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Synthesis of some 2, 6-bis (1-coumarin-2-yl)-4-(4-substituted phenyl) pyridine derivatives as potent biological agents

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Abstract A convenient one-pot, three-component synthesis of 2, 6-bis (1-coumarin-2-yl)-4-(4-substituted phenyl) pyridine derivatives (**3a–k**) by Chichibabin reaction has been reported. These compounds were synthesized by the reaction of 3-acetyl coumarin (**1a**) or 5-bromo 3-acetyl coumarin (**1b**) with substituted aromatic aldehydes (**2a–k**) and ammonium acetate under acidic conditions and the structure was confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic methods. The newly synthesized compounds (**3a–k**) were evaluated for antimicrobial activity, DPPH free radical scavenging activity and ferrous ion-chelating ability. The mode of action of these active compounds was carried out by docking receptor GlcN6P synthase. Compounds **3a**, **3b**, **3c**, and **3d** have displayed potential antimicrobial activity and some of the compounds have shown promising antioxidant properties.

1. Introduction

Coumarin derivatives containing pyridine heterocycle (Brahmbhatt and Pandya, 2001, 2003; Brahmbhatt et al., 2004, 2005, Brahmbhatt et al., 2007; Niraj et al., 2010) are known to exhibit anticoagulant, antibacterial and antifungal activities (Brahmbhatt et al., 2010). Pyridine scaffolds have been found in numerous naturally occurring compounds and are also frequently used in functional materials (Muller and Bunz, 2007; McKillop and Boulton, 1984; Jones, 1996; Spitzner, 2004).

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Pyridine-diol derivatives are of particular interest as building blocks for the construction of dendritic nanostructures in supramolecular chemistry (Christinat et al., 2007), whereas N-protected pyridine-3, 4-diols find applications as potent chelating agents in medicinal chemistry (Dehkordi et al., 2008). Furthermore, perfluorinated heteroaromatic compounds are interesting synthetic intermediates for the development of novel pharmaceuticals (Negwer, 1994). Coumarin nucleus has been the aim of many researchers as most of its derivatives were proved to be bioactive as antibacterial, antifungal, antioxidant (Murray et al., 1982; Nermien et al., 2011; Hamdi et al., 2008; Kenchappa et al., 2013) anticoagulant (Miky and Farrag, 1997), anticancer (Via et al., 1999; Bhattacharyya et al., 2009), anthelmentic (Lee et al., 1998) and antitumor (Tegginamath et al., 2011). Much research has been focused on the inhibition of bacterial growth by naturally occurring coumarins (xanthoxin, herniarin, umbelliferone, and scopoletin) and on the antifungal activity of umbelliferone, scopoletin, and coumarin itself (Hamdi et al., 2008).

In modern drug designing, molecular docking is routinely used for understanding drug-receptor interaction. This method has been frequently used to predict the binding affinity and orientation of small drug molecules at the active site of their protein targets (Vijesh et al., 2013). When designing novel antimicrobial agents, enzymes involved in the biosynthesis of microbial cell walls are generally good targets. In this regard, glucosamine-6-phosphate synthase, which is liable for the synthesis of D-glucosamine-6-phosphate, is particularly attractive because it is involved in the first step for the formation of the core aminosugar, *N*-acetyl glucosamine that is an essential part of the unique peptidoglycan and chitin components of the cell walls of bacteria and fungi, respectively (Marshall et al., 2003).

The concept of divergence and convergence in organic synthesis is very useful. Convergent synthesis pathways generally show advantages over linear or divergent approaches with respect to speed, time, yield, and reproducibility. Among organic reactions, multicomponent reactions are highly convergent (Beck et al., 2000). During a multicomponent reaction, more than two starting materials are assembled to afford a complex product. Up to seven-component reactions have been described so far (Domling and Ugi, 1993). Therefore, they constitute a superior tool for diversity-oriented and complexity-generating synthesis for drug discovery (Lee et al., 2000).

In view of the above facts, in the present work we have developed a simple and convenient method for the synthesis of 2, 6-bis (1-coumarin-2-yl)-4-(4-substituted-phenyl) pyridine derivatives and evaluated for antimicrobial and antioxidant activities.

2. Materials and methods

2.1. Chemistry

Melting points were recorded on electro thermal melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer and chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. LC–MS were obtained using Agilent LC/MS instrument. The FT-IR spectra were taken in KBr pellets (100 mg) using Shimadzu FT-IR spectrophotometer. Column chromatography was performed using silica gel (230–400 mesh), silica gel GF254 plates from Merck were used for TLC and spots were identified either by UV or dipping the plates in potassium permanganate solution.

General procedure for the synthesis of 2, 6-bis (1-coumarin-2-yl)-4-(4-substituted phenyl) pyridine derivatives (**3a–k**).

Two equivalents of 3-acetyl coumarin (**1a**) or 5-bromo 3-acetyl coumarin (**1b**), one equivalent of aromatic aldehydes (**2a–k**) and 1.5 equivalent of ammonium acetate were dissolved in acetic acid and the reaction mixture was refluxed for about 8–10 h in an oil bath at the temperature of 110–115 °C. After completion of the reaction, the reaction mixture was kept at room temperature overnight, and poured into ice cold water. The solid separated was filtered and washed with sodium bicarbonate solution and recrystallized using ethanol.

*2.1.1. 3-(6-(2-Oxo-2H-chromen-3-yl)-4-phenylpyridin-2-yl)-2H-chromen-2-one (**3a**)*

Yield 79%; m.p; 251–253 °C, (lit. m.p; 253–254 °C); white solid (Anil et al., 2010).

*2.1.2. 3-(4-(4-Chlorophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3b**)*

Yield 90%; m.p; 162–165 °C; white solid; mol. formula; $C_{29}H_{16}ClNO_4$; IR (KBr): 3063 (Ar–H), 1718 (coumarin C=O), 1613 (C=N), 1559, 749, 693 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.33–7.81 (12H, m, Ar–H), 8.64 (2H, s, 3-H and 5-H of pyridine ring), 8.87 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 116.4, 119.5, 121.8, 124.6, 125.3, 127.4, 128.9, 129.1, 129.2, 132.3, 138.0, 142.8, 149.8, 151.4, 154.0, 160.3; MS: *m/z* 477.89 [M^+], 479.50 [M + 2].

