gel F 254 plates (Merck) with viewing by UV (254 nm) chamber. Through the use of column chromatography on silica gel (100-200 mesh, Merck), all of the cyanopyridines have been purified.

Experimenting
Standard protocol for 6-(4-aminophenyl)-4-(substituted aryl)-3-cyano-2-aminopyridine synthesis (4a-h)

A 50 ml round-bottom flask was charged with 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 pure alcohol (15 ml). The reaction mixture was then refluxed for two to three
h. After the reaction was finished, the reaction mixture was placed into crushed ice while being constantly stirred. The TLC was used to monitor the reaction's progress using Silica gel-G. After being separated, the solid was filtered and dried. It was purified using column chromatography on silica gel (100–200 mesh) with a mobile phase consisting of a combination of ethylacetate and hexane. After purification, the pyridine derivatives 4a–h were produced as fine powder with 30–70% yields, ranging from orange red to yellowish red.

2-cyano-4-(2-chlorophenyl) 2-amino-3- -6-(4-aminophenyl) (4a) Pyridine: solid orange-yellow; Yield 54%; mp 176-178 °C; IR (KBr) cm-1: 3415, 3346, 2205, 1617, 1568, 1367, 822; 1H-NMR (DMSO-d6) 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); 13C-NMR (DMSO-d6) δ: 85.92, 107.96, 117.41, 118.65, 125.23, 127.40, 128.61, 129.59, 130.58, 131.29, 136.61, 152.13, 159.06, 159.99; LC-MS m/z: 321.32 [{M+H}+]. C18H13N4Cl's anal. calcd is C, 67.40; H, 4.04; and N, 17.48. C, 68.83; H, 4.16; N, 17.95 were found. 3-cyano-4-(4-chlorophenyl) 2-amino-3- Orange-yellow solid -6-(4-aminophenyl)pyridine (4b): Yield 62%; mp 188-189 °C; IR (KBr) cm-1:

3402, 3338, 2200, 1618, 1574, 1367, 820; 1H-NMR (DMSO-d6): 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), and 7.87 (2H, d, J = 8.4 Hz); 13C-NMR (DMSO-d6) δ: 85.89, 107.34, m/z for LC-MS: 321.32 [{M+H}+]. Anal. Calculated for C18H13N4Cl: C, 67.40; H, 4.04; N, 17.48; Found: C, 68.59; H, 4.18; N, 17.87. 2-cyano-4-(2,4-dichlorophenyl) 2-amino-3- -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; 1H-NMR (DMSO-d6) δ: 5.67 (2H, br s), 6.65-7.44 (3H, m), and 7.84 (2H, d, J = 8.8 Hz); 13C-NMR (DMSO-d6) δ: 7.00 (1H, s). Anal. Calculated for C18H12N4Cl2: 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): mp 192-194 °C; yield 70%; IR (KBr) cm-1: 3466, 3425, 2200, 1615, 1574, 1371, 1238; 1H-NMR (DMSO-d6) δ: 5.63 (2H, d, J = 8.8 Hz), 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); 13C-NMR (DMSO-d6) δ: 90.82, 109.45, 114.37, 117.29, 118.71, 126.43, 136.23, 145.65, 153.76, 154.64, 163.97, 164.21; LC-MS m/z: 305.11 [{M+H}+]. For C18H13N4F, the anal. calculation was C, 71.11; H, 4.31; N, 18.41; the result was C, 72.35; H, 4.76; N, 19.12. 4-(3-bromophenyl)-6-(4-aminophenyl)-2-amino-3-cyano pyridine (4e): Orange red solid; Yield 50%; mp 205-207 °C; IR (KBr) cm-1: 3424, 3348, 2203, 1629, 1571, 1367, 639; 1H-NMR (DMSO-d6) 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); 13C- NMR (DMSO-d6) 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}+]. C, 60.17; H, 3.64; N, 16.21; Anal. Calcd for C18H13N4Br: C, 59.23; H, 3.59; N, 15.34. 3-cyano-4-(4-methoxyphenyl) 2-amino-3- -6-(4-aminophenyl) Pyridine (4f): solid orange-yellow; Yield 19%; mp 178-179 °C; IR (KBr) cm-1: 3468, 3358, 2200, 1610, 1577, 1373, 1251; 1H-NMR (DMSO-d6) 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); 13C-NMR (DMSO-d6) δ: 59.67, 92.45, 108.34, 115.22, 158.21, 162.27; LC-MS m/z: 317.14 [{M+H}+]. C, 73.05; H, 5.29; N, 17.90; Anal. Calcd for C19H16N4O: C, 72.11; H, 5.31; N, 17.71. 4-(4-methylphenyl)-6-(4-aminophenyl)-2-amino-3-cyano 40% yield; mp 172-174 °C; IR (KBr) cm-1: 3462, 3363, 2202, 1622, 1583, 1371; pyridine (4g): Yellow solid; 13C-NMR (DMSO-d6) 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; 1H-NMR (DMSO-d6) 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), and 7.68 (2H, d, J = 8.6 Hz); LC-MS m/z: 301.14 [{M+H}+]. The C19H16N4 anal. calcd is C, 75.98; H, 5.37; and N, 18.65. C, 76.86; H, 5.39; N, 19.16 were found. 2-amino-3-cyano -4-(4-dimethylaminophenyl) -6-(4-aminophenyl) Pyridine (4h): solid crimson scarlet; 44% yield; mp 208-209 °C; IR (KBr) cm-1: 3444, 3328, 2212, 1614, 1561, 1360; 1H-NMR (DMSO- d6) 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); 13C-NMR (DMSO-d6) 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}+]. Calculated anal values for C20H19N5 are C, 73.01; H, 5.81; and N, 21.26. Found: H, 5.86; N, 21.97; C, 73.94.

