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Synthesis and antimicrobial activity of several substituted pyridin-1-yl-4H-1,2,4-triazole-3-thioles

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ABSTRACT

Several 5-substitutedphenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2,3-b] pyridin-1-yl]4H-1,2,4-triazole-3-thioles ***4a-f*** *have been synthesised and screened for antibacterial and antifungal activities. Structures of the synthesized derivatives were characterized by IR, [1]H-NMR, Mass and elemental analysis (C, H, N). Compound* ***4f*** *displayed significant antimicrobial activity.*

Keywords: Antibacterial, antifungal, substituted pyridin-1-yl-4H-1,2,4-triazole-3-thioles.

INTRODUCTION

1,2,4-triazole containing ring system received much attention due to its versatile biological utility and facilitating the scientific approaches to derive useful pharmacophores. Frequent attempts have been made to develop new substituted 1,2,4-triazoles possessing more effective antimicrobial activity. 1,2,4-triazole containing ring systems exhibit a range of biological activities including antiseptic, analgesic, anti-convulsant, antibiotic [1], antiallergic [1], anti-inflammatory [1-10,13], diuretic[1,5,8], fungicidal [3,4,10-13], insecticidal [3,10,13], herbicidal [3,10,13], antibacterial [3-6,11,12], antiviral [2-5,7,8,10], antidepressant [2,5,9], antimicrobial [2-5,7,10-12], antitumor [3,6,9-10], antihypertensive [5,8-9] and antimigraine compounds [7]. Several bearing the 1,2,4-triazole nucleus are also under clinical trials viz.. anastrozole, rizatriptan, nefazodone, vorozole, ribavirin, fluconazole, letrozole and uniconazole. In addition several triazoles also demonstrated their versatility as precursors commercially for photosensitive materials as inks and toners [14], polymer chemistry [11], and others [15-16]. Literature survey revealed the importance of fused pyridines in the field of heterocyclic compounds [17-18] and play significant role in the medicinal utility of the molecules [19-20]. In the present study, synthesis and the antimicrobial activity of several substituted pyridin-1-yl-4H-1,2,4-triazole-3-thiol have been reported.

MATERIALS AND METHODS

Material

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.

Measurement

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), 7.46 (d, 1H), 7.75 (d, 1H), 8.31 (d, 1H). MS (m/z, %): 153.57.

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

A stirred mixture of compound **2** (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), 7.60 (s, 1H), 7.98 (d, 1H). MS (m/z, %): 149.15.

General method of preparation of 3-substitutedphenyl-4-amino-5-mercapto triazoles 3a-f

Substituted 1,2,4-triazoles were prepared according to the reported method [23-26].

General preparation of 5-substitutedphenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene) hydrazinyl}1H-pyrrolo[2,3-b]pyridin-1-yl]4H-1,2,4-triazole-3-thioles 4a-f

A dimethylformamide solution of 3-Aryl-4-amino-5-mercapto triazoles [22-26] **3a-f** (0.01mol) and 6-[2-{1-(3-thiophen-2-yl)phenyl}ethyllidene]hydrazinylfuro[2,3-b]pyridine **2** (0.01 mol) was refluxed for 3-5 h. Excess of solvent was distilled off under reduced pressure, residue dumped in ice-water, stirred, washed, filtered, dried to afford products which were recrystallised with appropriate solvents to yield the products **4a-f**.

5-Phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene) hydrazinyl}1H-pyrrolo[2,3-b]pyridin-1-yl]4H-1,2,4-triazole-3-thiol 4a: Yield: 61%; m.p.: 131 ^{0}C; R_f : 0.63. Anal. Calcd. For $C_{27}H_{21}N_7S_2$: C, 63.88; H, 4.17; N, 19.31. Found: C, 63.66; H, 4.11, N, 19.40. IR (KBr, cm^{-1}): 1250 (C-N), 1520 (N-N), 1573 (C—C of aromatic), 1628 (C=N), 2710 (SH), 3033 (aromatic CH), 3419 (OH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.16 (m, 3H), 5.17 (s, 1H), 6.50-6.70 (m, 3H), 7.20-8.10 (m, 11H), 8.76 (d, 2H), 11.38 (bs, 1H). MS (m/z, %): 507.13.

