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DESIGN AND CHARACTERIZATION PYRIDO[2,3-D]PYRIMIDINES SUBSTITUTED CHALCONE DERIVATIVES FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Chalcones are very desirable starting materials in medicinal chemistry since they can be produced artificially or naturally and are known to display a variety of biological functions. Chalcones have been found to possess a reactive α,β-unsaturated keto functional group, which is the source of their biological action. It is well known that pyrido[2,3-d]pyrimidines are pharmacophoric components in a wide range of active compounds with diverse pharmacological activities. By using the Claisen-Schmidt condensation method, several aromatic aldehydes (2a-k) were combined with 4-aminoacetophenone (1) to create chalcones 3a-k. When guanidine hydrochloride was used to cyclize these 4-aminochalocones under basic alcoholic conditions, 2,4,6-trisubstituted pyrimidines 4a–k were produced with quantifiable quantities. A straightforward regioselective cyclization was used to create the novel 4-amino-5,7-disubstituted pyrido[2,3-d]pyrimidines 6a-g. This was accomplished by aromatizing 2-amino-3-cyano-4,6-disubstituted pyridines 5a-k, which were derived from 4-aminochalcones 3a-k. All the synthesized compounds were characterized by spectroscopic means (FT-IR, 1H & 13C NMR) and elemental analysis and screened for antimicrobial activity. All the molecules were found to possess better activity against microbes.

KEYWORDS: Chalcone, Pyrimidine, bacteria, fungal, antimicrobial activity

INTRODUCTION

A material that destroys or stops the growth of microorganisms like bacteria, fungus, or protozoa is known as an antimicrobial. Antimicrobial medications have two effects: they either eradicate bacteria (microbicidal) or stop them from growing (microbistatic). Pasteur and Joubert's observations—that one species of bacteria may stop the growth of another—mark the beginning of the history of antimicrobials. At the time, they were unaware that the other bacterium was making an antibiotic, which was the reason why one bacterium was unable to grow. According to technical definitions, antibiotics are only compounds that a certain bacteria produces that either kill or stop the growth of another microorganism. Naturally, the word "antibiotic" now refers to nearly any medication that aims to cure a bacterial illness in the body. (2) Chalcones are particularly appealing starting materials in medicinal chemistry since they can be found naturally or artificially and are known to exhibit a variety of biological functions. Chalcones' biological activity has been linked to the existence of a reactive α,β-unsaturated keto functional group (3). Numerous pyridine-containing synthetic and naturally occurring compounds have significant biological effects; as a result, the literature has documented a number of effective methods for creating functionalized pyridines (4–7). Among all, 2-amino-3-cyanopyridines with different alkyl, aryl and heteroaryl groups were found to have antihypertensive (8), antitumor (9), antimicrobial (7), anti-inflammatory, cardiovascular analgesic and antipyretic (10), antiparkinsonism properties (11), as well as Ikk-β inhibitor properties (12). Many active compounds, including anticancer, anticonvulsant, antibacterial, calcium channel antagonists, antimicrobial, antileishmanial, anti-inflammatory and analgesic, antitubercular, diuretic, and CNS depressive medicines, are known to contain pyrido[2,3-d]pyrimidines as pharmacophoric components (13). Two broad methods are typically used to create pyrido[2,3-d]pyrimidines: 1) forming the pyridine ring by cyclizing appropriate pyrimidine substituents, and 2) forming the pyrimidine ring by cyclizing appropriate pyrimidine substituents (14). Hence, we are interested for the synthesis Pyrido[2,3-D]Pyrimidines Substituted Chalcone Derivatives for antimicrobial activity.

2. MATERIALS AND METHODS

Melting points (MP) were determined on a standard Boetius apparatus and are uncorrected. The IR spectra were recorded in Perkin-Elmer BXF1 FT-IR spectrophotometer using KBr disc method. ^{1}H and ^{13}C NMR spectra were recorded in the indicated solvent on a Bruker AMX 400 and 100 MHz respectively with tetramethylsilane (TMS) as internal standard (chemical shifts in δ ppm). The elemental analyses of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within ± 0.4% of the theoretical values. Analytical TLC was performed on Silica Gel F_{254} plates (Merck) with visualization by UV (254 nm) chamber with protective filters. All the cyanopyridines and pyrido[2,3-d]pyrimidines have been purified by column chromatography performed on silica gel (100-200 mesh, Merck). All reagents and solvents were used as without further purification.

Synthesis and Characterization of compound:

The synthesis of a new series of 2-amino-3-cyanopyridines and their pyrido[2,3-d]pyrimidines is now disclosed in light of these reports. The 4-aminochalcones (3a-k) and malononitrile were refluxed together in an alcoholic ammonium acetate through the Michael reaction to provide the desired target compounds, 2-amino-3-cyano-4,6-disubstituted pyridines (5a-k), with the elimination of 1 mol of hydrogen and water. The equivalent pyrido[2,3-d]pyrimidines (6a-g) are formed when the cyanopyridines (5a-k) condense with formamide at 170°C (16–28 hours) (Scheme 2, Table 2). 2,4,6-trisubstituted pyrimidines are synthesised from corresponding 4-aminochalcones. Schemes 1 and 2 depict the synthetic

routes taken to produce the final compounds, and spectral and analytical data were used to confirm the structures.