Standard protocol for 6-(4-hydroxyphenyl)-4-(substituted aryl)-3-cyano-2-aminopyridine synthesis (4i-p)

A 50 ml round-bottom flask was charged with 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 pure alcohol (15 ml). The reaction mixture was then refluxed for two to three hours. The TLC used Silica gel-G to track the reaction's progress. After the reaction was finished, the reaction mixture was continuously stirred as it was dumped onto crushed ice. After being separated, the solid was filtered and dried. It was purified by employing a combination of ethylacetate and hexane as the mobile phase in column chromatography on Silica gel (100-200 mesh, Merck). After purification, the pyridine derivatives 4i–p yielded fine powders that ranged from orange to yellowish red, with yields of 35-75%.

IR (KBr) cm-1: 3420, 3358, 2210, 1610, 1570, 1377, 824; mp 165-167 °C; 2-amino-3-cyano-4-(2-chlorophenyl)-6-(4-hydroxyphenyl)pyridine (4i): Yellow solid; Yield 68%; 1.45 (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); 13C-NMR (DMSO-d6) 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}+]. Found: C, 68.19; H, 3.89; N, 13.85; Anal. Calcd for C18H12N3OCl: C, 67.20; H, 3.76; N, 13.06. 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; 1H-NMR (DMSO-d6) [+]: 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), 7.77 (2H, d, J = 8.6 Hz), 13.65 (1H, br s); 13C-NMR (DMSO-d6) δ: 85.44, 112.48, 116.86, 121.26, 123.64, 127.64, 134.64, 130.68, 134.64, 150.87, 158.88, 159.45, 163.65; LC-MS m/z: 322.04 [{M+H}+]. Found: C, 68.04; H, 3.90; N, 13.54; Anal. Calcd for C18H12N3OCl: C, 67.19; H, 3.76; N, 13.06. 2. Amino -3-cyano -4 (dichlorophenyl-2,4) -6-(4-hydroxyphenyl) Pyridine (4k): IR (KBr) cm-1: 3423, 3348, 2210, 1628, 1575, 1347, 832; 1H-NMR (DMSO-d6) 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); 13C-NMR (DMSO-d6) 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. for C18H11N3OCl2: 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) IR (KBr) cm-1: 3413, 3326, 2202, 1620, 1553, 1358, 1274; 1H-NMR (DMSO-d6) δ: 6.58 (2H, d, J = 8.6 Hz), 6.82 (2H, br s), 7.16 (1H, s), 7.67 (2H, dd, J = 9.4 Hz, J = 8.6 Hz), and 7.80 (2H, d, J = LC-MS m/z: 306.2 [{M+H}+]. C, 71.41; H, 4.06; N, 14.17; Anal. Calcd for C18H12N3OF: C, 70.82; H, 3.96; N, 13.76. 2-amino-3-cyano-4-(3-bromophenyl)-6-(4-hydroxyphenyl) orange red solid pyridine (4m); yield 43 percent; mp 200-201 °C; IR (KBr) cm-1: 3447, 3378, 2210, 1643, 1564, 1364, 631; 1H-NMR (DMSO-d6) 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); LC-MS m/z: 366.02 [{M+H}+); 13C-NMR (DMSO-d6) 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.

