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SYNTHESIS AND ANTIMICROBIAL ACTIVITY EVALUATION OF SOME (3*E*)-2,6-DIARYL-N-PYRIDINYL-4,5-DIHYDROPYRIDAZIN-3(2*H*)-IMINES

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ABSTRACT

Some (3*E*)-2,6-diaryl-N-pyridinyl-4,5-dihydropyridazin-3(2*H*)-imines were prepared by the reaction of 2-(aryl)-6-phenyl-4,5-dihydropyridazin-3(2*H*)-ones with appropriate pyridinyl amine using acetic acid as a solvent. The structures of the representative compounds were confirmed on the basis of their FTIR, ^{1}H-NMR, and ^{13}C-NMR. These compounds were evaluated for their antimicrobial activity by the cup plate method against two Gram positive bacteria, namely, *Staphylococcus aureus* and *Bacillus subtilis*; three Gram negative bacteria, namely, *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*; and one fungus, *Candida albicans*. These compounds showed mild to moderate antimicrobial activity with respect to the standard drugs Ofloxacin and Ketoconazole. However, it has been identified that the presence of 2-pyridinyl group is an essential requirement in the chemical structure of these types of compounds to show better antimicrobial activity. It is also believed that the replacement of the phenyl, 4-nitropheny, and 2,4-dinitrophenyl groups at position 2 of the pyridazinone ring with other similar isosteres or bioisosteres along with the presence of 2-pyridinyl moiety may provide better antimicrobial agents.

KEYWORDS: Pyridazinone, 2-Pyridinyl, Antibacterial activity, Antifungal activity, Structure activity relationship.

INTRODUCTION

Antimicrobial resistance has been recognized as a global problem as it increases the rate of mortality and morbidity.[1] The literature reveals that the cause of antibiotic resistance includes the irrational use of the antibiotics and the failure to discover new antimicrobial agents since the late 1980s. Accordingly, there is a need to develop new antimicrobial agents to combat antimicrobial resistance problems.[1-3] Pyridazinone derivatives have gained substantial attention within the field of medicinal chemistry. In recent years, this chemical moiety has been investigated extensively for its diverse biological activities[4] including antiinflammatory activity, analgesic activity, anticancer activity, antiviral activity, antimicrobial activity, cardiovascular activity, antitubercular activity, antiobesity activity, antidiabetic activity, and neuroprotective activity. Some reviews of the chemistry and biological importance of pyridazinone derivatives have also been published,[4-8] wherein pyridazinone compounds are reported to possess very good antimicrobial activity. Encouraged by these observations and in continuation of our search for the potent heterocyclic antimicrobial agents,[9-13] we decided to prepare some (3*E*)-2,6-diaryl-N-pyridinyl-4,5-dihydropyridazin-3(2*H*)-imines as antimicrobial agents.

MATERIALS AND METHODS

Melting points were measured in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FT-IR spectrometer (Browser Morner, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 FT NMR (Bruker, Germany) spectrophotometer using TMS as internal reference (chemical shift in δ ppm). The purity of the compounds was checked on silica gel G plates using iodine vapours as visualizing agent. The R_f value of the compounds was determined by using a mixture of toluene, ethyl acetate and formic acid (5:4:1). All the reagents used in the present work were of analytical grade. The synthetic pathway for the preparation of (3*E*)-2,6-diaryl-N-pyridinyl-4,5-dihydropyridazin-3(2*H*)-imines **(5-13)** is provided in Figure 1.

