International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 1 | JAN-MAR | 2017 | 134-140
Original Research Article – Pharmaceutical Sciences
UGC Approved Journal

SYNTHESIS AND EVALUATION OF NOVEL 4,4'-(4-SUBSTITUTED PHENYL PYRIDINE-2,6-DIYL) BIS (N-SUBSTITUTED BENZYLIDENE ANILINE) DERIVATIVES AS ANTIMICROBIAL AGENTS

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ABSTRACT

A series of new 4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) derivatives (6a-o) were synthesized. The structures of title molecules (6a-o) have been confirmed by various spectral techniques (IR, ^{1}HNMR, MS) and elemental analysis. The newly synthesized compounds were screened for antibacterial and antifungal activity and most of the compounds showed significant activity comparable with that of the standard drug. The results revealed that 6m, 6h, 6l, 6c, 6g, 6k and 6b showed good antibacterial activity towards all bacterial strains (Bacillus subtilis, Streptococcus pneumonia, Escherichia coli and Proteus vulgaris) when compared to standard drug Ampicillin. Amongst all the compounds, 6m showed moderate antifungal activity against Saccharomyces cerevisiae and Candida albicans when compared to standard Ketoconazole.

KEY WORDS

Antibacterial activity, Antifungal activity, Pyridine and Benzylidine Aniline.

Received on: 15.11.2016
Accepted on: 25.12.2016
Published on: 01.01.2017

INTRODUCTION

Pyridine is a simple aromatic heterocyclic organic compound with the chemical formula C_5H_5N used as a precursor to agrochemicals and pharmaceuticals and is also an important solvent and reagent and common substructures in numerous natural products and functional materials[1]. Poly substituted pyridines possess important biological and pharmacological activities and could be used as potential antimicrobial and anticancer agents. Schiff bases are the compounds which are mainly formed by the condensation of the aldehydes and amines. These compounds can be synthesized by various synthetic routes. Some of those are easily synthesized from the various heterocyclic rings like Pyridine [2], Furan [3], Thiophene [4] and Triazoles [5]. Pharmacological actions of Schiff compounds which have been reported in previous studies are antibacterial [6], antifungal [7], cytotoxic [8], antimalarial [9], antitubercular [10], anti-helmintic [11], analgesic [12], anti-inflammatory [13] and antioxidant [14] etc.

In the present investigation, we are attempting to synthesize a new series of 4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) derivatives (6a-o) were synthesized by an efficient condensation reaction of 4-Substituted Phenyl-2, 6-bis (4-Amino Phenyl) Pyridine (5a-c), with Substituted Aromatic Aldehydes. Further, the synthesized compounds were screened for their antimicrobial activity.

MATERIALS AND METHODS

General

The melting points were determined on a Stuart SMP3 melting point apparatus. IR spectra were recorded in Bruker FT-IR Opus Spectroscopic Software Version 2.0 using KBr disc method. The NMR spectra were recorded in $CDCl_3$-d_6 or $CDCl_3$. ^1H NMR spectra were obtained on a Bruker Advance 400 (400 MHz), The chemical shifts were expressed in values parts per million (ppm scale) and the J values were reported in Hertz (Hz). The peak patterns were indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet, and Composition of C, H and N elemental analyses were performed on Elementar vario micro CHNS Analyzer.

Chemicals

All chemicals, reagents and solvents were obtained from Sigma-Aldrich, Merck and Hi-media and Finar chemical companies and were used without further purification. Analytical TLC was performed on Silica Gel F 254 plates (Merck) with visualization by UV (254 nm) chamber. All the pyridines have been purified by column chromatography performed on silica gel (100-200 mesh, Merck).