Anal. Calculated for C18H12N3OBr: C, 59.03; H, 3.30; N, 11.45; Found: C, 60.05; H, 3.42; N, 11.84.

3-cyano-4-(4-methoxyphenyl) 2-amino-3- IR (KBr) cm-1: 3456, 3354, 2206, 1614, 1589, 1370, 1258; mp 172-173°C; -6-(4-hydroxyphenyl)pyridine (4n): Orange yellow solid; Yield 50%; 13C-NMR (DMSO-d6) 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 1H-NMR (DMSO-d6) 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); m/z: 318.12 [{M+H}+]. For C19H15N3O2, the anal.

calculation was C, 71.91; H, 4.76; N, 13.24; the result was C, 72.56; H, 4.82; N, 13.24. 4-(4-methylphenyl)-6-(4-hydroxyphenyl)-2-amino-3-cyano Yellow solid pyridine (4o); yield of 57%; mp 168-170 °C; IR (KBr) cm-1: 3444, 3346, 2207, 1622, 1580, 1376; 1H-NMR (DMSO-d6) 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 = 8.43, 126.43, 128.25, 13C-NMR (DMSO-d6) δ: 30.56 [{M+H}+]. C, 76.54; H, 5.09; N, 14.13. 2 Anal. Calcd for C19H15N3O: C, 75.73; H, 5.02; N, 13.94 -amino -3-cyano -4-(4-dimethylaminophenyl) -6-(4-hydroxyphenyl) pyridine (4p): a solid that is crimson; IR (KBr) cm-1: 3456, 3350, 2210, 1615, 1567, 1354; yield 40%; mp 202-203 °C; 1H-NMR (DMSO- d6) 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); 13C-NMR (DMSO-d6) 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}+]. Calculated anal values for C20H18N4O are C, 72.71; H, 5.49; and N, 16.96. C, 73.50; H, 5.63; and N, 17.24 were found.

Pharmacology

Animals

For the tests, albino mice (20–25 g) of either sex and Wistar albino rats (150–200 g) of both sexes were chosen from M/S Ghosh Enterprises, Calcutta, West Bengal, India. Prior to the trial, the animals were given two weeks to acclimate to our laboratory setting. The animals lived in polypropylene cages with six animals each, and were kept in standard laboratory conditions, which included a 12:12 light and dark cycle, a temperature of 25±2°C, and a relative humidity of 35–60%. They were fed a standard rat pellet diet (Hindustan Liver Ltd., Mumbai) and given unlimited water. Our institutional animal ethics committee has granted permission (439/PO/A/01/CPCSEA) to carry out the experiments.