General procedure for synthesis of substituted 2-amino-3-cyanopyridines, 5a-k:A flask with a reflux condenser and a water separator was filled with a mixture of 4-aminochalcones (3a-k) (0.01 mol), malononitrile (0.01 mol), ethanol (5 ml), and ammonium acetate (0.015 mol). After shaking the reaction mixture to guarantee mixing, it was left to reflux for two to six hours. TLC was used to determine reaction completeness using Silica gel-G (15). After the reaction was finished, it was cooled to room temperature and continuously stirred with broken ice. After separation, the solid was filtered and dried. It was refined using column chromatography using Merck's 100–200 mesh silica gel, with a mobile phase consisting of a combination of ethyl acetate and hexane to produce pure cyanopyridines (5a–k). (16)

2-amino-3-cyano-4-(4-chlorophenyl)-6-(4-aminophenyl)pyridine, 5a: Yield 37%, MP 212±2 °C. IR (λmax, cm-1): 3402, 3338, 2200, 1574, 1367 and 820. 1H NMR (DMSO-d6): δ 5.65 (2H, br s, C-4-NH2); 6.61 (2H, d, J = 8.4 Hz, C-3 and 5-H); 6.78 (2H, br s, C-2-NH2); 7.08 (1H, s, C-5-H); 7.60 (2H, d, J = 8.4 Hz, C-2 and 6-H); 7.66 (2H, d, J = 8.4 Hz, C-2 and 6-H); 7.87 (2H, d, J = 8.4 Hz, C-3 and 5-H). Anal. Calcd for C18H13N4Cl: C, 67.41; H, 4.04; N, 17.49. Found: C, 67.43; H, 4.05; N, 17.49.

2-amino-3-cyano-4-(2,4-dichlorophenyl)-6-(4-aminophenyl)pyridine, 5b: Yield 20%, mp 227±2 °C. IR (λmax, cm^{-1}): 3425, 3352, 2202, 1630, 1580, 1368 and 829. 1H NMR (DMSO-d6): δ 5.67 (2H, br s, C-4-NH2); 6.60 (2H, d, J = 8.8 Hz, C-3 and 5-H); 6.85 (2H, br s, C-2-NH2); 7.00 (1H, s, C-5-H); 7.65-7.44 (3H, m, C-3,5 and 6-H); 7.84 (2H, d, J = 8.8 Hz, C-2 and 6-H). Anal. Calcd for C18H12N4Cl2: C, 60.90; H, 3.41; N, 15.77. Found: C, 60.84; H, 3.41; N, 15.77.

2-amino-3-cyano-4-(4-fluorophenyl)-6-(4-aminophenyl)pyridine, 5c: Yield 22%, mp 192±2 °C. IR (δmax, cm^{-1}): 3466, 3425, 2200, 1605, 1574, 1371 and 1238. 1H NMR (DMSO-d6): δ 5.63 (2H, br s, C-4-NH2); 6.61 (2H, d, J = 8.8 Hz, C-3 and 5-H); 6.76 (2H, br s, C-2-NH2); 7.08 (1H, s, C-5-H); 7.37 (2H, dd, J = 10.2 Hz, J = 8.8 Hz, C-2 and 6-H); 7.70 (2H, dd, J = 9.2 Hz, J = 8.6 Hz, C-3 and 5-H); 7.87 (2H, d, J = 8.4 Hz, C-2 and 6-H). Anal. Calcd for C18H13N4F: C, 71.11; H, 4.31; N, 18.41. Found: C, 71.05; H, 4.31; N, 18.42.

2-amino-3-cyano-4-(3-bromophenyl)-6-(4-aminophenyl)pyridine, 5d: Yield 18%, mp 237±2 °C. IR (λmax, cm-1): 3424, 3348, 2203, 1629, 1571, 1367 and 639. 1H NMR (DMSO-d6): δ 5.65 (2H, br s, C-4-NH2); 6.62 (2H, d, J = 8.4 Hz, C-3 and 5-H); 6.80 (2H, br s, C-2-NH2); 7.12 (1H, s, C-5-H); 7.52-7.48 (1H, t, J = 7.1 Hz, C-5-H); 7.64 (1H, d, J = 7.6 Hz, C-4-H); 7.72 (1H, d, J = 7.6 Hz, C-6-H); 7.83 (1H, s, C-2-H); 7.90 (2H, d, J = 8.4 Hz, C-2 and 6-H). Anal. Calcd for C18H13N4Br: C, 59.23; H, 3.59; N, 15.34. Found: C, 59.17; H, 3.59; N, 15.34.