5-(2-Hydroxy)phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2, 3-b]pyridin-1-yl]4H-1,2,4-triazole-3-thiol 4b: Yield: 57%; m.p.: 166 ^{0}C; R_f : 0.75. Anal. Calcd. For $C_{27}H_{21}N_7OS_2$: C, 61.93; H, 4.04; N, 18.72. Found: C, 62.05; H, 4.10, N, 18.60. IR (KBr, cm^{-1}): 1258 (C-N), 1515 (N-N), 1578 (C—C of aromatic), 1622 (C=N), 2700 (SH), 3027 (aromatic CH), 3415 (OH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.11 (m, 3H), 5.05 (s, 1H), 6.50-6.75 (m, 3H), 7.15-8.00 (m, 10H), 8.80 (d, 2H), 11.20 (bs, 1H), 12.50 (ss, 1H, exchangeable with D_2O). MS (m/z, %): 523.12.

5-(3-Hydroxy)phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2,3-b] pyridin-1-yl]4H-1,2,4-triazole-3-thiol 4c: Yield: 55%; m.p.: 149 ^{0}C; R_f : 0.68. Anal. Calcd. For $C_{27}H_{21}N_7OS_2$: C, 61.93; H, 4.04; N, 18.72. Found: C, 61.95; H, 4.06, N, 18.75. IR (KBr, cm^{-1}): 1254 (C-N), 1520 (N-N), 1581 (C—C of aromatic), 1627 (C=N), 2708 (SH), 3021 (aromatic CH), 3412 (OH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.06 (m, 3H), 5.10 (s, 1H), 6.53-6.70 (m, 3H), 7.24-8.10 (m, 10H), 8.90 (d, 2H), 11.10 (bs, 1H), 12.55 (ss, 1H, exchangeable with D_2O). MS (m/z, %): 523.12.

5-(4-Hydroxy)phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2,3-b] pyridin-1-yl]4H-1,2,4-triazole-3-thiol 4d: Yield: 50%; m.p.: 168 ^{0}C; R_f : 0.70. Anal. Calcd. For $C_{27}H_{21}N_7OS_2$: C, 61.93; H, 4.04; N, 18.72. Found: C, 61.88; H, 4.00, N, 18.64. IR (KBr, cm^{-1}): 1248 (C-N), 1518 (N-N), 1588 (C—C of aromatic), 1639 (C=N), 2720 (SH), 3030 (aromatic CH), 3420 (OH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.20 (m, 3H), 5.16 (s, 1H), 6.50-6.72 (m, 3H), 7.18-8.11 (m, 10H), 8.88 (d, 2H), 11.22 (bs, 1H), 12.40 (ss, 1H, exchangeable with D_2O). MS (m/z, %): 523.12.

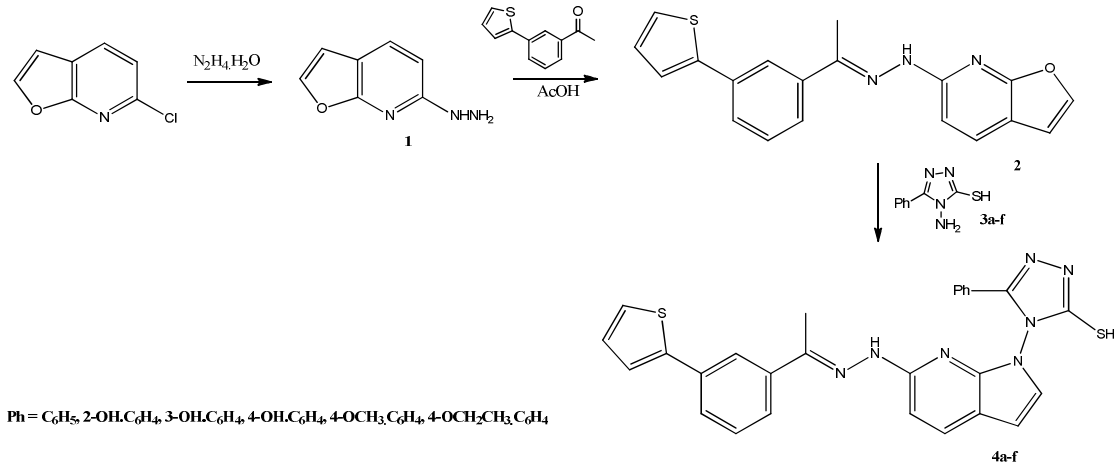
5-(4-Methoxy)phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2,3 -b]pyridin-1-yl]4H-1,2,4-triazole-3-thiol 4e: Yield: 49%; m.p.: 127 ^{0}C; R_f : 0.66. Anal. Calcd. For $C_{28}H_{23}N_7OS_2$: C, 62.55; H,

4.31; N, 18.24. Found: C, 62.52; H, 4.32, N, 18.25. IR (KBr, cm^{-1}): 1255 (C-N), 1524 (N-N), 1576 (C—C of aromatic), 1623 (C=N), 3032 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 3.11 (m, 3H), 3.95 (s, 3H), 5.12 (s, 1H), 6.48-6.69 (m, 3H), 7.12-8.10 (m, 10H), 8.77 (d, 2H), 11.25 (bs, 1H). MS (m/z, %): 537.14.