Anti-inflammatory activity

Albino rats were used to investigate the compounds' anti-inflammatory properties using the carrageenan-induced rat paw edema technique [15,16]. The test chemicals and standard medication were suspended in a sodium CMC (1% W/V) suspension. To cause inflammation in rats, a 1% W/V suspension of carrageenan sodium salt was made. Nineteen groups of six albino rats weighing between 150 and 200 grams apiece were created, and each group was given a unique number. Every group was given unlimited access to water and fasted throughout the whole night. For each group, 0.05 ml of a 1% carrageenan suspension was subcutaneously injected into the right hind paw's sub-plantar region to cause inflammation, and 0.05 ml of saline was injected into the left hind paw's sub-plantar region. Group 1 is regarded as a sham control group. As carrageenan-treated control animals, group-2 received 1% sodium CMC gel (1 ml/kg, p.o.) one hour before the carrageenan injection. Ibuprofen (10 mg/kg, p.o.) was administered as a regular medication to Group 3, and the group 4 to 19 were given synthetic cyanopyridines (10 mg/kg, p.o.) in that order. Using a digital plethysmometer apparatus, the thickness of each rat's two paws was measured before and after the carrageenan injection at intervals of 0, 0.5, 1, 2, and 4 hours. The graduated microscale attached to the mercury column allowed for the direct reading of mercury displacement caused by the dipping of the paw, magnifying the slight variations in paw thickness throughout the experiment. Calculations were made to determine the percentage inhibition of paw edema thickness in control, reference medication, and compound-treated mice. Table 2 displays the findings and statistical analysis of the anti-inflammatory activity of the substances tested, the reference medication, and the control.

Analgesic activity

In accordance with D'Amour et al. [17] and Kulkarni [18], the tail flick (tail-withdrawal from the radiant heat) method was used to assess the analgesic activity of the test compounds and standard using an analgesiometer. By inserting the tip (last 1-2 cm) of each animal's tail separately, the basal reaction time to radiant heat has been measured for the control, standard, and test groups. The termination point is considered to be the tail-withdrawal from the heat. A sodium CMC (1% W/V) aqueous suspension was used to suspend the test compounds and ibuprofen. Eighteen groups of six albino mice each, of either sex (20–25 g), were created and assigned unique numbers. After a 24-hour fast, the medication was given to each group along with unlimited water. As a control, just 1% w/v sodium CMC suspension (1 ml/kg, p.o.) was given to Group 1. As a benchmark, ibuprofen (10 mg/kg, p.o.) was given to Group 2. Test compounds were given to groups three through eighteen (10 mg/kg, p.o.) correspondingly. With their tails sticking out, all of the creatures were securely tied. The reaction time was measured in seconds at 0, 0.5, 1, 2, and 4 hours after the compounds were administered. To avoid damaging the tail, a 10-second cutoff limit was noted. Calculations were made to determine the proportion of protection in the control, conventional medication, and compound-treated animals. Table 3 displays the results and statistical analysis of the analgesic activity of the substances tested, ibuprofen, and the control.

Antimicrobial activity

All of the produced compounds' in vitro antibacterial activity was tested using the broth microdilution method [19, 20]. The compound suspension for the test bacteria was grown and diluted using Mueller Hinton broth, whereas the fungal nutrition was achieved using Sabouraud Dextrose broth. By comparing the turbidity, the inoculum size for the test strain was set at 108 CFU [Colony Forming Unit] per milliliter. [MTCC-Micro Type Culture Collection] Institute of Microbial Technology, Chandigarh, India, provided the strains used in the activity. The antibacterial and antifungal properties of compounds 4a–p were tested against Salmonella typhi (MTCC 98), Aspergillus fumigates (MTCC 3008), Streptococcus pneumonia (MTCC 3906), Escherichia coli (MTCC 443), Bacillus subtilis (MTCC 441), and Clostridium tetani (MTCC 449). To obtain the appropriate concentration of chemicals to test on microbiological strains, DMSO (dimethyl sulphoxide) was utilized as a vehicle. For each drug, the minimum inhibitory concentration (MIC) was defined as the concentration at which no discernible growth occurred following spot subculture. The standard medicines utilized for comparison in this study were griseofulvin and nystatin for antifungal activity and ampicillin for antibacterial activity. Table 4 provides a summary of the findings.