*2.1.3. 6-Bromo-3-(6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-phenylpyridin-2-yl)-2H-chromen-2-one (**3c**)*

Yield 86%; m.p; 158–162 °C; light yellow solid; mol. formula; $C_{29}H_{15}Br_2NO_4$; IR (KBr): 3055, 1723, 1606, 1531, 823 cm^{-1}; ^{1}H NMR($CDCl_3$, 400 MHz) δ: 7.38–7.95 (11H, m, Ar–H), 8.65 (2H, s, 3-H and 5-H of pyridine ring), 8.88 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 116.4, 119.5, 121.5, 124.6, 125.4, 127.2, 128.9, 129.8, 132.3, 135.1, 139.4, 142.8, 149.8, 151.4, 154.0, 160.3; MS: *m/z* = 601.24 [M^+], 603.25 [M + 2], 605.24 [M + 4].

*2.1.4. 6-Bromo-3-(6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-(4-chlorophenyl) pyridin-2-yl)-2H-chromen-2-one (**3d**)*

Yield 83%; m.p; 160–164 °C; light yellow solid; mol. formula; $C_{29}H_{14}Br_2ClNO_4$; IR (KBr): 3055, 1720, 1608, 1535, 825 cm^{-1}; ^{1}H NMR($CDCl_3$, 400 MHz) δ: 7.37–7.96 (10H, m, Ar–H), 8.64 (2H, s, 3-H and 5-H of pyridine ring), 8.82 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 116.4, 119.5, 121.5, 124.6, 125.4, 127.2, 128.9, 129.8, 132.3, 135.1, 139.4, 142.8, 149.8, 151.4, 154.0, 160.3; MS: *m/z* = 635.68 [M^+], 637.68 [M + 2], 639.68 [M + 4].

*2.1.5. 3-(6-(2-oxo-2H-chromen-3-yl)-4-p-tolylpyridin-2-yl)-2H-chromen-2-one (**3e**)*

Yield 85%; m.p; 266–268 °C; (lit. m.p; 269–270 °C); white solid (Anil et al., 2010).

*2.1.6. 3-(4-(4-(Dimethylamino) phenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3f**)*

Yield 76%; m.p; 161–164 °C, light yellow solid; mol. formula; $C_{31}H_{22}N_2O_4$; IR (KBr): 3055, 1720, 1608, 1535, 825 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.97 (6H, s, $N(CH_3)_2$), 7.34–7.91

(12H, m, Ar–H), 8.59 (2H, s, 3-H and 5-H of pyridine ring), 8.78 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 20.4, 115.4, 121.5, 123.3, 124.0, 125.1, 126.6, 128.9, 131.4, 134.1, 136.5, 140.5, 143.9, 149.8, 151.4, 152.0, 160.2; MS: m/z 486.51 [M$^+$].

2.1.7. 3-(4-(2-Fluorophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3g**)

Yield 91%; m.p; 75–80 °C, light yellow solid; Mol. Formula; $C_{29}H_{16}FNO_4$; IR (KBr): 3048, 1723, 1605, 1538, 821 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.39–7.96 (12H, m, Ar–H), 8.55 (2H, s, 3-H and 5-H of pyridine ring), 8.71 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 116.4, 120.5, 122.3, 123.5, 125.6, 126.1, 127.9, 131.8, 133.1, 136.5, 142.5, 143.9, 150.8, 151.6, 152.6, 162.2; MS: m/z 461.44 [M$^+$].

2.1.8. 3-(4-(3-Fluorophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3h**)

Yield 75%; m.p; 155–158 °C, light yellow solid; Mol. Formula; $C_{29}H_{16}FNO_4$; IR (KBr): 3035, 1719, 1610, 1540, 826 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.28–7.85 (12H, m, Ar–H), 8.57 (2H, s, 3-H and 5-H of pyridine ring), 8.75 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 115.4, 121.0, 122.1, 123.6, 125.8 (C), 126.6, 128.7, 132.6, 133.1, 137.3, 141.9, 143.6, 150.2 (C), 151.6, 152.6, 161.0; MS: m/z 461.44 [M$^+$].

2.1.9. 1.9. 3-(4-(4-Fluorophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3i**)

Yield 84%; m.p; 95–98 °C, light yellow solid; mol. formula; $C_{29}H_{16}FNO_4$; IR (KBr): 3031, 1710, 1625, 1543, 821 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.21–7.78 (12H, m, Ar–H), 8.56 (2H, s, 3-H and 5-H of pyridine ring), 8.70 (2H, s, 4'-H and 4″-H of coumarin rings); ^{13}C NMR: δ 114.4, 120.3, 121.1, 123.6, 124.2, 125.4, 127.3, 131.5, 133.1, 136.7, 140.6, 142.4, 150.2, 151.6, 153.6, 161.0 (C); MS: m/z 461.44 [M$^+$].

2.1.10. 3-(4-(4-Nitrophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3j**)

Yield 81%; m.p; 97–99 °C, light yellow solid; mol. formula; $C_{29}H_{16}N_2O_6$; IR (KBr): 3069, 1718, 1625, 1525, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.03–7.81 (12H, m, Ar–H), 8.65 (2H, s, 3-H and 5-H of pyridine ring), 8.86 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 52.5, 113.0, 115.5, 116.6, 120.4, 121.1, 123.2, 125.4, 127.6, 128.4, 130.2, 133.2, 145.8, 151.3,153.0, 161.3, 160.7; MS: m/z 488.44 [M$^+$].