2-amino-3-cyano-4-(4-methoxyphenyl)-6-(4-aminophenyl)pyridine, 5e: Yield 19%, mp 228±2 °C. IR (λmax, cm-1): 3468, 3428, 2200, 1610, 1577, 1373 and 1251. 1H NMR (DMSO-d6): δ 3.83 (3H, s, C-4-OCH3); 5.62 (2H, br s, C-4-NH2); 6.55 (2H, d, J = 8.4 Hz, C-3 and 5-H); 6.68 (2H, br s, C-2-NH2); 7.05 (1H, s, C-5-H); 7.09 (2H, d, J = 8.4 Hz, C-3 and 5-H); 7.61 (2H, d, J = 8.8 Hz, C-2 and 6-H); 7.86 (2H, d, J = 8.8 Hz, C-2 and 6-H). Anal. Calcd for C19H16N4O: C, 71.11; H, 4.31; N, 18.41. Found: C, 71.05; H, 4.30; N, 18.40.

2-amino-3-cyano-4-(3,4-dimethoxyphenyl)-6-(4-aminophenyl)pyridine, 5f: Yield 28%, mp 234±2 °C. IR (λmax, cm-1): 3465, 3430, 2205, 1614, 1582, 1368 and 1259. 1H NMR (DMSO-d6): δ 3.74 (3H, s, C-3-OCH3); 3.80 (3H, s, C-4-OCH3); 5.62 (2H, br s, C-4-NH2); 6.59 (2H, d, J = 8.4 Hz, C-3 and 5-H); 6.75 (2H, br s, C-2-NH2); 6.99 (1H, s, C-5-H); 7.55 (2H, d, J = 8.8 Hz, C-2 and 6-H); 6.93-6.79 (3H, m, C-2, 5 and 6-H). Anal. Calcd for C20H18N4O2: C, 69.36; H, 5.2; N, 16.18. Found: C, 69.34; H, 5.21; N, 16.18.

2-amino-3-cyano-4-(4-methylphenyl)-6-(4-aminophenyl)pyridine, 5g: Yield 35%, mp 218±2 °C. IR (λmax, cm-1): 3462, 3363, 2202, 1622, 1583 and 1371. 1H NMR (DMSO-d6): δ 2.39

(3H, s, C-4-CH3); 5.62 (2H, br s, C-4-NH2); 6.55 (2H, d, J = 8.8 Hz, C-3 and 5-H); 6.70 (2H, br s, C-2-NH2); 7.05 (1H, s, C-5-H); 7.17 (2H, d, J = 8.0 Hz, C-3 and 5-H); 7.53 (2H, d, J = 8.0 Hz, C-2 and 6-H); 7.68 (2H, d, J = 8.6 Hz, C-2 and 6-H). Anal. Calcd for C19H16N4: C, 76.07; H, 5.37; N, 18.65. Found: C, 76.00; H, 5.36; N, 18.66.

2-amino-3-cyano-4-(4-dimethylaminophenyl)-6-(4-aminophenyl)pyridine, 5h: Yield 34%, mp 208±2 °C. IR (λmax, cm-1): 3444, 3328, 2212, 1614, 1561 and 1360. 1H NMR (DMSO-d6): δ 3.10 [6H, s, C-4-N(CH3)2]; 5.86 (2H, br s, C-4-NH2); 6.84 (2H, d, J = 10.2 Hz, C-3 and 5-H); 7.07 (1H, s, C-5-H); 7.26 (2H, d, J = 9.8 Hz, C-3 and 5-H); 7.83 (2H, d, J = 10.0 Hz, C-2 and 6-H); 8.02 (2H, br s, C-2-NH2); 8.13 (2H, d, J = 9.8 Hz, C-2 and 6-H). Anal. Calcd for C20H19N5: C, 73.01; H, 5.81; N, 21.26. Found: C, 72.94; H, 5.80; N, 21.27.

2-amino-3-cyano-4-(9-anthracenyl)-6-(4-aminophenyl)pyridine, 5i: Yield 33%, mp 266±2°C. IR (λmax, cm-1): 3468, 3335, 2217, 1630, 1601 and 1355. 1H NMR (DMSO-d6): δ 6.19 (2H, br s, C-4-NH2); 6.63 (2H, d, J = 8.8 Hz, C-3 and 5-H); 7.61-7.55 (4H, m, Anthracenyl-H); 7.62 (1H, s, Anthracenyl-H); 7.66 (1H, s, C-5-H); 7.90 (2H, d, J = 8.4 Hz, C-2 and 6-H); 8.25-8.14 (4H, m, Anthracenyl-H); 8.66 (2H, br s, C-2-NH2). Anal. Calcd for C26H18N4: C, 80.90; H, 4.69; N, 14.50. Found: C, 80.86; H, 4.70; N, 14.52.