Experimental Procedure

4-Substituted Phenyl-2,6-bis (4-Nitro Phenyl) Pyridine 4(a-c): In a round-bottomed flask (250 ml) equipped with a reflux condenser, a mixture of Substituted Benzaldehyde (1a-c, 0.06 mol), *p*-Nitroacetophenone (2, 20 g, 0.12 mol), Ammonium Acetate (3, 60 g), and Glacial Acetic Acid (150 ml) was refluxed for 2 hrs. Upon cooling, crystals separated, which were filtered and washed first with Acetic Acid (50%) and then with cold ethanol. These dark yellow crystals were recrystallized from absolute ethanol, and then dried at 60 ºC under vacuum. The product was 4-Substituted Phenyl-2,6-bis (4-Nitro Phenyl) Pyridine (4a-c) [15].

4-Substituted Phenyl-2,6-bis (4-Amino Phenyl) Pyridine 5(a-c): In a two-necked round-bottomed flask (1000 ml) equipped with a reflux condenser and a dropping funnel, a suspension of 4-Substituted Phenyl-2,6- bis(4-Nitro Phenyl) Pyridine (4a-c) (13.75 g, 0.033 mol), Palladium on Carbon 5% (1.4 g), and Ethanol (500 ml) was prepared. The mixture was warmed, and while being stirred magnetically, hydrazine hydrate 85% (35 ml) in ethanol (50 ml) was added dropwise over a 1.5 hr period through the dropping funnel while maintaining the temperature at about 50 °C. The reaction mixture was then refluxed for 2 hrs and filtered while hot. On cooling, the filtrate gave white-cream colored crystals of the title diamine compound 5(a-c), which were recrystallized from ethanol and vacuum dried [15].

4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) 6(a-o): 4-Substituted Phenyl-2, 6-bis (4-Amino Phenyl) Pyridine (5a-c, 0.005 mol), Substituted Aromatic Aldehydes (0.005 mol) dissolved in minimum volume of ethanol, and then 2-3 drops of Concentrated Sulphuric acid are added to the reaction mixture. The solution was stirred for 4-5 hrs at room temperature and the progress of the reaction was followed by TLC until the reaction was complete. It was cooled to 0°C, the precipitate was filtered, washed with ethanol, and recrystallized from methanol [16].

Spectral Data

4,4'-(4-Phenyl Pyridine-2,6-diyl) bis (N-Benzylidene Aniline) (6a): mp (°C): 210-212, IR (KBr) cm^{-1}: 3098 (CH=CH, Aromatic), 3031(CH=N, Olefinic), 1665(C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.412-7.423 (d, 1H, aromatic proton), 7.512-7.538 (m, 10H, aromatic protons), 7.684-7.692 (d, 4H, aromatic protons), 7.831-7.840 (d, 4H, aromatic proton), 8.302-8.309 (d, 4H, aromatic protons), 8.646 (s, 2H, -CH- proton). LC-MS: (M^+) peak at m/z 513.74, Analysis Calculated for $C_{37}H_{27}N_3$; C (86.52/86.64), H (5.30/5.38), N (8.18/8.26).

4,4'-(4-Phenyl Pyridine-2,6-diyl) bis (N-4-Cyano Benzylidene Aniline) (6b): mp (°C): 220-222. IR (KBr) cm^{-1}: 3110 (CH=CH, Aromatic), 3064 (CH=N, Olefinic), 2251 (C≡N, Cyano), 1682 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.418-7.521 (m, 5H, aromatic protons), 7.545-7.549 (d, 4H, aromatic protons), 7.680-7.688 (d, 4H, aromatic protons), 8.015-8.023 (d, 4H, aromatic proton), 8.208 (s, 2H, aromatic protons), 8.316-8.324 (d, 4H, aromatic proton), 8.642 (s, 2H, -CH- proton). LC-MS: Molecular ion (M^+) peak at m/z 563.52; Analysis Calculated for $C_{39}H_{25}N_5$; C (83.10/83.22), H (4.47/4.56), N (12.43/12.54).