2.1.11. 3-(4-(4-Methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2-yl)-2H-chromen-2-one (**3k**)

Yield 87%; m.p; 275–277 °C; light yellow solid; mol. formula; $C_{30}H_{19}NO_5$; IR (KBr): 3065, 1710, 1615, 1520, 823 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.90 (3H, s, OCH_3), 7.02–7.80 (12H, m, Ar–H), 8.62 (2H, s, 3-H and 5-H of pyridine ring), 8.88 (2H, s, 4′-H and 4″-H of coumarin rings); ^{13}C NMR: δ 55.5, 113.9, 114.5, 116.4, 119.5, 121.1, 124.6, 125.4, 128.6, 128.9, 130.2, 132.2, 142.8, 151.3, 154.0, 160.3, 160.7; MS: m/z 473.47 [M$^+$].

2.2. Pharmacology

2.2.1. Antimicrobial activity

The newly synthesized compounds were screened for antibacterial activity against one gram-positive bacterium, *Bacillus subtilis* and three Gram-negative bacteria *Pseudomonas aeruginosa*, *E. coli* and *Salmonella typhi*. Antifungal activity was screened against four fungal strains, *Aspergillus flavus*, *Candida albicans*, *Microspora griseous*, and *Aspergillus terreus*. Dimethylsulfoxide was used as solvent control. The bacterial culture was inoculated on nutrient agar (Merck) and fungal culture was inoculated on potato dextrose agar media (20 mL) and poured into sterilized Petri dishes (99 mm). Media plates were inoculated with liquid cultures homogeneously by spread plate method.

All the test compounds were dissolved in dimethylsulfoxide (DMSO) to get a concentration of 100 μL and loaded into the wells of agar plates directly. Plates inoculated with the bacteria were incubated at 37 °C for 24 h and the fungal culture was incubated at 25 °C for 72 h. All determinations were done in triplicates. Amoxicillin and Gentamicin (1.64, 0.64, and 0.25 mg/mL) were used as standard drugs for antibacterial and antifungal activities respectively.

2.2.2. In silico molecular docking studies

The newly synthesized compounds were subjected to molecular docking studies using Auto Dock (version 4.2) with Lamarckian genetic algorithm (Roseman, 2001; Milewski, 2002; Milewski et al., 2006; Whelan and Ballou, 1975).The synthesized ligand molecules having 2D structure were converted into energy minimized 3D structures and were further used for in silico protein–ligand docking. The docking of receptor GlcN-6-P with newly synthesized ligands exhibited well established bonds with one or more amino acids in the receptor active pocket. The active pocket was considered to be the site where glucosamine-6-phosphate complexes with GlcN-6-P of 2VF5. The active pocket consisted of 12 amino acid residues as Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347, and Lys603 (Wallace et al., 1995).

The crystal structure of GlcN-6-P synthase (PDB ID 2VF5) from the PDB (http://www.pdb.org/pdb/home/home.do) was selected and edited by removing the heteroatoms and adding C-terminal oxygen (Binkowski et al., 2003). The graphical user interface program "Auto Dock Tools" was used to prepare, run and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged.

2.2.3. Antioxidant activity

2.2.3.1. Scavenging effect on stable radical 1, 1-diphenyl-picrylhydrazyl (DPPH). Free radical-scavenging capacities of different compounds were determined according to the reported procedure (Braca et al., 2001). Freshly prepared DPPH solution was taken in test tubes and newly synthesized compounds were added (100 μg) to each test tube so that the final volume will be 3 mL. After 10 min, the absorbance was recorded at 517 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan). Butylatedhydroxytoluene (BHT) dissolved in distilled water was used as a reference. Control sample was prepared using the same volume without any compound and reference BHT, 95% methanol served as blank. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitutions. Test

was performed in triplicate and the results were averaged. Radical scavenging activity was calculated using the formula.

$$\% \text{ of Radical scavenging activity} = [(A_{control} - A_{test})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control sample and A_{test} is the absorbance of the test sample.

2.2.3.2. Chelating effects on ferrous ions. The chelating effect was determined according to the method of Decker and welch (1990), (Nevcihan et al., 2010). In brief, 2 mL of different concentrations (0.5–2.0 mg/mL) of the compound in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and total volume was adjusted to 5 mL with methanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm.

The inhibition percentage of ferrozine-Fe^{+2} complex formations was calculated using the formula given below.

$$\text{Metal chelating effect } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of control (the control contains $FeCl_2$ ferrozine complex formation molecules) and A_{sample} is the absorbance of the test compound. Ascorbic acid is used as control.

Table 1 Zone of inhibition of synthesized compounds (**3a–k**).

