Annals of Science and Allied Research Vol. 1 (1) December, 2023: 147-152.
doi: https://doi.org/10.5281/zenodo.10521542

SYNTHETIC APPROACH TO DESIGN SOME SUBSTITUTED FURO-[2,3-B] PYRIDINES AS ANTIBACTERIAL AND ANTIFUNGAL AGENTS

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(Received on December 10, 2023; Revised on December 22, 2023; Accepted on December 30, 2023)

ABSTRACT

Several novel 1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylenanilinyl]furo[2,3-b]pyridines (4a-e) have been synthesised from furopyridine and pyrazole moieties by using conventional synthetic strategy. Structures of the synthesized Schiff bases were characterized by IR, ^{1}H-NMR, Mass and elemental analysis (C, H, N). Synthesised Schiff bases were screened for antibacterial and antifungal activities

Keywords : Antibacterial, antifungal, substituted furo[2,3-b]pyridines

INTRODUCTION

Drug resistance to pathogens is a blow to human beings and the only solution is the continual development of new antibacterial agents with unique structure and action mechanism on pathogens from the existing antibacterial therapeutics [1-2]. Literature is evident to explore the significance of fused pyridine compounds in the synthetic field of heterocyclic compounds [3-4] and play dominant role in bio potency of the molecules [5-6]. Since last few decades bulk of synthesis has been reported on various furopyridines [7-15]. The hetero pyridines displayed antiviral [16], antibacterial [17], anti-HIV [18] and antinociceptive [19] activities. Among the hetero pyridines, substituted furopyridines exhibited its pharmacological versatility as protease kinase inhibitor [20], antipsychotic [21], antihypertensive [22], diuretic [23], skin diseases [24] and in the treatment of depression and cerebral ischemia [25]. Furthermore, pyrazole and its substituted derivatives have been directed towards the pharmacological active class compounds. They are used as antitumor [26], antimicrobial [27-28], antiviral [29], cytotoxic [30], antimiotic [31], anti-inflammatory and ulcerogenic [32]. In the light of above study, we have focussed on two moieties, fused pyridine i.e. furopyridine and pyrazole to develop some newer Schiff bases with the hope to possess better pharmacological profile and lesser amount of toxicity. Herewith in the continual synthesis program [33-35], here we are reporting synthesis of some Schiff bases of fused pyridine i.e. furopyridines (Scheme-1).

MATERIALS & METHODS

Materials

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. Reference drugs ampicillin trihydrate and fluconazole were used.

Measurements

The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds was routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyser. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and ^{1}H-NMR spectra on Bruker DPX 200 using TMS as internal standard.

Synthesis

Preparation of 6-hydrazinylfuro[2,3-b]pyridine (1)

An ethanolic mixture of 6-chlorofuro[2,3-b]pyridine (0.01 mol) and 99% hydrazine hydrate (0.012 mol) was refluxed for 4 h. Excess of ethanol was distilled under reduced pressure. The obtained residue triturated with petroleum ether (40-60 ^{0}C), recystallized from absolute ethanol to yield the compound 1. Yield: 60%; m.p.: 93 ^{0}C; R_f : 0.60. Anal. Calcd. For C_7H_4ClNO: C, 54.75; H, 2.63; N, 9.12. Found: C, 54.62; H, 2.66, N, 9.10. IR (KBr, cm^{-1}): 670 (C-O-C), 1605 (C=N). ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.95 (d, 1H, J = 4.0 Hz), 7.46 (d, 1H, J = 16.0 Hz), 7.75 (d, 1H, J = 4.0 Hz), 8.31 (d, 1H, J = 16.0 Hz). MS (m/z, %): 153.57.

Preparation of 6-[2-{1-(3-thiophen-2-yl)phenyl}ethyllidene]hydrazinylfuro[2,3-b]pyridine (2)

A stirred mixture of compound **1** (0.01 mol) and 1-[3-(thiophen-2-yl)phenyl]ethanone (0.01 mol) in ethanol containing a drop of glacial acetic acid was refluxed for 1 h. Appeared solid cooled, filtered, washed, dried and recrystallized from ethanol to obtain compound 2. Yield: 65%; m.p.: 113 ^{0}C; R_f : 0.66. Anal. Calcd. For $C_7H_7N_3O$: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.44; H, 4.76, N, 28.12. IR (KBr, cm^{-1}): 670 (C-O-C), 1605 (C=N). ^{1}H-NMR ($CDCl_3$, δ/ppm): 4.00 (brs, 1H), 4.93 (brs, 2H), 6.70 (s, 1H), 6.94 (d, 1H, J=6.0 Hz), 7.60 (s, 1H), 7.98 (d, 1H, J=6.0 Hz). MS (m/z, %): 149.15.