2-amino-3-cyano-4-(2-pyridinyl)-6-(4-aminophenyl)pyridine, 5j: Yield 12%, mp 245±2 °C. IR (λmax, cm-1): 3423, 3330, 2215, 1644, 1576 and 1352. 1H NMR (DMSO-d6): δ 5.68 (2H, br s, C-4-NH2); 6.65 (2H, d, J = 8.2 Hz, C-3 and 5-H); 6.85 (2H, br s, C-2-NH2); 7.05 (1H, s, C-5-H); 7.63-7.47 (4H, m, C-3, 4, 5 and 6-H); 7.89 (2H, d, J = 8.2 Hz, C-2 and 6-H). Anal. Calcd for C17H13N5: C, 71.14; H, 4.56; N, 24.37. Found: C, 71.08; H, 4.56; N, 24.39.

2-amino-3-cyano-4-(4-pyridinyl)-6-(4-aminophenyl)pyridine, 5k: Yield 13%, mp 251±2 °C. IR (□max, cm-1): 3425, 3330, 2216, 1644, 1575 and 1352. 1H NMR (DMSO-d6): δ 5.61 (2H, br s, C-4-NH2); 6.60 (2H, d, J = 8.8 Hz, C-3 and 5-H); 6.70 (2H, br s, C-2-NH2); 7.10 (1H, s, C-5-H); 7.35 (2H, d, J = 9.8 Hz, C-2 and 6-H); 7.73 (2H, d, J = 9.2 Hz, C-3 and 5-H); 7.88 (2H, d, J = 8.0 Hz, C-2 and 6-H). Anal. Calcd for C17H13N5: C, 71.15; H, 4.57; N, 24.39. Found: C, 71.08; H, 4.56; N, 24.40.

General procedure for synthesis of substituted pyrido[2,3-d] pyrimidines, 6a-g: A reaction flask equipped with a reflux condenser and a water separator was filled with a mixture of 2-amino-3-cyano-4,6-disubstituted pyridines (5a-k) (0.001 mol), formamide (0.001 mol), and dimethyl formamide (5 mL). After reflux at 170°C on an oil bath for 22–24 hours, corresponding pyrido[2,3–d] pyrimidines 27 were formed (Scheme 2). TLC utilising silica gel-G allowed for the identification of the reaction's completion. A rotary vacuum evaporator was used to evaporate the solvent. To obtain pure pyridopyrimidines (6a-g), the residue was added to crushed ice, and the resulting precipitate was separated by suction, cleaned with water, dried, and purified using column chromatography on silica gel with a mobile phase consisting of a combination of ethyl acetate and hexane.

4-amino-5-(4-chlorophenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6a: Yield 55%, mp 315±2 °C. IR (λmax, cm-1): 3402, 3342, 1681, 1605, 1365 and 821. 1H NMR (DMSO-d6): δ 7.00 (2H, br s, C-4-NH2); 7.25 (1H, s, C-6-H); 7.31 (2H, d, J = 8.0 Hz, C-3 and 5-H); 7.63 (2H, d, J = 8.4 Hz, C-2 and 6-H); 7.64 (2H, br s, C-4-NH2); 7.70 (2H, d, J = 8.0 Hz, C-2 and 6-H); 8.12 (2H, d, J = 8.0 Hz, C-3 and 5-H); 8.33 (1H, s, C-2-H). Anal. Calcd for C19H14N5Cl: C, 65.67; H, 4.06; N, 20.13. Found: C, 65.60; H, 4.02; N, 20.14.

4-amino-5-(2,4-dichlorophenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6b: Yield 34%, mp 338±2 °C. IR (λmax, cm-1): 3403, 3337, 1684, 1600, 1366 and 827. 1H NMR (DMSO-d6): δ 7.07 (2H, br s, C-4-NH2); 7.20 (1H, s, C-6-H); 7.54 (1H, s, C-3-H); 7.62-7.56 (2H, m, C-5 and C-6-H); 7.70 (2H, d, J = 8.4 Hz, C-3 and 5-H); 7.85 (2H, br s, C-4-NH2); 8.09 (2H, d, J = 8.4 Hz, C-2 and 6-H); 8.33 (1H, s, C-2-H). Anal. Calcd for C19H13N5Cl2: C, 59.74; H, 3.43; N, 18.32. Found: C, 59.68; H, 3.43; N, 18.33.

4-amino-5-(4-fluorophenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6c: Yield 51%, mp 306±2 °C. IR (λmax, cm-1): 3406, 3300, 1682, 1604, 1366 and 1239. 1H NMR (DMSO-d6): δ 6.96 (2H, br s, C-4-NH2); 7.24 (1H, s, C-6-H); 7.30 (2H, d, J = 8.4 Hz, C-3 and 5-H); 7.42-7.37 (2H, m, C-2 and 6-H); 7.72 (2H, br s, C-4-NH2); 7.75-7.73 (2H, m, C-3 and 5-H); 8.12 (2H, d, J = 8.4 Hz, C-2 and 6-H); 8.34 (1H, s, C-2-H). Anal. Calcd for C19H14N5F: C, 68.94; H, 4.26; N, 21.14. Found: C, 68.88; H, 4.26; N, 21.12.