Comp no.	Conc mg/mL	Zone of inhibition in mm (mean ± S.D.) $n = 3$							
		Antibacterial				Antifungal			
		P.a ± S.D	*B.s* ± S.D*	*S.t* ± S.D*	*E.c* ± S.D*	*A.f* ± S.D*	*C.a* ± S.D*	*M.g* ± S.D*	*A.t* ± S.D*
3a	1.6	20 ± 0.20	34 ± 0.10	35 ± 0.30	–	22 ± 0.20	19 ± 0.30	30 ± 0.50	20 ± 0.20
	0.64	30 ± 0.20	32 ± 0.20	33 ± 0.20	–	21 ± 0.50	18 ± 0.40	19 ± 0.20	12 ± 0.10
	0.25	32 ± 0.20	28 ± 0.30	32 ± 0.10	–	17 ± 0.10	15 ± 0.20	16 ± 0.10	11 ± 0.20
3b	1.6	18 ± 0.10	24 ± 0.10	21 ± 0.20	22 ± 0.40	20 ± 0.20	14 ± 0.10	22 ± 0.20	17 ± 0.50
	0.64	14 ± 0.20	14 ± 0.20	17 ± 0.10	16 ± 0.10	12 ± 0.30	13 ± 0.20	18 ± 0.10	12 ± 0.20
	0.25	13 ± 0.10	13 ± 0.10	14 ± 0.10	10 ± 0.20	08 ± 0.20	09 ± 0.20	10 ± 0.20	07 ± 0.20
3c	1.6	20 ± 0.10	36 ± 0.30	35 ± 0.30	16 ± 0.10	21 ± 0.50	16 ± 0.50	34 ± 0.10	19 ± 0.20
	0.64	15 ± 0.20	32 ± 0.20	35 ± 0.10	13 ± 0.30	19 ± 0.20	18 ± 0.20	21 ± 0.30	12 ± 0.20
	0.25	14 ± 0.20	27 ± 0.10	22 ± 0.20	10 ± 0.10	13 ± 0.20	11 ± 0.20	15 ± 0.40	09 ± 0.20
3d	1.6	18 ± 0.20	36 ± 0.20	34 ± 0.30	17 ± 0.50	21 ± 0.10	21 ± 0.10	19 ± 0.20	20 ± 0.20
	0.64	14 ± 0.10	34 ± 0.30	34 ± 0.10	14 ± 0.30	17 ± 0.20	20 ± 0.30	15 ± 0.10	12 ± 0.30
	0.25	13 ± 0.20	29 ± 0.20	20 ± 0.20	–	14 ± 0.30	15 ± 0.20	12 ± 0.20	10 ± 0.20
3e	1.6	18 ± 0.30	17 ± 0.10	16 ± 0.10	17 ± 0.40	09 ± 0.20	03 ± 0.20	–	10 ± 0.10
	0.64	14 ± 0.10	15 ± 0.20	16 ± 0.10	15 ± 0.30	05 ± 0.10	01 ± 0.30	–	01 ± 0.20
	0.25	09 ± 0.20	10 ± 0.10	10 ± 0.20	12 ± 0.10	–	–	–	–
3f	1.6	–	–	–	–	13 ± 0.20	01 ± 0.20	12 ± 0.30	01 ± 0.30
	0.64	13 ± 0.20	12 ± 0.30	18 ± 0.10	18 ± 0.50	–	–	–	–
	0.25	08 ± 0.10	10 ± 0.10	12 ± 0.20	10 ± 0.30	–	–	–	–
3g	1.6	19 ± 0.30	18 ± 0.30	17 ± 0.30	18 ± 0.10	15 ± 0.10	03 ± 0.10	–	01 ± 0.20
	0.64	17 ± 0.10	18 ± 0.20	14 ± 0.20	18 ± 0.40	13 ± 0.20	02 ± 0.20	–	01 ± 0.10
	0.25	12 ± 0.20	10 ± 0.10	09 ± 0.10	12 ± 0.20	10 ± 0.30	02 ± 0.10	–	–
3h	1.6	20 ± 0.30	20 ± 0.30	–	18 ± 0.10	12 ± 0.50	03 ± 0.20	–	03 ± 0.10
	0.64	17 ± 0.10	18 ± 0.10	12 ± 0.20	17 ± 0.30	12 ± 0.20	03 ± 0.20	–	01 ± 0.10
	0.25	10 ± 0.10	12 ± 0.20	0.8 ± 0.10	1.0 ± 0.50	04 ± 0.10	–	–	–
3i	1.6	18 ± 0.30	20 ± 0.30	17 ± 0.10	20 ± 0.20	07 ± 0.20	–	–	12 ± 0.10
	0.64	16 ± 0.20	19 ± 0.10	17 ± 0.20	12 ± 0.40	05 ± 0.30	–	–	10 ± 0.20
	0.25	10 ± 0.10	08 ± 0.20	09 ± 0.30	10 ± 0.10	–	–	–	06 ± 0.10
3j	1.6	20 ± 0.30	18 ± 0.10	21 ± 0.20	19 ± 0.20	10 ± 0.20	–	–	13 ± 0.20
	0.64	18 ± 0.10	16 ± 0.10	20 ± 0.30	16 ± 0.50	10 ± 0.10	–	–	10 ± 0.30
	0.25	12 ± 0.20	10 ± 0.30	13 ± 0.20	10 ± 0.30	07 ± 0.40	–	–	05 ± 0.20
3k	1.6	18 ± 0.30	17 ± 0.20	21 ± 0.10	17 ± 0.20	02 ± 0.30	–	–	03 ± 0.10
	0.64	16 ± 0.20	16 ± 0.10	19 ± 0.20	15 ± 0.10	01 ± 0.20	–	–	10 ± 0.10
	0.25	13 ± 0.10	10 ± 0.20	12 ± 0.10	10 ± 0.10	–	–	–	–
Stand[a]	1.6	26 ± 0.30	36 ± 0.30	36 ± 0.20	24 ± 0.20	–	–	–	–
	0.64	22 ± 0.20	28 ± 0.10	30 ± 0.40	20 ± 0.30	–	–	–	–
	0.25	19 ± 0.10	20 ± 0.20	23 ± 0.10	16 ± 0.20	–	–	–	–
Stand[b]	–	–	–	–	–	24 ± 0.20	20 ± 0.10	34 ± 0.30	22 ± 0.30
	–	–	–	–	–	20 ± 0.50	16 ± 0.20	28 ± 0.20	20 ± 0.10
	–	–	–	–	–	20 ± 0.10	18 ± 0.30	20 ± 0.40	20 ± 0.20

P.a, *Pseudomonas aeruginosa*; *B.s*, *Bacillus subtilis*; *S.t*, *Salmonella typhi*; *E.c*, *Escherichia coli*; *A.f*, *Aspergillus flavus*; *C.a*, *Candida albicans*; *M.g*, *Microspora griseous*; *A.t*, *Aspergillus terreus*.

[a] Stand – Amoxicillin.

[b] Stand – Gentamicin.

* S.D – Standard deviation.

Table 2 Minimum inhibitory concentration (μg/ml) of compounds (**3a–k**).

Comp. no.	Minimum inhibitory concentration (MIC μg/mL)							
	P.a	*B.s*	*S.t*	*E.c*	*A.f*	*C.a*	*M.g*	*A.t*
3a	16.02	15.90	16.09	–	12.60	12.65	12.65	13.95
3b	20.32	23.56	18.26	19.45	12.60	15.51	17.55	22.54
3c	15.80	15.90	16.91	15.94	21.50	25.50	21.50	18.57
3d	15.88	15.90	16.91	15.96	52.32	65.36	75.12	63.12
3e	30.21	35.56	36.65	28.85	NT	NT	NT	NT
3f	NT	NT	NT	NT	NT	NT	NT	NT
3g	100.65	95.26	96.95	95.26	95.34	68.24	56.15	39.45
3h	102.32	82.36	98.59	102.76	22.5	59.64	72.19	56.14
3i	49.36	59.46	76.36	65.36	NT	NT	NT	NT
3j	30.25	28.85	35.46	33.19	NT	NT	NT	NT
3k	120.32	110.36	104.26	96.37	NT	NT	NT	NT
Amoxicillin	15.50	15.50	15.50	15.50	–	–	–	–
Gentamicin	–	–	–	–	12.50	12.50	12.50	12.50

NT – not tested.