Preparation of 1-[3-{3-(thiophen-2-yl)phenyl}-4-carbaldehyde-1*H*-pyrazolyl]furo[2,3-b] pyridine (3)

A Vilsmeier-Haack reagent was prepared from N, N`-DMF (10 ml) and phosphorous oxy chloride (1.1 ml). Compound 2 (0.01 mol) was charged lot wise to the Vilsmeier-Haack reagent and allowed to stir at 60-65^{0}C for 2 h. rapidly and then poured into ice-cold water. The reaction mixture cooled and neutralized with 10% sodium bicarbonate solution to get solid which was filtered, washed properly with water and recrystallized from ethanol to furnish compound **3**. Yield: 50%; m.p.: 169 ^{0}C; R_f : 0.70. Anal. Calcd. For $C_{19}H_{15}N_3OS$: C, 68.45; H, 4.53; N, 12.60. Found: C, 68.49; H, 4.55, N, 12.64. IR (KBr, cm^{-1}): 670 (C-O-C), 1605 (C=N). ^{1}H-NMR ($CDCl_3$, δ/ppm): 2.35 (s, 3H), 6.30 (d, 1H, J =16.0 Hz), 6.60 (d, 1H, J=8.0 Hz), 7.00-7.18 (m, 3H), 7.31 (d, 1H, J=16.0 Hz), 7.50 (d, 1H), 7.69 (brs, 1H), 7.83 (d, 2H, J=2.0 Hz), 8.01 (s, 1H), 8.18 (d, 1H, J=8.0 Hz). MS (m/z, %): 333.41.

General preparation of 1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylenanilinyl]furo [2,3-b] pyridines (4a-e)

The solution of compound 3 (0.02 mol) in ethanol was refluxed with different substituted anilines (0.02 mol) for 4-6 h. On completion of the reaction, excess of solvent was distilled off and the residue thus obtained was cooled, poured into ice cold water, triturated with petroleum ether (40-60 ^{0}C) and recrystallised with appropriate solvents to furnish the products **4a-e**.

1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylenanilinyl]furo[2,3-b] pyridine 4a: Yield: 54%; m.p.: 151 ^{0}C; R_f : 0.69. Anal. Calcd. For $C_{27}H_{18}N_4OS$: C, 72.63; H, 4.06; N, 12.55. Found: C, 72.44; H, 4.10, N, 12.47. IR (KBr, cm^{-1}): 1250 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1620 (C=N), 3033 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.40 (d, 1H, J=8.0 Hz), 6.60-6.82 (m, 3H), 7.00-7.45 (m, 11H), 7.61 (d, 1H, J=10.0 Hz), 8.05 (s, 1H), 8.76 (s, 1H). MS (m/z, %): 446.52.

1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylene-4-methoxyanilinyl]furo[2,3-b] pyridines 4b: Yield: 50%; m.p.: 123 ^{0}C; R_f : 0.73. Anal. Calcd. For $C_{28}H_{20}N_4O_2S$: C, 70.57; H, 4.23; N, 11.76. Found: C, 72.41; H, 4.17, N, 12.00. IR (KBr, cm^{-1}): 1255 (C-N), 1524 (N-N), 1570 (C—C of aromatic), 1622 (C=N), 3028 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.56 (s, 3H), 6.35 (d, 1H, J=8.4 Hz), 6.58-6.77 (m, 3H), 7.03-7.51 (m, 10H), 7.68 (d, 1H, J=10.6 Hz), 8.10 (s, 1H), 8.84 (s, 1H). MS (m/z, %): 476.55.

1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylene-2-aminoanilinyl]furo[2,3-b] pyridines 4c: Yield: 42%; m.p.: 169 ^{0}C; R_f : 0.77. Anal. Calcd. For $C_{27}H_{19}N_5OS$: C, 70.26; H, 4.15; N, 15.17. Found: C, 70.40; H, 4.14, N, 15.21. IR (KBr, cm^{-1}): 1256 (C-N), 1521 (N-N), 1578 (C—C of aromatic), 1626 (C=N), 3035 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.14 (bs, 2H), 6.38 (d, 1H, J=8.1 Hz), 6.54-6.71 (m, 3H), 7.01-7.40 (m, 10H), 7.64 (d, 1H, J=10.2 Hz), 8.11 (s, 1H), 8.80 (s, 1H). MS (m/z, %): 461.54.

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doi: https://doi.org/10.5281/zenodo.10521542

1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylene-3-aminoanilinyl]furo[2,3-b] pyridines 4d: Yield: 50%; m.p.: 149 ^{0}C; R_f : 0.73. Anal. Calcd. For $C_{28}H_{19}N_5OS$: C, 70.26; H, 4.15; N, 15.17. Found: C, 70.32; H, 4.16, N, 15.20. IR (KBr, cm^{-1}): IR (KBr, cm^{-1}): 1252 (C-N), 1527 (N-N), 1580 (C—C of aromatic), 1621 (C=N), 3033 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.11 (bs, 2H), 6.40 (d, 1H, J=8.4 Hz), 6.58-6.75 (m, 3H), 7.10-7.49 (m, 10H), 7.70 (d, 1H, J=10.4 Hz), 8.20 (s, 1H), 8.84 (s, 1H). MS (m/z, %): 461.54.