4-amino-5-(3-bromophenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6d: Yield 64%, mp 342±2 °C. IR (λmax, cm-1): 3407, 3339, 1684, 1606, 1360 and 701. 1H NMR (DMSO-d6): δ 7.00 (2H, br s, C-4-NH2); 7.28 (1H, s, C-6-H); 7.54-7.50 (1H, t, J = 8.8 Hz, C-5-H); 7.68 (2H, d, J = 8.4 Hz, C-3 and 5-H); 7.73 (2H, d, J =10.2 Hz, C-4 and 6-H); 7.87 (2H, br s, C-4-NH2); 8.13 (1H, s, C-2-H); 8.15 (2H, d, J = 7.8 Hz, C-2 and 6-H); 8.33 (1H, s, C-2-H). Anal. Calcd for C19H14N5Br: C, 58.21; H, 3.60; N, 17.85. Found: C, 58.16; H, 3.59; N, 17.84.

4-amino-5-(4-methoxyphenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6e: Yield 34%, mp 315±2 °C. IR (λmax, cm-1): 3402, 3336, 1681, 1605, 1365 and 1251. 1H NMR (DMSO-d6): δ 3.76 (3H, s, C-4-OCH3); 7.03 (2H, br s, C-4-NH2); 7.34 (1H, s, C-6-H); 7.29 (2H, d, J = 8.0 Hz, C-3 and 5-H); 7.71 (2H, d, J = 8.4 Hz, C-2 and 6-H); 7.68 (2H, br s, C-4-NH2); 7.76 (2H, d, J = 8.0 Hz, C-2 and 6-H); 8.06 (2H, d, J = 8.0 Hz, C-3 and 5-H); 8.42 (1H, s, C-2-H). Anal. Calcd for C20H17N5O: C, 69.97; H, 4.95; N, 20.40. Found: C, 69.96; H, 4.95; N, 20.40.

4-amino-5-(4-methylphenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6f: Yield 43%, mp 312±2 °C. IR (λmax, cm-1): 3403, 3300, 1681, 1607 and 1368. 1H NMR (DMSO-d6): δ 2.40 (3H, s, C-4-CH3); 6.91 (2H, br s, C-4-NH2); 7.22 (1H, s, C-6-H); 7.36 (2H, d, J = 7.8 Hz, C-3 and 5-H); 7.57 (2H, d, J = 7.6 Hz, C-2 and 6-H); 7.65 (2H, br s, C-4-NH2); 7.70 (2H, d, J = 8.8 Hz, C-3 and 5-H); 8.11 (2H, d, J = 8.4 Hz, C-2 and 6-H); 8.33 (1H, s, C-2-H). Anal. Calcd for C20H17N5: C, 73.46; H, 5.23; N, 21.43. Found: C, 73.39; H, 5.20; N, 21.40.

4-amino-5-(4-dimethylaminophenyl)-7-(4-aminophenyl)pyrido[2,3-d]pyrimidine, 6g: Yield 24%, mp 302±2 °C. IR (λmax, cm-1): 3457, 3364, 1682, 1597 and 1356. 1H NMR (DMSO-d6): δ 2.95 {6H, s, N(CH3)2}; 6.98 (2H, br s, C-4-NH2); 7.23 (1H, s, C-6-H); 7.33 (2H, d, J = 8.8 Hz, C-3 and 5-H); 7.40-7.37 (2H, J = 8.2 Hz, C-2 and 6-H); 7.75 (2H, br s, C-4-NH2); 7.84 (2H, d, J = 8.2 Hz, H-3 and H-5); 8.08 (2H, d, J = 8.4 Hz, H-2 and H-6); 8.30 (1H, s, H-2). Anal. Calcd for C21H20N6: C, 70.78; H, 5.61; N, 23.59. Found: C, 70.76; H, 5.60; N, 23.61.

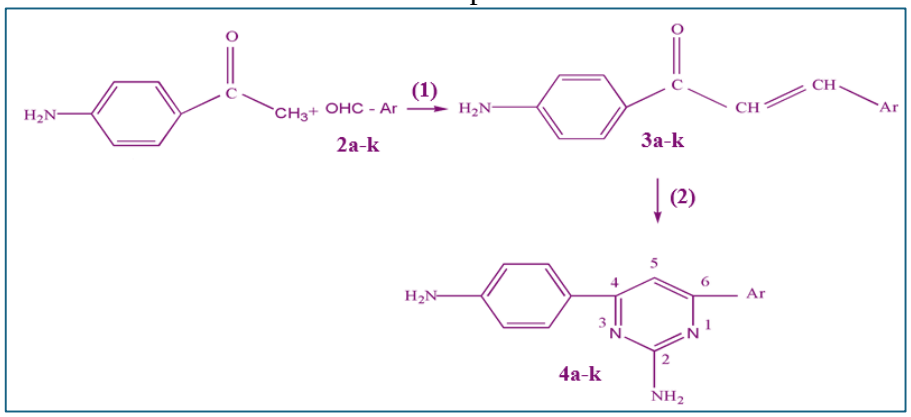
Antimicrobial studies: Using the disc diffusion method, the antibacterial activity of the synthesised compounds is assessed against two bacterial strains, S. aureus and E. coli, and two fungal pathogens, A. niger and C. albicans. The acquired results were compared to the antibacterial and antifungal activity of conventional drugs, namely chloramphenicol and clotrimazole, respectively. (17, 18)