P.a, *Pseudomonas aeruginosa*; *B.s*, *Bacillus subtilis*; *S.t*, *Salmonella typhi*, *E.c*, *Escherichia coli*; *A.f*, *Aspergillus flavus*; *C.a*, *Candida albicans*; *M.g*, *Microspora griseous*; *A.t*, *Aspergillus terreus*.

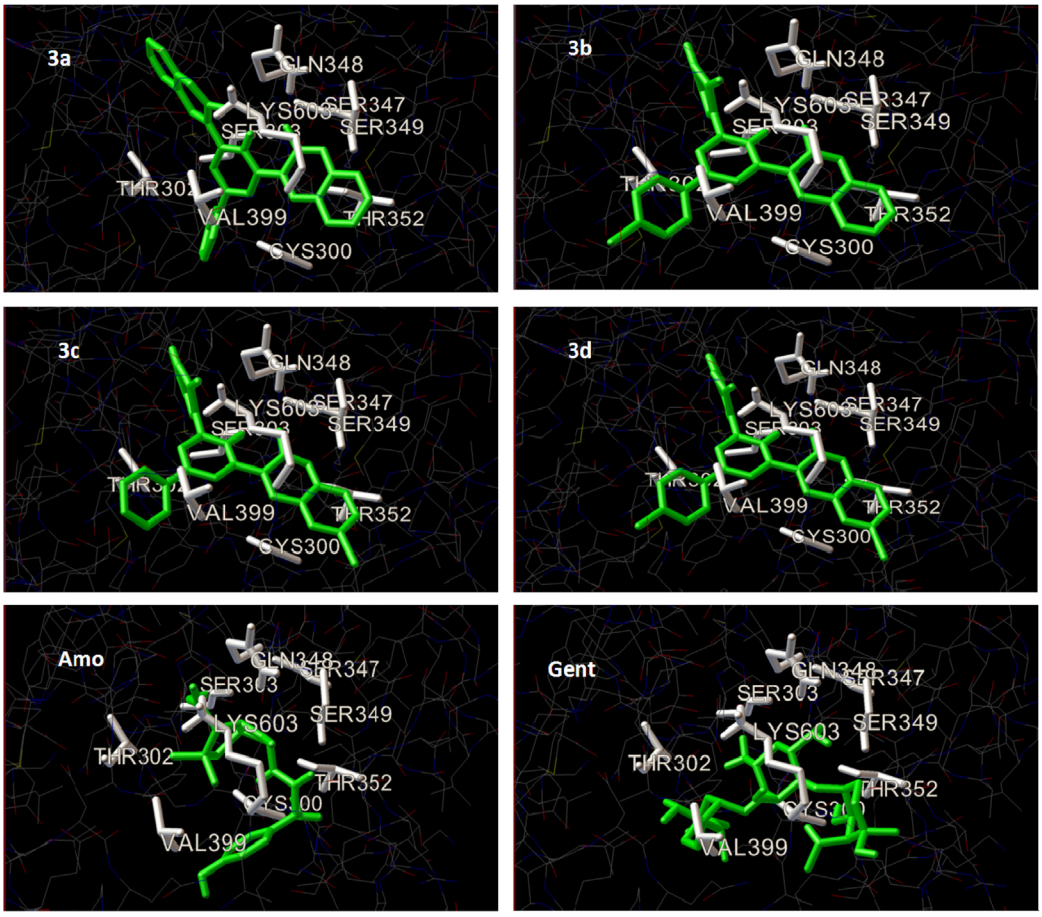


Figure 1 Interaction of ligand molecules **3a**, **3b**, **3c**, **3d**, **Amo**, **Gent**, with GlcN-6-P, **Amo**, **Gent**, represents interaction of amoxicilin and gentamicin (standards) with GlcN-6-P respectively. (In all the images the ligands are represented in green color and the target protein GlcN-6-P in Secondary structure and secondary colorization).

3. Results and discussion

3.1. Chemistry

We report herein, a simple and expeditious synthesis of highly substituted pyridines in excellent yield. The starting compounds (**1a–b)** were synthesized according to the literature method (Vijesh et al., 2010). In a typical procedure a 2:1:1.5 mixture of 3-acetyl coumarin (**1a**) or 5-bromo 3-acetyl coumarin (**1b**), substituted aromatic aldehydes (**2a–k**) and ammonium acetate were refluxed in acetic acid at 110 °C to furnish the Chichibabin products (**3a–k**) in good yields. The method involves the formation of imine by the reaction of 3-acetyl coumarin with ammonia. In the second step aldol condensation takes place between substituted aldehyde and enolate ion of 3 acetyl coumarin and in the final step Michael addition reaction takes place between coumarin chalcone and imines. From a mechanistic standpoint (Farley and Eliel, 1956; Frank and Seven, 1949; Frank and Riener1950), ammonia serves two functions and occurs through the formation of enamine with a third equivalent of aldehyde. The Michael addition of enamine to enolate followed by cyclization gives rise to a final compound (see Scheme 1).

IR Spectroscopic analysis of the newly synthesized compounds (**3a–k**) revealed that the strong absorption band at 3053–3073 cm^{-1} corresponds to the Ar–H stretching vibration. The sharp absorption bands at 660–662 and 740–748 cm^{-1} correspond to the presence of C–Br, C–Cl group on the phenyl ring. Compound **3j** exhibited the strong absorption band at 1625 and 1525 cm^{-1} which correspond to symmetric and asymmetric frequency of the NO_2 group on the phenyl ring.