1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylene-2-bromoanilinyl]furo[2,3-b] pyridines 4e: Yield: 42%; m.p.: 151 ^{0}C; R_f : 0.69. Anal. Calcd. For $C_{27}H_{17}N_4OSBr$: C, 61.72; H, 3.26; N, 10.66. Found: C, 61.70; H, 3.20, N, 10.67. IR (KBr, cm^{-1}): 1248 (C-N), 1521 (N-N), 1569 (C—C of aromatic), 1621 (C=N), 3036 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.31 (d, 1H, J=8.2 Hz), 6.51-6.73 (m, 3H), 7.12-7.70 (m, 10H), 7.95 (d, 1H, J=10.4 Hz), 8.29 (s, 1H), 8.90 (s, 1H). MS (m/z, %): 525.42.

Antimicrobial test

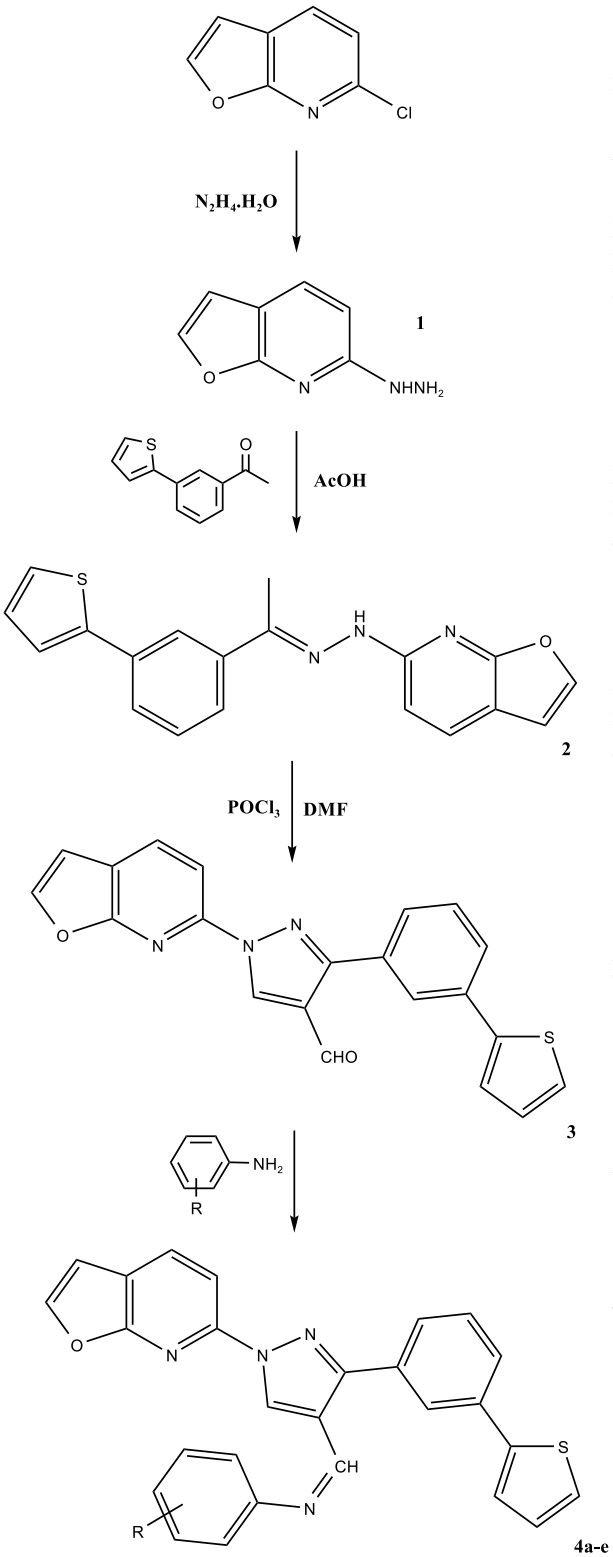
All the newly synthesized compounds were screened for their antibacterial and antifungal activity. Microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method [37-38] was used for determination of the preliminary antibacterial activity. Disks measuring 6 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 ^{0}C for an hour. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 ^{0}C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The bacterial inhibition values of the tested compounds against the tested bacteria strains are recorded in mm (Table-**I)**. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata*, *Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method 39-40]. All the fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 ^{0}C for 1 h. Using an agar punch wells were made into each well labelled. A control was also prepared in triplicate and maintained at 37 ^{0}C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The fungal inhibition values of the tested compounds against the tested fungal strains are recorded in mm (Table-**I)**.