Disc diffusion assay: figuring out a compound's antibacterial susceptibility at specific concentration doses using the Kirby-Bauer method. A few colonies of organisms were produced for 2.5 hours after being introduced in 2-4 cc of broth. To stop the inoculation material from leaking onto the agar plates during incubation, they were dried for thirty minutes prior to inoculation. The diluted culture is evenly dispersed across the agar surface using a sterile cotton swab dipped into the bacterial suspension. Whatman filter paper No. 1 discs, each with a diameter of 5 mm, were autoclaved before being dried for an hour at 60 °C. Using flame-powered forceps, the sterile filter paper discs impregnated with 400 µg (10 ml) and 800 µg (10 ml) of compound were deposited on the pre-inoculated surface. Within thirty minutes, the disc bearing plates were incubated for twenty-four hours at 37°C. The inhibition zone diameter was measured following incubation. The degree of sensitivity of the bacterial or fungal strain and the concentration of the drug being tested are directly correlated with the zone of inhibition. That is evident from a methodical review of antibiotic activity data. A rise

in the petridish zone of inhibition corresponds with an increase in drug concentration. (19, 20, 21, 22)

RESULTS AND DISCUSSION

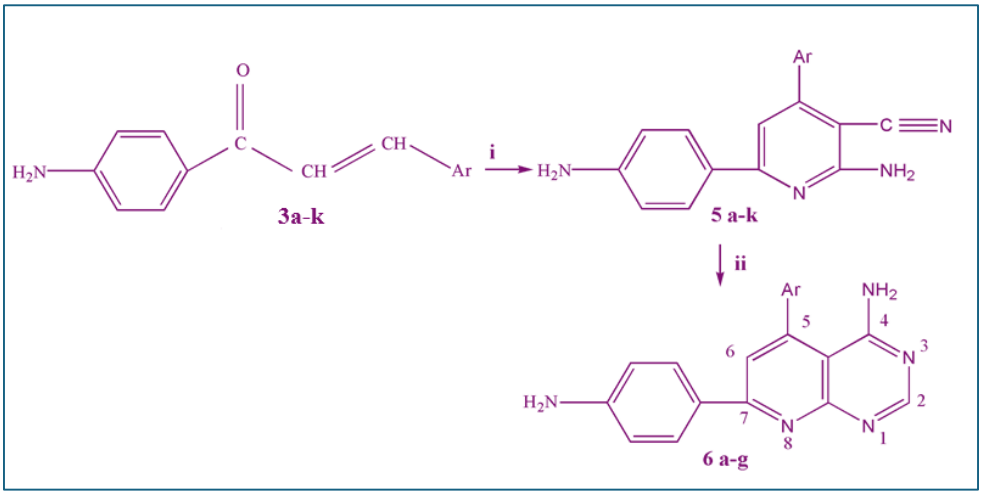
Synthesis: Chalcones 3a-k of 4-aminoacetophenone 1 were produced via the Claisen-Schmidt condensation reaction from 4-aminoacetophenone 1 utilising different aldehydes 2a-k. Pyrimidines 4a–k were created by cyclizing the oxo group after the amino group of the guanidine was added 1,2-or 1,4 times (Scheme 1, Table 1). We have previously disclosed the spectroscopic determination of the structures of products 3a-k and 4a-k.



SCHEME 1: Synthesis of chalcones 3a-k and 2,4,6-trisubstituted pyrimidines 4a-k. Reagents and conditions: (1) ethanol, aqueous potassium hydroxide, room temperature (16-20 h); (2) guanidine hydrochloride, ethanol, solid KOH, reflux (2 to 6 h) at 75-80 ºC.

Table 1: 4-aminochalcones (3a-k) and 2,4,6-Trisubstituted Pyrimidines (4a-k)

Compound	Ar	Compound	Ar
3a, 4a	4-Chlorophenyl	3g, 4g	3,4,5-Trimethoxyphenyl
3b, 4b	2,4-Dichlorophenyl	3h, 4h	4-Methylphenyl
3c, 4c	4-Fluorophenyl	3i, 4i	4-Dimethylaminophenyl
3d, 4d	3-Bromophenyl	3j, 4j	9-Anthracenyl
3e, 4e	4-Methoxyphenyl	3k, 4k	2-Pyridinyl
3f, 4f	3,4-Dimethoxyphenyl		



SCHEME 2: Synthesis of 2-amino-3-cyanopyridines 5a-k and pyrido[2,3-d]pyrimidines 6a-g. Reagents and conditions: i) malononitrile, ethanol, ammonium acetate, reflux (4 to 6 h) at 75-80 °C; (ii) formamide, DMF, reflux (24 to 30 h) at 170-180 °C.