The chemical shift (^{1}H NMR) observed in the range of δ 7.03 and 7.96 corresponds to the aromatic protons, the singlet in the range of 8.55 and 8.66 ppm corresponds to two protons of the pyridine ring and another singlet at 8.70–8.89 ppm corresponds to two protons (4′-H and 4″-H) of the coumarin ring. The structure was further confirmed by ^{13}C NMR which was in agreement with the synthesized compounds. The mass spectra of these compounds displayed a molecular ion peak at appropriate m/z values corresponding to the respective molecular formulae.

3.2. Pharmacology

3.2.1. In vitro antibacterial and antifungal activities

The newly synthesized compounds (**3a–k**) were evaluated for their antimicrobial activity using agar-well diffusion method at three different concentrations and the results are given in Table 1. The investigation of antimicrobial screening revealed that, test compounds showed varying degrees of activity against all the tested microorganisms. The activity of these compounds was found to be concentration dependent.

Further, the compounds which showed a good zone of inhibition were studied for minimum inhibitory concentration (MIC) using serial broth-dilution method to quantify the antimicrobial potency of the compounds. MIC was performed at different concentrations i.e. 1, 10, 25, 50,100, and 150 μg/mL and the results have been given in Table 2. It is clear from our present findings that heterocyclic systems with electron withdrawing and donating groups on the phenyl ring and the

Table 3 Molecular docking results of synthesized compounds **(3a–k)** with glucosamine-6-phosphate synthase.

Comp. no.	Binding energy (kJ mol^{-1})	Inhibition constant (μM)	RMSd	Ligand efficiency	No. of hydrogen bonds	Bonding residues	Bond length (Å)
3a	−5.07	193.05	0.0	−0.14	2	2VF5: GLN348: HE22: Ligands/3a:::O	2.078
						2VF5: LYS603: HZ1: Ligands/3a:::O	1.989
3b	−4.97	226.14	0.0	−0.14	2	2VF5: LN348:HE22: Ligands/3b:::O	2.186
						2VF5:LYS603:HZ1: Ligands/3b:::O	2.201
3c	−4.84	281.02	1.08	−0.13	1	2VF5:GLN348: HE22: Ligands/3c:::O	2.241
3d	−4.69	367.79	0.0	−0.14	2	2VF5:GLN348:HE22: Ligands/3d:::O	1.848
						2VF5:LYS603:HZ1: Ligands/3d:::O	1.874
Amoxicillin	−3.8	163.28	0.0	−0.15	3	2VF5:THR352:OG1: Ligands/Amoxicillin:::H	1.985
						2VF5:SER349:HG:Ligands/Amoxicillin:::O 2VF5:LYS603:HZ1: Ligands/Amoxicillin:::O	1.732
							1.699
Gentamicin	−1.41	215.90	0.0	−0.04	5	2VF5:THR302:OG1:Ligands/Gentamicin:::H	2.03
						2VF5:SER349: HG: Ligands/Gentamicin:::O	1.906
						2VF5:THR352:OG1: Ligands/Gentamicin:::H	2.019
						2VF5: SER349:OG: Ligands/Gentamicin:::H	1.882
						2VF5: SER303:OG: Ligands/Gentamicin:::H	2.046

coumarin moiety play an important role in varying the efficacy of antimicrobial activity.

The role of electron withdrawing group in improving antimicrobial activities had been reported in the literature (Sharma et al., 2004).

The investigation of MIC of the tested compounds revealed that the compound **3a** with no substitution on phenyl ring, compounds **3c** and **3d** substituted with electron withdrawing groups have shown MIC's at lowest concentration ranging from 15.80–16.91 μg/mL against tested bacterial organisms as compared with the standard Amoxicillin. Compound **3c** showed MIC value of 21.50 μg/mL against tested fungal organisms *A. flavus*, *M. griseous*; compound **3a** is inactive against *E. coli*, compounds **3a** and **3b** (**Cl**-group on phenyl ring) have shown a MIC value of 12.60 μg/mL against *A. flavus* and compound **3b** have shown good inhibition with a MIC value of 12.65 and 12.68 μg/mL against *C. albicans* and *M. griseous* respectively, when compared with the standard Gentamicin. Compound **3b** exhibited moderate to good inhibition with a MIC value of 18.26 and 15.51 μg/mL against *S. typhi* and *C. albicans* respectively. Compound **3h** have shown a MIC value of 22.50 μg/mL against *A. flavus*. Remaining tested compounds showed varying degrees of MIC values ranging from 30.21 to 120.32 μg/mL.

To compare the binding affinity of the newly synthesized compounds, they were subjected for molecular docking using Auto Dock (version 4.2) with Lamarckian genetic algorithm. The newly synthesized compounds **3a**, **3b**, **3c**, and **3d** were analyzed for their mechanism of antimicrobial action.

The estimated binding energy between the protein and ligand for each molecule was compared to substantiate the experimental results. The compounds which have exhibited the bonding with one or the other amino acids in the active pockets are shown in Fig. 1.

The docking of GlcN-6-P synthase with **3a**, **3b**, **3c**, and **3d** revealed that the image **3d** with least binding energy (6.05 kJ mol^{-1}) and which also establishes two hydrogen bonds between coumarin lactone with gln 348, and another coumarin lactone with lys 603 amino acids in the active site of the target protein with minimum bond length (1.848 and 1.874 Å) has the highest affinity and hence is the best dock conformation Binding energy inhibition constant, RMS value, ligand efficiency, number of hydrogen bonds, and bond length of four tested compounds and two standards were recorded in each ligand confirmations and are given in Table 3.

By comparing antimicrobial activity and docking results, we concluded that heterocyclic derivatives containing oxygen in the coumarin seem to be potentially active drugs.

Table 4 Scavenging effect of synthesized compounds **(3a–k)** on stable radical 1, 1-diphenyl-picrylhydrazyl (DPPH).