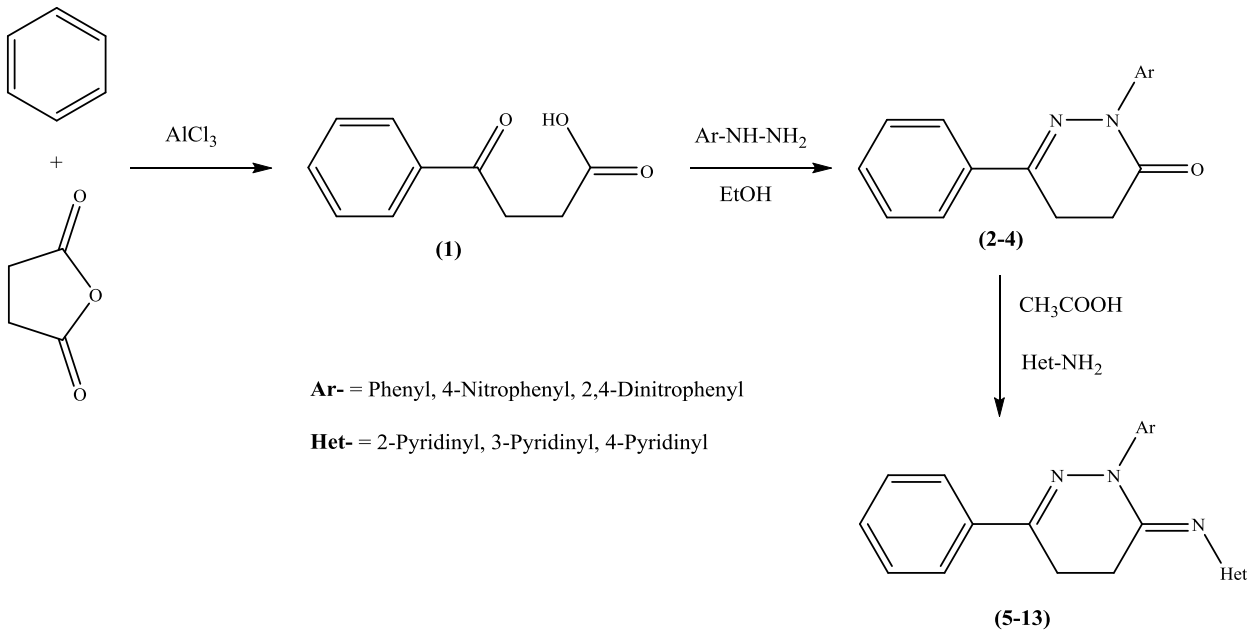

Figure 1: The synthetic pathway for the preparation of (3*E*)-2,6-diaryl-N-pyridinyl-4,5-dihydropyridazin-3(2*H*)-imines **(5-13)**

The compound **(1)** and the compounds **(2-4)** were prepared by the same methods as provided in our previous report.[14]

Synthesis of 3-Benzoylpropionic acid (1)

To a solution of succinic anhydride (0.1 mole) in benzene (50 mL), anhydrous aluminium chloride (0.125 mole) was added in small portions with stirring over a period of 2 hours. The reaction mixture was refluxed for 2 hours. After completion of the reaction, excess of benzene was removed by steam distillation. The reaction mixture was dissolved in sodium hydroxide solution, filtered, and hydrochloric acid was added to it. The solid mass obtained was filtered, washed with cold water, dried and recrystallized from methanol to give a colorless product which gave effervescence with sodium bicarbonate solution.

General procedure for the preparation of 2-(aryl)-6-phenyl-4,5-dihydropyridazin-3(2*H*)-one (2-4)

A mixture of 3-benzoylpropionic acid (0.01 mole) and appropriate phenylhydrazine (0.01 mole) in ethanol (30 mL) was refluxed for about 8 hours to 10 hours. The solid obtained was hot filtered, washed with a dilute solution of sodium bicarbonate and then recrystallized from ethanol.

General procedure for the preparation of (3*E*)-2,6-diaryl-N-(pyridinyl)-4,5-dihydro pyridazin-3(2*H*)-imines (5-13)

The compound **(2)** or compound **(3)** or compound **(4)** (0.01 mole) and appropriate pyridinyl amine (0.01 mole) were taken in a 100 mL flask containing 25 mL of glacial acetic acid. The mixture was refluxed for about 6 hours to 10 hours. After completion of the reaction, the reaction mixture was cooled. The solid obtained was filtered and recrystallized from acetic acid.

Antimicrobial activity

The *in vitro* antimicrobial activity was carried out against 24 hours old cultures of five bacteria, namely, *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 700603); and one fungus, *Candida albicans* (ATCC 2091) using cup plate method.[15,16] Nutrient agar medium and Sabouraud dextrose medium were used for antibacterial activity and antifungal activity, respectively. The compounds were tested at concentrations of 10 µg/mL in sterile dimethylformamide using Ofloxacin and Ketoconazole as standard drugs for antibacterial and antifungal activity, respectively. Inhibition was recorded by measuring the diameter of the inhibition zone after 24 hours for bacteria and 48 hours for fungus. Each experiment was repeated thrice and an average of the three independent determinations was recorded. The percentage antimicrobial activity was calculated for each compound with respect to the standard drugs.

Statistical Analysis

All data (n = 3) are presented as Mean ± Standard Error Mean (SEM). The data were analyzed by One-Way Analysis of Variance (ANOVA) with Dunnett's Multiple Comparison Test with respect to control group and standard group using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The results were considered significantly different at $p < 0.05$ as compared with control group as well as standard drug groups.

RESULTS AND DISCUSSION

The compounds **(5-13)** were prepared according to the method outline in Figure 1. The compounds **(1)** and the compounds **(2-4)** were prepared by the same methods as provided in our previous report.[14] The physical constant data of the compounds **(5-13)** is provided in Table 1.