5-(4-Ethoxy)phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2,3-b]pyridin-1-yl]4H-1,2,4-triazole-3-thiol 4f: Yield: 53%; m.p.: 148 ^{0}C; R_f : 0.70. Anal. Calcd. For $C_{29}H_{25}N_7OS_2$: C, 63.14; H, 4.57; N, 17.77. Found: C, 63.20; H, 4.51, N, 17.70. IR (KBr, cm^{-1}): 1250 (C-N), 1519 (N-N), 1582 (C—C of aromatic), 1625 (C=N), 3030 (aromatic CH). ^{1}H-NMR ($CDCl_3$, δ/ppm): 2.55 (m, 5H), 4.00 (s, 3H), 5.06 (s, 1H), 6.50-6.71 (m, 3H), 7.20-8.15 (m, 10H), 8.89 (d, 2H), 11.10 (bs, 1H). MS (m/z, %): 551.16.

RESULTS AND DISCUSSION

6-Hydrazinylfuro[2,3-b]pyridine **1** was prepared by the hydrazinolysis of 6-chlorofuro[2,3-b]pyridine according to reported method [21]. Reaction of compound **1** with 1-[3-(thiophen-2-yl)phenyl] ethanone furnished 6-[2-{1-(3-thiophen-2-yl)phenyl}ethylidene]hydrazinylfuro[2,3-b]pyridine **2**. Targeted 5-substitutedphenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene)hydrazinyl}1H-pyrrolo[2,3-b]pyridin-1-yl]4H-1,2,4-triazole-3-thioles **4a-f** have been synthesised by the refluxing of compound **2** with 3-substitutedphenyl-4-amino-5-mercapto triazoles **3a-f** [23-26] in DMF solution (Scheme-**1**). All the prepared moieties were evaluated by using the cup plate method for antimicrobial activity against selected pathogens. Standard drugs ampicillin trihydrate and fluconazole were used respectively for antibacterial and antifungal activity. Compound 1 and 2 showed no inhibition. Biological testing results cleared that conversion of 6-[2-{1-(3-thiophen-2-yl)phenyl}ethylidene] hydrazinylfuro[2,3-b]pyridine **2** into 5-substituted phenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene) hydrazinyl}1H-pyrrolo[2,3-b]pyridin-1-yl]4H -1,2,4-triazole-3-thioles **4a-f** resulted into significant microbial inhibition. Compounds **4a-f** showed mild to moderate antimicrobial activity. Their microbial inhibition potential was in of order 4a< 4c< 4b< 4d< 4e< 4f. Among all the screened derivative **4f** claimed broader and significant antimicrobial activity. On the basis of S.A.R. (structure activity relationship), it can be concise that incorporation of 3-substitutedphenyl-4-amino-5-mercaptotriazoles brought significant microbial inhibition (Table-**1**).



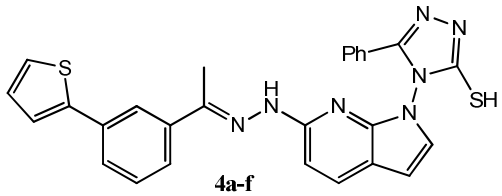
Scheme-1

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 [27-28] 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-**1)**. 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 29-30]. 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-**1)**.

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



4a-f

Compound	Antibacterial activity (mm)				Antifungal activity (mm)			
	S. aureus	*E. coli*	*P. vulgaris*	*K. pneumoniae*	*A. fumigatus*	*A. flavus*	*C. albicans*	*C. glabrata*
1.								
2.								
4a.	-	-	6	-	6	6	-	-
4b.	-	6	6	-	8	6	-	-
4c.	-	6	6	-	6	6	-	-
4d.	-	6	-	-	8	8	-	6
4e.	10	10	8	8	10	6	-	8
4f.	15	10	12	16	15	18	10	15
Ampicillin trihydrate	16	16	20	20	-	-	-	-
Fluconazole	-	-		-	20	20	15	15
DMF (control)	-	-		-	-	-	-	-

- means no activity.

CONCLUSION

5-substitutedphenyl-4-[6-{2-(1-(3-(thiophen-2-yl)phenyl)ethylidene) hydrazinyl}1H-pyrrolo[2,3-b]pyridin-1-yl]4H-1,2,4-triazole-3-thioles **4a-f** were synthesised by conventional synthetic methodology and evaluated for antibacterial and antifungal testing against the selected microbes. Among all the tested compounds, compound **4f** displayed significant antimicrobial potential.

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