Comp. no.	DPPH assay in %	Comp. no.	DPPH assay in %
3a	26.3 ± 0.23	**3g**	35.3 ± 0.28
3b	68.6 ± 0.61	**3h**	78.3 ± 0.16
3c	52.5 ± 0.45	**3i**	56.2 ± 0.35
3d	74.1 ± 0.14	**3j**	27.3 ± 0.12
3e	38.7 ± 0.35	**3k**	28.7 ± 0.11
3f	37.2 ± 0.26	BHT	90.42 ± 0.25

Each value is expressed as mean ± SD of three replicates. Butylatedhydroxytoluene (BHT) used as standard.

3.3. Scavenging effect on stable radical 1, 1-diphenyl-picrylhydrazyl (DPPH)

The newly synthesized compounds (**3a–k**) were tested for 2, 2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity with butylatedhydroxytoluene (BHT) as standard and the results are tabulated in Table 4. It has been reported that the heterocyclic systems with halogen substituent have shown better antioxidant properties than other substituent (Hossain et al., 2009; Ferreira et al., 2006).

The investigation of (DPPH) radical scavenging activity revealed that compounds with electron withdrawing substituents such as −Br and −Cl (**3b**, **3d**, and **3h**) displayed very good antioxidant activity. The other tested compounds showed moderate to good antioxidant activity.

3.4. Chelating ability of metal ion

It has been well known that transition metal ions such as those of iron and copper are important catalysts for the generation of free radicals to initiate the radical chain reaction or the radical mediates lipid peroxidation (Nawar, 1996).It has been reported that the compounds containing two or more of the following functional groups: OH, COOH, C═O, NR_2 and O in a favorable structure–function configuration can show the activity of metal chelation (Yuan et al., 2005).The ferrous ion-chelating activities of the newly synthesized compounds are shown in Fig. 2.The range and mean of Fe^{+2} chelating capacities varied significantly among different compounds on the basis of substitution on the phenyl ring.

Due to extra stabilization, compounds **3d** and **3h** would have higher aptitude to trap radical in a faster rate than the other similar type of molecules. Compounds **3a**, **3c**, and **3g** showed very good chelating ability, whereas compounds **3i** and **3j** have shown comparable activity with the standard EDTA. The compounds substituted with electron donating groups (**3f**, **3e**, and **3k**) showed significantly lesser activity. The metal-chelating effect of the tested compounds with respect to electron withdrawing substituent on the phenyl ring is in the order of EDTA > 2-F > 4-F > 4-NO_2 > 4-Cl > 3-F, in case of electron donating substituent it is in the order of EDTA > 4-OCH_3 > 4-CH_3 > 4-$N(CH_3)_2$.

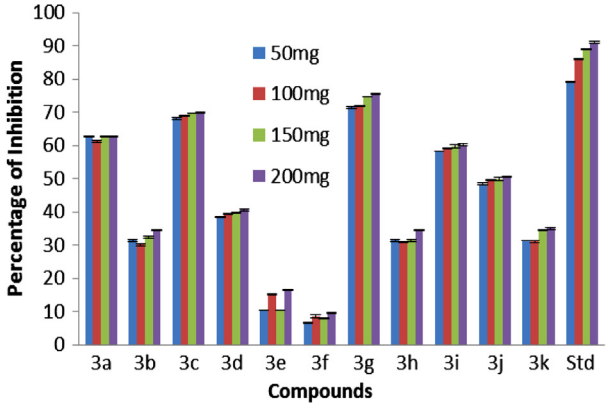
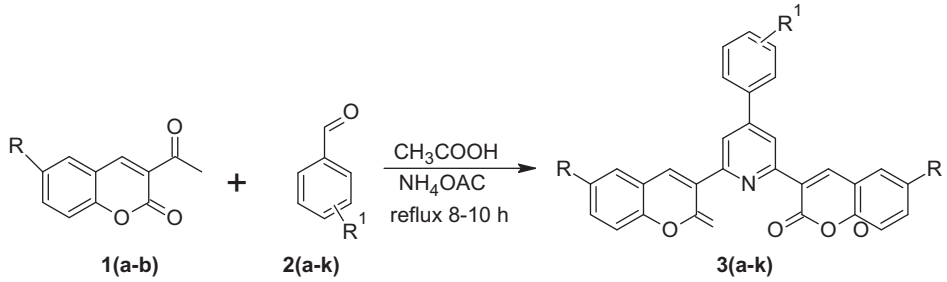


Figure 2 Ferrous ion chelating assay in % of test compounds (**3a–k**). Each value is expressed as mean ± SD of three replicates. EDTA is used as a standard



Comp	R	R^1	Comp	R	R^1
3a	H	H	3g	H	2-F
3b	H	4-Cl	3h	H	3-F
3c	Br	H	3i	H	4-F
3d	Br	4-Cl	3j	H	4-NO_2
3e	H	4-CH_3	3k	H	4-OCH_3
3f	H	4-$N(CH_3)_2$			

Scheme 1 Synthesis of 2, 6-bis (1-coumarin-2-yl)-4-(4-substituted phenyl) pyridine derivatives (**3a–k**).

4. Conclusion

In conclusion, we have designed an efficient synthetic route for the synthesis of compounds **3a–k**. The antimicrobial results revealed that the compounds containing the halogen or electron withdrawing substituents either on the coumarin or phenyl ring exhibited potent antimicrobial and antioxidant activities. In the antibacterial studies, compounds bearing a bromo group in addition to a chloro group exhibited greater activity than those bearing only the chloro group. This observation suggests that di-substitution in the target compounds by halogens enhanced the antimicrobial and antioxidant potential.

References

Anil, K.P., Niraj, H.P., Mehul, A.P., Brahmbhatt, D.I., 2010. Arkivoc 11, 28–38.

Beck, B., Hess, S., Dömling, A, 2000. Bioorg. Med. Chem. Lett. 10, 1701.

Bhattacharyya, S.S., Paul, S., Mandal, S.K., Banerjee, A., Boujedaini, N., Khuda-Bukhsh, A.R., 2009. Eur. J. Pharmacol. 614, 128–136.

Binkowski, A., Naghibzadeg, S., Liang, J., 2003. Nucleic Acids Res. 31, 3352–3355.

Braca, A., Tommasi, N.D., Bari, L.D., Pizza, C., Politi, M., Morelli, I., 2001. J. Nat. Prod. 64, 892–895.