4,4'-(4-Phenyl Pyridine-2,6-diyl) bis (N-4-Chloro Benzylidene Aniline) (6c): mp (°C): 244-246 IR (KBr) cm^{-1}: 3108 (CH=CH, Aromatic), 3046 (CH=N, olefinic), 1674 (C=N, Pyridine), 1262 (-C-N-), 682 (-C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.416-7.526 (m, 9H, aromatic protons), 7.683-7.688 (d, 4H, aromatic protons), 7.773-7.782 (d, 4H, aromatic proton), 8.208 (s, 2H, aromatic protons), 8.304-8.313 (d, 4H, aromatic proton), 8.642 (s, 2H, -CH- proton). LC-MS: Molecular ion (M^+) & (M+2) peak at m/z 582.50 & 584.61. Analysis Calculated for $C_{37}H_{25}N_3Cl_2$; C (76.29/76.38), H (4.33/4.46), N (7.21/7.30).

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Table 1: Physical Data of intermediates (4a-c & 5a-c) and 4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl)bis(N-Benzylidene Aniline) derivatives 6 (a-o).

S.No.	Comp	Substituent		Mol. Formula	Mol. wt.	MP (°C)	Yield (%)	R_f
		R	R^1					
1	4a	H	NA	$C_{23}H_{15}N_3O_4$	397.38	182-184	62	0.33
2	4b	4-CN	NA	$C_{24}H_{14}N_4O_4$	422.39	196-198	58	0.42
3	4c	4-Cl	NA	$C_{23}H_{14}ClN_3O_4$	431.83	200-202	66	0.41
4	5a	H	NA	$C_{23}H_{19}N_3$	337.42	172-174	60	0.36
5	5b	4-CN	NA	$C_{24}H_{18}N_4$	362.43	184-186	64	0.38
6	5c	4-Cl	NA	$C_{23}H_{18}ClN_3$	371.87	190-192	59	0.44
7	6a	H	H	$C_{37}H_{27}N_3$	513.63	210-212	78	0.41
8	6b	H	4-CN	$C_{39}H_{25}N_5$	563.65	220-222	72	0.38
9	6c	H	4-Cl	$C_{37}H_{25}N_3Cl_2$	582.52	244-246	77	0.32
10	6d	H	2,3-OH	$C_{37}H_{27}N_3O_4$	577.63	216-218	64	0.29
11	6e	H	2,4-OH	$C_{37}H_{27}N_3O_4$	577.63	222-224	68	0.36
12	6f	4-CN	H	$C_{38}H_{26}N_4$	538.64	230-232	74	0.33
13	6g	4-CN	4-CN	$C_{40}H_{24}N_6$	588.66	234-236	62	0.3
14	6h	4-CN	4-Cl	$C_{38}H_{24}N_4Cl_2$	607.53	254-256	65	0.37
15	6i	4-CN	2,3-OH	$C_{38}H_{26}N_4O_4$	602.64	226-228	68	0.31
16	6j	4-CN	2,4-OH	$C_{38}H_{26}N_4O_4$	602.64	238-240	62	0.38
17	6k	4-Cl	H	$C_{37}H_{26}N_3Cl$	548.08	248-250	64	0.34
18	6l	4-Cl	4-CN	$C_{39}H_{24}N_5Cl$	598.09	256-258	58	0.36
19	6m	4-Cl	4-Cl	$C_{37}H_{24}N_3Cl_3$	616.97	262-264	53	0.33
20	6n	4-Cl	2,3-OH	$C_{37}H_{26}N_3O_4Cl$	612.07	258-260	60	0.32
21	6o	4-Cl	2,4-OH	$C_{37}H_{26}N_3O_4Cl$	612.07	252-254	62	0.43