Table 1: The physical constant data of the compounds (5-13)

Compound	Ar-	Het-	Molecular Formula	M.P. (±2 °C)	Yield (%)	R_f Value
5	Phenyl	2-pyridinyl	$C_{21}H_{18}N_4$	177-179	70	0.68
6	Phenyl	3- pyridinyl	$C_{21}H_{18}N_4$	165-167	65	0.66
7	Phenyl	4- pyridinyl	$C_{21}H_{18}N_4$	191-193	70	0.71
8	2,4-Dinitropheny	2- pyridinyl	$C_{21}H_{16}N_6O_4$	172-174	70	0.69
9	2,4-Dinitropheny	3- pyridinyl	$C_{21}H_{16}N_6O_4$	188-190	65	0.77
10	2,4-Dinitropheny	4- pyridinyl	$C_{21}H_{16}N_6O_4$	201-203	75	0.68
11	4-Nitrophenyl	2- pyridinyl	$C_{21}H_{17}N_5O_2$	174-176	80	0.73
12	4-Nitrophenyl	3- pyridinyl	$C_{21}H_{17}N_5O_2$	169-171	70	0.66
13	4-Nitrophenyl	4- pyridinyl	$C_{21}H_{17}N_5O_2$	175-177	75	0.69

The spectral data of the representative compounds are provided below.

2,6-Diphenyl-4,5-dihydropyridazin-3(2*H*)-one (3)

IR (KBr) cm^{-1}: 1685 (C=O), 1600 (C=N), 1510 (C=C); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.72 (t, J = 8 Hz, 2H, H-4 of pyridazinone), 3.11 (t, J = 8 Hz, 2H, H-5 of pyridazinone), 7.28 (t, J = 8Hz, 1H, Ar-H), 7.42-7.46 (m, 5H, Ar-H,), 7.57 (d, J = 11 Hz, 2H, Ar-H), 7.84 (t, J = 8 Hz, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 22.2 (C-4 of pyridazinone), 27.3 (C-5 of pyridazinone), 124.8 (2C, Ar-C), 126.0 (2C, Ar-C), 126.1 (Ar-C), 128.2 (2C, Ar-C), 128.5 (2C, Ar-C), 129.7 (Ar-C), 135.4 (Ar-C), 141.4 (Ar-C), 151.8 (Ar-C), 165.2 (C=O of pyridazinone).

2-(2,4-Dinitrophenyl)-6-phenyl-4,5-dihydropyridazin-3(2*H*)-one (4)

IR (KBr) cm^{-1}: 1625 (C=O), 1600 (C=N), 1510 (C=C), 1310 and 1340 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.36 (t, J = 8 Hz, 2H, H-4 of pyridazinone), 3.02 (t, J = 8 Hz, 2H, H-5 of pyridazinone), 7.42-7.66 (m, 4H, Ar-H), 7.84-7.93 (m, 2H, Ar-H), 8.21-8.26 (m, 1H, Ar-H), 8.78 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 23.6 (C-4 of pyridazinone), 33.5 (C-5 of pyridazinone), 116.4 (Ar-C), 123.0 (Ar-C), 123.4 (Ar-C), 126.5 (2C, Ar-C), 128.5 (3C, Ar-C), 129.6 (Ar-C), 136.1 (Ar-C), 143.3 (Ar-C), 144.4 (Ar-C), 146.5 (Ar-C), 168.0 (C=O of pyridazinone).

(*E*)-2,6-diphenyl-N-(pyridin-2-yl)-4,5-dihydropyridazin-3(2*H*)-imine (5)

IR (KBr) cm^{-1}: 1610, 1615 and 1625 (C=N), 1505, 1510 and 1520 (C=C); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.51 (t, J = 8 Hz, 2H, H-4 of pyridazinone), 2.98 (t, J = 8 Hz, 2H, H-5 of pyridazinone), 6.88 (d, J = 10 Hz, 1H, H-3 of pyridine), 7.08 (t, J = 7 Hz, 1H, H-5 of pyridine), 7.30-7.97 (m, 11H, Ar-H), 8.33 (d, J = 10 Hz, 1H, H-6 of pyridine); ^{13}C-NMR

(DMSO-d_6, 100 MHz) δ ppm: 21.3 (C-4 of pyridazinone), 25.2 (C-5 of pyridazinone), 114.0 (Ar-C), 117.5 (3C, Ar-C), 120.3 (Ar-C), 126.0 (2C, Ar-C), 122.6 (2C, Ar-C), 127.4 (2C, Ar-C), 130.2 (Ar-C), 134.4 (Ar-C), 135.1 (Ar-C), 143.4 (Ar-C), 146.2 (2C, Ar-C), 156.4 (C-3 of pyridazinone), 158.4 (C-2 of pyridine).