Acute toxicity test

Lethal dose [41] (LD_{50}) of test compounds were determined in albino mice. After 24 h of drug administration, percent mortality in each group was observed from the data obtained LD_{50}. Data revealed that compound 4h does not show any toxicity up to dose of 9.18 mg/ml body weight in mice.

RESULT AND DISCUSSION

Synthetic strategy started with the 6-chlorofuro[2,3-b]pyridine. 6-Hydrazinylfuro[2,3-b]pyridine (**1)** was prepared by the hydrazinolysis of 6-chlorofuro[2,3-b]pyridine according to the method in literature [36]. Reaction of compound **1** with 1-[3-(thiophen-2-yl)phenyl] ethanone yielded 6-[2-{1-(3-thiophen-2-yl)phenyl}ethyllidene] hydrazinylfuro[2,3-b]pyridine (**2**). Vilsmeier-Haack reagent ($POCl_3$-DMF) furnished 1-[3-{3-(thiophen-2-yl) phenyl}-4-carbaldehyde-1*H*-pyrazolyl]furo[2,3-b] pyridine (**3)** in good yields. The synthesis of target Schiff bases (**4a-e**) were afforded by the reaction of different aromatic amines with compound (**3**) in presence of 2% sodium hydroxide solution (Scheme-**1**). All the prepared moieties were evaluated by using the cup plate method for antimicrobial activity against selected pathogenic strains.



R = H, 4-OCH_3, 2-NH_2, 3-NH_2, 2-Br

Scheme-1

The screening results were compared with standard ampicillin trihydrate and fluconazole respectively for antibacterial and antifungal testing. Furthermore, the most potent Schiff base was also tested for lethal dose. Derivative 3 was found active against only *P. vulgaris*. Antimicrobial screening data of compounds 4a-e, cleared that conversion of 1-[3-{3-(thiophen-2-yl) phenyl}-4-carbaldehyde-1*H*-pyrazolyl]furo[2,3-b] pyridine i.e. 3 into Schiff bases of furo[2,3-b]pyridine 4a-e resulted into significant pathogenic inhibition. Compounds 4a-h displayed mild to moderate antibacterial and antifungal activity. Out of the tested Schiff bases 4a-e, Schiff base 4f, 4g and 4h illustrated noteworthy antimicrobial potency against the selected panel of pathogens. Comparative antimicrobial study revealed the microbial inhibition potential order as 4a< 4c< 4b< 4d< 4e. Among all the screened substituted furo[2,3-b]pyridines, derivative **4e** elicited broader and significant antimicrobial potential. Based on structure activity relationship, it can be concluded that incorporation of 2/3/4-bromo substituted aniline is responsible for encouraging antimicrobial potential but 4-bromo substituted aniline claimed remarkable antimicrobial spectrum (Table-**I**).

CONCLUSION

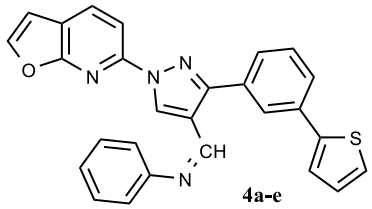
1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylenanilinyl]furo[2,3-b]pyridines (4a-e) were synthesised by conventional synthetic methodology and screened for their antimicrobial activity against the selected panel of pathogens. Among all the tested compounds, bromo substitution bearing mimic 4e displayed broad antimicrobial potential against the used all pathogens with lesser toxicity. More derivatisation required for better biological profiling.

Acknowledgement

We are thankful for the Department of Botany, Pt. L.M.S. Campus, Rishikesh, U.K., India for biological activities.

Annals of Science and Allied Research Vol. 1 (1) December, 2023: 147-152.
doi: https://doi.org/10.5281/zenodo.10521542

***Table-I*:** Antimicrobial screening of 1-[3-[{3-(thiophen-2-yl)phenyl}-1*H*-pyrazol-4-yl]methylenanilinyl]furo[2,3-b] pyridines **(4a-e)**.



4a-e

Compound	Antibacterial activity (mm)				Antifungal activity (mm)			
	S. aureus	*E. coli*	*P. vulgaris*	*K. pneumoniae*	*A. fumigatus*	*A. flavus*	*C. albicans*	*C. glabrata*
3.	-	-	6	-	-	-	-	-
4a.	-	-	6	-	6	6	-	-
4b.	-	6	6	-	8	6	-	-
4c.	-	6	6	-	6	6	-	-
4d.	-	6	-	-	8	8	-	6
4e.	12	10	8	8	14	14	6	8
Ampicillin trihydrate	16	16	20	20	-	-	-	-
Fluconazole	-	-	-	-	20	20	15	15
DMF (control)	-	-	-	-	-	-	-	-

- means no activity.

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