4,4'-(4-Phenyl Pyridine-2,6-diyl) bis (N-2,3-dihydroxy Benzylidene Aniline) (6d): mp (°C): 216-218. IR (KBr) cm^{-1}: 3378 (-OH, Phenolic), 3102 (CH=CH, Aromatic), 3028 (CH=N, olefinic), 1683 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 5.361-5.366 (d, 4H, hydroxyl protons), 6.823-6.846 (d, 4H, aromatic protons), 7.221-7.230 (d, 2H, aromatic protons), 7.426-7.430 (d, 1H, aromatic proton), 7.536-7.545 (m, 4H, aromatic protons), 7.676-7.682 (d, 4H, aromatic protons), 8.204-8.315 (m, 6H, aromatic protons), 8.768 (s, 2H, -CH-proton). LC-MS: Molecular ion (M^+) peak at m/z 577.48; Analysis Calculated for $C_{37}H_{27}N_3O_4$; C (76.93/76.85), H(4.71/4.80), N(7.27/7.39).

4,4'-(4-Phenyl Pyridine-2,6-diyl) bis (N-2,4-dihydroxy Benzylidene Aniline) (6e): mp (°C): 222-224. IR (KBr) cm^{-1}: 3312 (-OH, Phenolic), 3114 (CH=CH, Aromatic), 3016 (CH=N, olefinic), 1671 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 5.352-5.358 (d, 4H, hydroxyl protons), 6.415-6.418 (d, 2H, aromatic protons), 7.411-7.417 (d, 1H, aromatic proton), 7.517-7.529 (m, 6H, aromatic protons), 7.656-7.682 (m, 6H, aromatic proton), 8.212-8.315 (m, 6H, aromatic protons), 8.643 (s, 2H, -CH-proton). LC-MS: Molecular ion (M^+) peak at m/z 577.60; Analysis Calculated for $C_{37}H_{27}N_3O_4$; C (76.93/76.87), H (4.71/4.83), N (7.27/7.36).

4-(2,6-bis (4-Benzylidene Amino) Phenyl) Pyridine-4-yl) Benzonitrile (6f): mp (°C): 230-232. IR (KBr) cm^{-1}: 3085

(CH=CH, Aromatic), 3045 (CH=N, olefinic), 2247 (C≡N, Cyano), 1652 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.482-7.552 (m, 4H, aromatic protons), 7.561-7.678 (m, 8H, aromatic protons), 7.801-7.818 (d, 2H, aromatic protons), 7.880-7.930 (m, 6H, aromatic protons), 8.310-8.320 (d, 2H, aromatic protons), 8.552 (s, 2H, aromatic protons), 8.874 (s, 2H, -CH- proton). LC-MS: Molecular ion (M^+) peak at m/z 538.61; Analysis Calculated for $C_{38}H_{26}N_4$; C (84.73/84.78), H(4.87/4.95), N(10.40/10.52).

4-(2,6-bis (4-Cyano Benzylidene Amino) Phenyl) Pyridine-4-yl) Benzonitrile (6g): mp (°C): 234-236. IR (KBr) cm^{-1}: 3123 (CH=CH, Aromatic), 3014 (CH=N, olefinic), 2251 (C≡N, Cyano), 1669 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.541-7.548 (d, 4H, aromatic protons), 7.686-7.694 (d, 4H, aromatic protons), 7.822-7.841 (m, 4H, aromatic protons), 8.011-8.023 (d, 4H, aromatic proton), 8.208-8.310 (m, 6H, aromatic proton), 8.642 (s, 2H, -CH- proton). LC-MS: Molecular ion (M^+) peak at m/z 588.62; Analysis Calculated for $C_{40}H_{24}N_6$; C (81.61/81.70), H (4.11/4.18), N (14.28/14.36).