(*E*)-2-(2,4-dinitrophenyl)-6-phenyl-N-(pyridin-2-yl)-4,5-dihydropyridazin-3(2*H*)-imine (8)

IR (KBr) cm^{-1}: 1615, 1620 and 1625 (C=N), 1510, 1515 and 1520 (C=C), 1305 and 1330 (NO_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.58 (t, J = 8 Hz, 2H, H-4 of pyridazinone), 2.99 (t, J = 8 Hz, 2H, H-5 of pyridazinone), 6.85 (d, J = 10 Hz, 1H, H-3 of pyridine), 7.09 (t, J = 7 Hz, 1H, H-5 of pyridine), 7.28-7.59 (m, 6H, Ar-H), 8.0 (d, J = 10 Hz, 1H, Ar-H), 8.38-8.44 (m, 3H, Ar-H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 21.4 (C-4 of pyridazinone), 25.4 (C-5 of pyridazinone), 114.0 (Ar-C), 116.0 (Ar-C), 117.3 (Ar-C), 118.8 (Ar-C), 126.1 (2C, Ar-C), 126.7 (2C, Ar-C), 128.3 (Ar-C), 129.1 (Ar-C), 134.1 (Ar-C), 135.1 (Ar-C), 136.0 (Ar-C), 136.5 (Ar-C), 143.1 (Ar-C), 144.4 (Ar-C), 145.5 (Ar-C), 156.5 (C-3 of pyridazinone), 158.5 (C-2 of pyridine).

The *in vitro* antimicrobial activity was carried out against five bacteria and one fungus using cup plate method.[15,16] The percentage antimicrobial activity data of the compounds **(5-13)** with respect to the standard drugs is provided in Table 2.

Table 2: The percentage antimicrobial activity of the compounds (5-13) with respect to the standard drugs

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
5	70.13	62.66	55.18	54.73	51.55	64.50
6	51.55	56.44	44.26	48.33	34.33	56.75
7	67.66	61.46	52.50	54.27	42.65	63.45
8	79.66	71.85	76.74	87.75	87.65	75.70
9	76.90	66.82	72.66	83.90	78.50	67.65
10	77.88	68.74	72.75	83.66	78.52	70.50
11	89.74	84.58	62.15	77.88	67.75	85.88
12	88.40	80.33	58.44	57.25	53.77	77.33
13	88.90	82.33	62.15	75.33	56.33	77.33
Control	0.0	0.0	0.0	0.0	0.0	0.0
Ofloxacin	100.00	100.00	100.00	100.00	100.00	-
Ketoconazole	-	-	-	-	-	100.00

*No zone of inhibition for dimethylformamide. Zone of inhibition of Ofloxacin (100%) = 26 mm (E. coli), 32 mm (S. aureus), 28 mm (B. subtilis),30 mm (P. aeruginosa), 35 mm (S. typhi). Zone of inhibition of Ketoconazole (100%) = 30 mm (C. albicans). n = 3, *p > 0.05.*

The antimicrobial activity of the compounds **(5-13)** revealed that compound **11** exhibited the highest activity of 89.74% and 84.58% against Gram positive bacteria, *S. aureus* and *B. subtilis* respectively, with respect to the standard drug, Ofloxacin. The compound **8** exhibited highest activity of 76.74%, 87.75%, and 87.65% against Gram negative bacteria, *E. coli, P. aeruginosa* and *K. pneumonia* respectively, with respect to the standard drug, Ofloxacin. The compound **11** also exhibited the highest activity of 85.88% against *C. albicans* with respect to the standard drug, Ketoconazole. The structure activity relationship of the compounds revealed that for better antibacterial activity against Gram positive bacteria and *C. albicans* presence of a nitro group is required. The increase in the number of nitro groups decreases the antibacterial activity against Gram positive bacteria and *Candida albicans*. The structure activity relationship of the compounds against Gram negative bacteria revealed that the antibacterial activity increases with the number of nitro group. The antimicrobial activity of the compounds **(5-13)** against Gram positive bacteria, Gram negative bacteria, and *C. albicans* revealed that the compounds containing 2-pyridinyl moiety provide better antimicrobial agents.

CONCLUSION

It is evident from the antimicrobial activity data of the compounds **(5-13)** that none of the compounds exhibited comparable activity against standard drugs Ofloxacin and Ketoconazole. However, it has been identified that the presence of 2-pyridinyl group is an essential requirement in these types of compounds to act as better antimicrobial agent. There is a possibility that the replacement of the phenyl, 4-nitropheny, and 2,4-dinitrophenyl groups at position 2 of the pyridazinone ring with other similar isosteres or bioisosteres may provide better antimicrobial agents. Accordingly, this study may be extended to acquire more information about the structure activity relationships of this series of compounds.

CONFLICT OF INTEREST

The author declares that no conflict of interest is associated with this work.

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