Results and Discussion

Chemistry

The method produced the target compounds, 4a-p, by first forming the arylidenemalononitrile (Michael reaction) from malononitrile and aromatic aldehydes. This reaction then combines with the aryl ketones 3a-b and ammonium acetate to produce the 2-amino-3-cyanopyridines. Spectroscopic analysis was used to determine the structure of the products, 4a-p. Bands at 3400-3200 cm-1 (NH2), 3450-3425 (OH) cm-1, and 2220-2210 were visible in the IR spectra of 4a-p. 1640-1600 cm-1 (C⁇N) and cm-1 (C›N). With a broad singlet at ppm 13.70-13.40 and 5.60-5.70 attributed to the hydroxylic proton and amino protons, respectively, and another broad singlet at ppm 6.85-6.70 attributed to the amino protons of the cyanopyridine nucleus that vanished when the deuteriodimethylsulphoxide solution was shaken with deuterium oxide, the 1H-NMR spectra of these compounds provided additional support for the structure of cyanopyridines. Additionally, the creation of the 2-amino-3-cyano-4, 6-disubstituted pyridine nucleus is further confirmed by the distinctive singlet peak seen at ppm 7.20-7.10, which shows the existence of a single proton at the C-5 position of

the pyridine ring. At ppm 6.0–8.0, additional aromatic proton signals were seen. The pyridine structure was confirmed by the 13C-NMR spectra, which showed distinctive peaks between ppm 180-160 for ring carbons next to the nitrogen atom in the pyridine nucleus, ppm 150-120 for other ring carbons, ppm 119-116 for cyanide carbon, and ppm 60-30 outside the ring carbons. The fragmentation patterns were typical of the associated pyridines, and the mass spectra displayed the corresponding molecular ion peak [M+H]+ as the base peak. All of the recently synthesized compounds' structures were validated by elemental analysis.

Anti-inflammatory activity

Using a rat paw edema model caused by carrageenan, the anti-inflammatory properties of all the synthesized compounds (4a-p) were examined. Prior to and 0.5, 1, 2, and 4 hours following carrageenan injection, the effects of the test compounds and ibuprofen, as a reference, were assessed. As shown in Table 2, the percentage of edema inhibition was computed in relation to the saline control group. Most of the drugs showed significant suppression of edema size ($p < 0.01$) when compared to ibuprofen. Compounds 4f and 4h in the 4-aminophenyl series carrying 4-methoxyphenyl and 4-dimethylaminophenyl substituent, and compounds 4m and 4n in the 4-hydroxyphenyl series carrying 3-bromophenyl and 4-methoxyphenyl substituent at the C-4 position of pyridine nucleus, respectively, demonstrated remarkable activity, while compounds 4c, 4e, 4k, and 4p were found to be the most potent anti-inflammatory compounds, as shown in Table 2. Additionally, compound 4k was discovered to be the lead structure out of all of them. The following structure-activity relationship (SAR) was obtained by analyzing the anti-inflammatory properties of the synthesized compounds 4a–p. The potency order of the five halogen-substituted pyridine derivatives 4a-e and 4i-m was determined to be 2,4-Cl2 > 3-Br > 4-Cl > 4-F > 2-Cl. The potency order among the three pyridine derivatives that were substituted with electron donors, 4f-h and 4n-p, was 4-N(CH3)2 > 4-OCH3 > 4-CH3.

Analgesic activity

The tail flick method, which uses heat as a source to cause pain in mice, was utilized to assess the analgesic activity of the synthesized compounds (4a–p). The test drugs' analgesic effectiveness is directly correlated with the increase in reaction time (time interval) over basal. Table 3 provides a summary of the findings. Because compounds 4c and 4p carry 2,4-dichlorophenyl at C-4 in 4-aminophenyl series and 4-dimethylaminophenyl (4p) at C-4 in 4-hydroxyphenyl series on the 2-amino-3-cyanopyridine nucleus, they demonstrated dose dependent activity with higher protection at 120 minutes, which is comparable to the reference standard. They also exerted their activity similarly to that of the well-known medication ibuprofen. Furthermore, compounds with 3-bromophenyl (4e) and 4-dimethylaminophenyl (4h) substituents in the 4-amino series and 2, 4-dichlorophenyl (4k) and 3-bromophenyl (4m) moieties in the 4-hydroxy series at the C-4 position in the cyanopyridine nucleus were found to have moderate analgesic activity. This activity increased at 60 minutes and peaked at 120 minutes.