4-(2,6-bis (4-Chloro Benzylidene Amino) Phenyl) Pyridine-4-yl) Benzonitrile (6h): mp (°C): 254-256. IR (KBr) cm^{-1}: 3120 (CH=CH, Aromatic), 3065 (CH=N, olefinic), 2232 (C≡N, Cyano), 1645 (C=N, Pyridine), 653 (C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.523-7.526 (d, 4H, aromatic protons), 7.681-7.686 (d, 4H, aromatic protons), 7.782-7.788 (d, 4H, aromatic proton), 7.828-7.843 (m, 4H, aromatic protons), 8.211 (s, 2H, aromatic protons), 8.312-8.318 (d, 4H, aromatic proton), 8.654 (s, 2H, -CH- protons). LC-MS: Molecular ion (M^+), (M+2) & (M+4) peak at m/z 607.45, 609.50 & 611.24. Analysis Calculated for $C_{38}H_{24}N_4Cl_2$; C (75.12/75.24), H (3.98/3.86), N (9.22/9.36).

4-(2,6-bis (2,3-dihydroxy Benzylidene Amino) Phenyl) Pyridine-4-yl) Benzonitrile (6i): mp (°C): 226-228. IR (KBr) cm^{-1}: 3352 (O-H, Phenolic), 3078 (CH=CH, Aromatic), 3039 (CH=N, olefinic), 2238 (C≡N, Cyano), 1653 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 5.361-5.366 (d, 4H, hydroxyl protons), 6.823-6.846 (d, 4H, aromatic protons), 7.221-7.230 (d, 2H, aromatic protons), 7.692-7.698 (d, 4H, aromatic protons), 7.836-7.847 (m, 4H, aromatic protons), 8.218-8.326 (m, 6H, aromatic protons), 8.882 (s, 2H, -CH-proton). LC-MS: Molecular ion (M^+) peak at m/z 602.48; Analysis Calculated for $C_{38}H_{26}N_4O_4$; C (75.73/75.85), H (4.35/4.47), N (9.30/9.41).

4-(2,6-bis (2,4-dihydroxy Benzylidene Amino) Phenyl) Pyridine-4-yl) Benzonitrile (6j): mp (°C): 238-240. IR (KBr) cm^{-1}: 3346 (O-H, Phenolic), 3143 (CH=CH, Aromatic), 3052 (CH=N, olefinic), 2238 (C≡N, Cyano), 1679 (C=N, Pyridine). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 5.364-5.369 (d, 4H, hydroxyl protons), 6.424-6.427 (d, 2H, aromatic protons), 7.523-7.528 (d, 2H, aromatic protons), 7.662-7.680 (m, 6H, aromatic protons), 7.823-7.842 (m, 4H, aromatic protons), 8.220-8.305 (m, 6H, aromatic protons), 8.864 (s, 2H, -CH-proton). LC-MS: Molecular ion (M^+) peak at m/z 602.53; Analysis Calculated for $C_{38}H_{26}N_4O_4$; C (75.73/75.83), H (4.35/4.44), N (9.30/9.39).

4,4'-(4-(4-Chloro Phenyl) Pyridine-2,6-diyl) bis(N-Benzylidene Aniline) (6k): mp (°C): 248-250. IR (KBr) cm^{-1}: 3126 (CH=CH, Aromatic), 3053 (CH=N, olefinic), 1653 (C=N, Pyridine), 1253 (-C-N-), 623 (-C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.425-7.484 (m, 6H, Aromatic protons), 7.654-7.689 (d, 2H, Aromatic protons), 7.743-7.779 (d, 4H, Aromatic protons), 7.761-7.895 (d, 4H, Aromatic protons), 8.023-8.123 (m, 8H, Aromatic protons), 8.577 (s, 2H, -CH- proton). LC-MS: Molecular ion (M^+) & (M+2) peak at m/z 548.16 & 550.04. Analysis Calculated for $C_{37}H_{26}ClN_3$; C (81.08/81.17), H (4.78/4.86), N (7.67/7.74).