Brahmbhatt, D.I., Pandya, U.R., 2001. Indian J. Chem. 40B, 419.

Brahmbhatt, D.I., Pandya, U.R., 2003. Indian J. Chem. 42B, 145.

Brahmbhatt, D.I., Patel, C.N., Pandya, V.P., Patel, M.A., 2004. Indian J. Chem. 43B, 2228.

Brahmbhatt, D.I., Pandya, V.P., Patel, C.N., Patel, M.A., 2005. Indian J. Chem. 44B, 1863.

Brahmbhatt, D.I., Gajera, J.M., Pandya, V.P., Patel, M.A., 2007. Indian J. Chem. 46B, 869–871.

Brahmbhatt, D.I., Ankit, R.K., Anil, K.P., Niraj, H.P., 2010. Indian J. Chem. 49B, 971–977.

Christinat, N., Scopelliti, R., Severin, K., 2007. J. Org. Chem. 72, 2192–2200. http://dx.doi.org/10.1021/jo062607p.

Dehkordi, L.S., Liu, Z.D., Hider, R.C., 2008. Eur. J. Med. Chem. 43, 1035–1047. http://dx.doi.org/10.1016/j.ejmech.2007.07.011.

Dömling, A., Ugi, I., 1993. Angew. Chem. Int. Ed. Engl. (32), 563.

Farley, C.P., Eliel, E., 1956. J. Am. Chem. Soc. 78, 3484.

Ferreira, I., Queiroz, M., Vilas-Boas, M., Estevinho, L., 2006. Bioorg. Med. Chem. Lett. 16, 1384–1387.

Frank, R.L., Seven, R.P., 1949. J. Am. Chem. Soc. 71, 2635.

Frank, R.L., Riener, E.F., 1950. J. Am. Chem. Soc. 72, 4183.

Hamdi, N., Puerta, M.C., Valerga, P., 2008. Eur. J. Med. Chem. 43, 2541–2548.

Hossain, M., Shaha, S., Aziz, F., 2009. Bangladesh Med. Res. Counc. Bull. 35, 49–52.

Jones, G., 1996. In: McKillop, A. (Ed.), Comprehensive Heterocyclic Chemistry, Pergamon, Oxford, U.K., 5, pp. 167–243.

Kenchappa, R., Yadav, D.B., Peethambar, S.K., Telkar, Sandeep, Venkatesh, K.B., 2013. Med. Chem. Res.. http://dx.doi.org/10.1007/s00044-013-0494-7.

Lee, D., Sello, J.K., Schreiber, S.L., 2000. Org. Lett. 2, 709–712.

Lee, B.H., Clothier, M.F., Dutton, F.E., Conder, G.A., Johnson, S.S., 1998. Bioorg. Med. Chem. Lett. 8, 3317–3320.

Marshall, N.J., Andruszkiewicz, R., Gupta, S., Milewski, S., Payne, J.W., 2003. J. Antimicrob. Chemother. 51, 821–831.

McKillop, A., Boulton, A.J., 1984. In: Rees, C.W., Katritzky A.R. (Eds.), Comprehensive Heterocyclic Chemistry, Pergamon, Oxford, U.K, 2, pp. 67–134.

Miky, J.A.A., Farrag, A.A., 1997. Indian J. Chem. 36 B, 357.

Milewski, S., 2002. Biochim. Biophy. Acta 1597, 173–192.

Milewski, S., Gabriel, I., Olchowy, J., 2006. Yeast 23 (1), 1–14.

Muller, T.J.J., Bunz, U.H.F., 2007. (Eds.), Functional Organic Materials; Wiley-VCH, Weinheim, Germany.

Murray, D.H., Mendez, J., Brown, S.A., 1982. The Natural Coumarins: Occurrence, Chemistry and Biochemistry. J. Wiley and Sons, New York.

Nawar, W.W., 1996. Lipids. In: Fennema O.R., (Ed.), Food Chemistry. Marcel Dekker, New York, pp. 225–313.

Negwer, M., 1994. Organic-Chemical Drugs and Their Synonyms, 7th ed. Akademie-Verlag, Berlin, Germany.

Nermien, M.S., Hany, M.M., Essam, S.A.E.H.K., Shymaa, S.M., Ahmed, M.E.A., 2011. Eur. J. Med. Chem. 46, 765–772.

Nevcihan, G., Cengiz, S., Bektas, T., Halil, S.M., 2010. Food Sci. Biotech. 19 (3), 691–696.

Niraj, H.P., Anil, K.P., Chirag, V.P., Apoorva, A.P., Brahmbhatt, D.I., 2010. Arkivoc 2, 283–291.

Roseman, S., 2001. J. Bio. Chem. 276 (45), 41527–41542.

Sharma, P., Rane, N., Gurram, V.K., 2004. Bioorg. Med. Chem. Lett. 14, 4185–4190.

Spitzner, D., 2004. Product class 1: pyridines. In: Black, D.St.C. (Ed.), Six-Membered Hetarenes with One Nitrogen or Phosphorus Atom Science of Synthesis. Thieme, Stuttgart, Germany, Vol. 15, pp 11–284.
Tegginamath, G., Ravindra, R.K., Pramod, P.K., Sheetal, B.M., 2011. Arab. J. Chem.
Via, L.S., Gia, O., Magno, S.M., Santana, L., Teijeira, M., Uriarte, E., 1999. J. Med. Chem. 42, 4405–4413.
Vijesh, A.M., Isloor, A.M., Vivek, P., Shaoib, A., Shridhar, M., 2010. Eur. J. Med. Chem. 45, 5460–5464.
Vijesh, A.M., Isloor, A.M., Telkar, Sandeep., Arulmoli, T., 2013. Arab. J. Chem. 6, 197–204.
Wallace, A.C., Laskowski, R.A., Thornton, J.M., 1995. Prot. Eng. 8, 127–134.
Whelan, W.L., Ballou, C.E., 1975. J. Bacteriol. 124, 1545–1557.
Yuan, Y.V., Bone, D.E., Carrington, M.F., 2005. Food Chem. 91, 485–494.