Table 2: Cyanopyridines 5a-k and Pyrido[2,3-d]pyrimidines 6a-g

Compound	**Ar**	**Compound**	**Ar**
5a	4-Chlorophenyl	6a	4-Chlorophenyl
5b	2,4-Dichlorophenyl	6b	2,4-Dichlorophenyl
5c	4-Fluorophenyl	6c	4-Fluorophenyl
5d	3-Bromophenyl	6d	3-Bromophenyl
5e	4-Methoxyphenyl	6e	4-Methoxyphenyl
5f	3,4-Dimethoxyphenyl	6f	4-Methylphenyl
5g	4-Methylphenyl	6g	4-Dimethylaminophenyl
5h	4-Dimethylaminophenyl		
5i	9-Anthracenyl		
5j	2-pyridinyl		
5k	4-pyridinyl		

Antimicrobial Activity: Tables 3 and 4 summarise the results of the evaluation of the antibacterial activity of all seven newly synthesised pyrido[2,3-d]pyrimidine derivatives (6a-g) against two bacterial and two fungal strains. The inclusion of 2,4-dichlorophenyl, 4-fluorophenyl, and 4-dimethylaminophenyl moieties at the C-5 position of the pyrido[2,3-d]pyrimidine nucleus may be the cause of Compounds 6b, 6c, and 6g's strong antibacterial action against bacterial and fungal strains. All of the remaining pyridopyrimidines were found to be active against every type of bacteria examined, despite their limited antimicrobial activity. All things considered, the primary antimicrobial drugs are those that have 2,4-dichlorophenyl, 4-fluorophenyl, and 4-dimethylamino moieties as substituents at the right place in chalcones, pyrimidines, and pyridopyrimidines.

Table 3: In vitro antifungal pyrido[2,3-d]pyrimidines (6a-g)

S. No.	Compound	Diameter of zone of inhibition in mm			
		Bacteria			
		S. aureus		E. coli	
		400 µg/disc	800 µg/disc	400 µg/disc	800 µg/disc
1	6a	11	12	12	12
2	6b	14	16	09	12
3	6c	13	13	10	16
4	6d	09	16	11	13
5	6e	10	14	13	15
6	6f	09	09	09	13
7	6g	17	17	12	17
8	Chloramphenicol	21	23	18	22

Table 4: In vitro antifungal pyrido[2,3-d]pyrimidines (6a-g)

S. No.	**Compound**	**Diameter of zone of inhibition in mm**			
		Fungi			
		A. niger		**C. albicans**	
		400 µg/disc	**800 µg/disc**	**400 µg/disc**	**800 µg/disc**
1	6a	12	15	13	13
2	6b	17	23	16	19
3	6c	12	13	13	9
4	6d	13	14	15	14

5	6e	10	14	14	15
6	6f	12	19	15	15
7	6g	16	14	16	17
8	Clotrimazole	21	29	23	28

CONCLUSION

Based on the observed results, it is inferred that pyridopyrimidines showed greater anticancer activity than chalcones themselves or pyrimidines cyclized from the same chalcones. The current investigation discovered new substituted pyrimidines, pyrido[2,3-d]-pyrimidines, and chalcones. Following the conversion of chalcones to their corresponding pyridopyrimidines, antimicrobial activity was significantly increased. This deduction showed that, in comparison to chalcones and pyrimidines, pyridopyrimidines have superior antibacterial action due to the presence of a nucleus that enhances antimicrobial activity. To fully understand their precise mode of action and their therapeutic potential as antimicrobial agents, more research is needed.

REFERENCES:

1. Jesmin M, Ali MM, Khanam JA. Thai, Pesticidal activities of some Schiff bases derived from benzoin, salicylaldehyde, aminophenol and 2,4 dinitrophenyl hydrazine. J Pharm Sci 2010;34:20-31.
2. Gangadasu B, Narender P, Raju BC, Rao VJ. Base induced Carbon–Nitrogen (C=N) double bond migration in schiff bases. Indian J Chem 2005;44(B):2598-600.
3. Powers DG, Casebier DS, Fokas D, Ryan WJ, Troth JR and Coffen DL: Automated parallel synthesis of chalcone-based screening libraries. Tetrahedron 1998; 54:4085-4096.
4. Rajendra PY and Srinivasa RA: A Study of anti-inflammatory and analgesic activity of new 2,4,6-trisubstituted pyrimidines. Chemical and Pharmaceutical Bulletin 2011; 59:1079-1082.
5. Temple CJr, Rener GA, Waud WR and Noker PE: Antimitotic agents: structure – activity studies with some pyridine derivatives. Journal of Medicinal Chemistry 1992; 35:3686-3690.
6. Gholap AR, Kiran ST, Shirazi F, Kumari R, Manoj Kumar B, Deshpande MV and Srinivasan KV: Synthesis and evaluation of antifungal properties of a series of the novel 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile and its analogues. Bioorganic and Medicinal Chemistry 2007; 15:6705-6715.
7. Baldwin JJ, Engelhardt EL, Hirschmann R, Ponticello GS, Atkinson JG, Wasson BK, Sweet CS and Scriabine A: Heterocyclic analogs of the antihypertensive beta adrenergic blocking agent (S)-2-[3-(tert-butylamino)-2-hydroxypropoxy]-3-cyanopyridine. Journal of Medicinal Chemistry 1980; 23:65-70.
8. Divyesh CM, Manish PP and Ranjan GP: An efficient one-pot synthesis and in vitro antimicrobial activity of new pyridine derivatives bearing the tetrazoloquinoline nucleus. Arkivoc 2009; Xiv:64-74.
9. Zhang F, Zhao Y, Li Sun, Lu Ding, Yucheng Gu and Ping Gong: Synthesis and anti-tumor activity of 2-amino-3-cyano-6-(1H-indol-3-yl)-4-phenylpyridine derivatives in vitro. European Journal of Medicinal Chemistry 2011; 46:3149-3157.
10. Manna F, Chimenti F, Bolasco A, Filippelli A, Palla A, Filippelli W, Lampa E and Mercantini R: Anti-inflammatory, analgesic and antipyretic 4,6-disubstituted-3-cyano pyridines-2-ones and 3-cyano-2-aminopyridines. European Journal of Medicinal Chemistry 1992; 27:627-632.
11. Mantri M, Graaf de O, Van Veldhoven J, Goblyos A, Kunzel JK Von FD, Mulder-Krieger T, Link R, de Vries H, Beukers MW, Brussee J and Ijzerman AP: 2-amino-6-

furan-2-yl-4-substituted nicotinonitriles as A2A adenosine receptor antagonists. Journal of Medicinal Chemistry 2008; 51:4449-4455.

12. 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 KB, Bacon KB and Lowinger TB: Synthesis and structure-activity relationships of novel IKK-beta inhibitors. Part 3: Orally active anti-inflammatory agents. Bioorganic and Medicinal Chemistry Letters 2004; 14:4019-4022.
13. Xiang-shan Wang, Zhao-sen Zeng, Da-qing Shi, Xian-yong Wei and Zhi-min Zong: KF-Alumina catalyzed one-pot synthesis of pyrido[2,3-d]pyrimidine derivatives. Synthetic Communications 2004; 34:4331-4338.
14. Wenyan Mo, Guihong Liao, Tao Wang and Hongwu He: Facile synthesis and biological activities of novel fluorine-containing pyrido[4,3-d]pyrimidines. Journal of Fluorine Chemistry 2008; 129:519-523.
15. Vazzana I, Terranova E, Mattioli F, Sparatore F. Aromatic schiff bases and 2,3 disubstituted 1,3 thiazolidin-4-one derivatives as antiinflammotory agents. ARKIVOC 2004;5:364-74.
16. Bairagi S, Bhosale A, Deodhar Meenakshi N. Design, Synthesis and evaluation of schiff bases of 4 chloro 3 coumarin aldehyde as antimicrobial agents. E J Chem 2009;6:759-62.
17. Kumar S, Dhar DN, Saxena PN. Synthesis, Characterization and antimicrobial properties of schiff bases derived from condensation of 8-formyl 7-hydroxy-4-methyl coumarin and substituted triazole derivatives. J Sci Ind Res 2009;68:181-7.
18. Ravichandran V, Mohan S, Kumar KS. Synthesis and antimicrobial activity of mannich bases of isatin and its derivatives with2-[(2,6 dichlorophenyl)amino] phenyl acetic acid. ARKIVOC 2007;14:51-7.
19. Satyanarayana VSV, Sreevani P, Sivakumar A, Vijayakumar V. Synthesis and antimicrobial activity ofnewschiff bases containing coumarinmoeity and their spectral characterization. ARKIVOC 2008;17:221-33.
20. Jarrahpour A, Khalili D. Synthesis, Antibacterial, Antifungal and antiviral activity evaluation. Molecules 2006;11:59-63.
21. Valentina P, Ilango K, Deepthi M, Harusha P, Pavani G, Sindhura KL, et al. Antioxidant activity of some substituted 1,2,4–triazo-5-thion schiff bases. J Pharm Sci Res 2009;1:74-7.
22. Ocal N, Yolacan C, Kaban S, Leonor Y, Vargas M, Kouznetsov VJ. Transformations of schiff bases derived from quinoline-8-carbaldehyde. Synthesis of C-8 sunbstituted quinoline. Heterocycl Chem 2001;38:233-6.