The following SAR was obtained by analyzing the analgesic activity of all compounds 4a–p. In both the 4-amino and 4-hydroxy series, the potency order of the five halogen-substituted cyanopyridines, 4a-e and 4i-m, was 2,4-Cl2 ≅ 3-Br > 4-F > 4-Cl ¯ 2-Cl. In both series, the potency order of the three electron-releasing substituted cyanopyridines, 4f-h and 4n-p, was 4-N(CH3)2 > 4-OCH3 > 4-CH3. At 120 minutes, compound 4p showed the strongest analgesic effect of all. According to these findings, 4k and 4p are more promising compounds as anti-inflammatory and analgesic drugs, respectively, and more research is needed to clarify the precise mode of action for their potential therapeutic use.

Antimicrobial action;
The broth microdilution method was used to test each produced compound's in vitro antibacterial properties. Compounds 4b and 4j demonstrated exceptional activity against Gram positive bacteria Streptococcus pneumoniae and Gram negative bacteria Escherichia coli, while compounds 4h and 4p were found to be highly active against Gram negative bacteria Escherichia coli only when compared to the standard antibiotic ampicillin, according to an analysis of the data (Table 4). Compounds 4b, 4d, 4f, 4h, 4j, 4l, 4n, and 4p are reported to be more effective against Gram-positive bacteria Bacillus subtilis than the conventional antibiotic ampicillin. It has been discovered that chemicals 4b, 4e, 4h, 4j, 4l, and 4p are more effective than ampicillin against the Gram-positive bacteria Clostridium tetani. The majority of the compounds didn't show enough strength to stop Salmonella typhi. Compounds 4b, 4f, 4h, 4j, and 4p are more effective against Candida albicans than typical fungicidal griseofulvin, according to an antifungal investigation. The majority of the substances were determined to be insufficiently strong to inhibit Aspergillus fumigatus.

In conclusion
A simple one-pot multi-component process has been used to create a number of novel 2-amino-3-cyano-4,6-disubstituted pyridine derivatives. This synthesis approach enables the incorporation of several aromatic and heteroaromatic substitutions into the 4- and 6-positions of pyridine as well as the development of a quite complex heterocyclic structure including nitrogen. Non-steroidal anti-inflammatory drugs (NSAIDs) and antimicrobial agents will be used in multi-target drug therapy, one of the recently developed combination therapies as multi-target lead discovery. These new agents may act by one of the mechanisms discussed in the introduction and can be further utilized for lead optimization purposes. The anti-inflammatory, analgesic, and antibacterial characteristics of the 4,6-diaryl-3-cyano-2-aminopyridines 4a–p were regulated by the electro negativity of the substituents and their displacement on the 4-aryl ring; these effects were stronger when electron-releasing groups were present. Additionally, it was found that substituting the 4-hydroxyphenyl group (4i-p) for the 4-aminophenyl group (4a-h) at the C-6 position of the 3-cyano-2-aminopyridine nucleus improved its effectiveness.

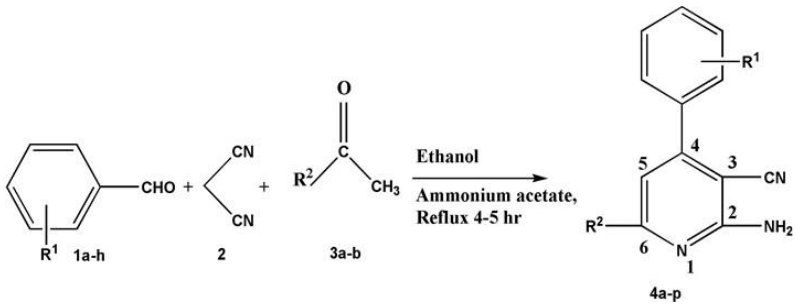

Figure 1. Multi-component synthesis of 4, 6-disubstituted-3-cyano-2-aminopyridines **4a-p**.

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	75.07.87	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

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