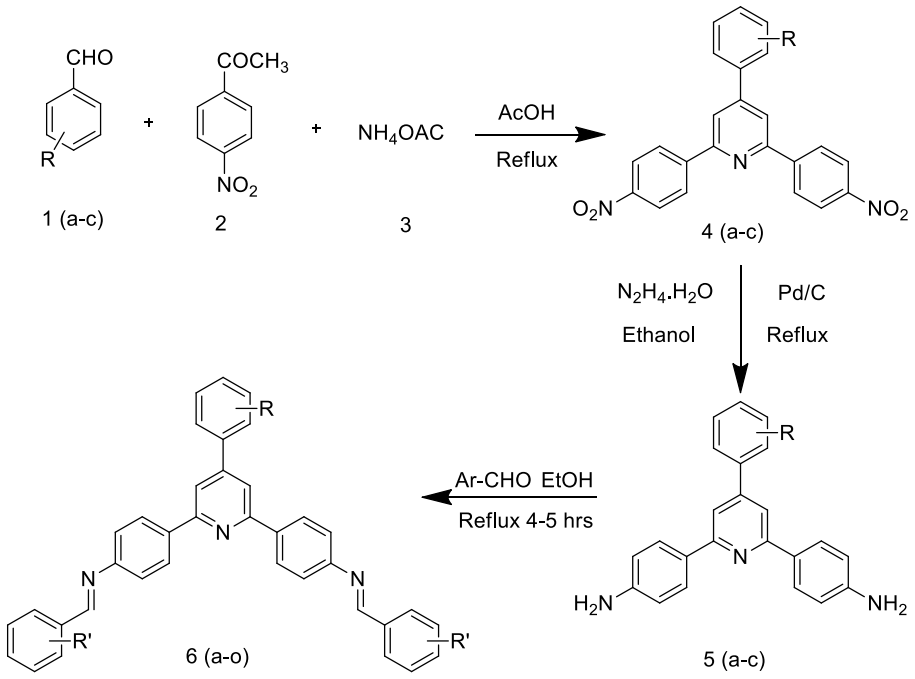
4,4'-(4-(4-Chloro Phenyl Pyridine-2,6-diyl) bis (N-4-Cyano Benzylidene Aniline) (6l): mp (°C): 256-258. IR (KBr) cm^{-1}: 3118 (CH=CH, Aromatic), 3029 (CH=N, olefinic), 2243 (C≡N, Cyano), 1652 (C=N, Pyridine), 653 (-C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.544-7.568 (m, 6H, aromatic protons), 7.684-7.697 (d, 4H, aromatic protons), 8.011-8.064 (d, 4H, aromatic protons), 8.118-8.304 (m, 8H, aromatic protons), 8.638 (s, 2H, -CH-protons). LC-MS: Molecular ion (M^+) & (M+2) peak at m/z 598.12 & 600.11. Analysis Calculated for $C_{39}H_{24}ClN_5$; C (78.32/78.45), H (4.04/4.12), N (11.71/11.84).

4,4'-(4-(4-Chloro Phenyl Pyridine-2,6-diyl) bis (N-4-Chloro Benzylidene Aniline) (6m): mp (°C): 262-264. IR (KBr) cm^{-1}: 3149 (CH=CH, Aromatic), 3078 (CH=N, olefinic), 1659 (C=N, Pyridine), 1323 (-C-N-), 745 (-C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 7.532-7.556 (m, 6H, aromatic protons), 7.675-7.680 (d, 4H, aromatic protons), 7.778-7.786 (d, 4H, aromatic protons), 8.123-8.304 (m, 8H, aromatic protons), 8.643 (s, 2H, -CH-protons). LC-MS: Molecular ion (M+2), (M+4) & (M+6) peaks at m/z 618.92, 620.79 & 622.68. Analysis Calculated for $C_{37}H_{24}Cl_3N_3$; C (72.03/72.11), H (3.92/3.84), N (6.81/6.95).

4,4'-(4-(4-Chloro Phenyl Pyridine-2,6-diyl) bis (N-2,3-dihydroxy Benzylidene Aniline) (6n): mp (°C): 258-260. IR (KBr) cm^{-1}: 3452 (O-H, Phenolic), 3075 (CH=CH, Aromatic), 3031 (CH=N, olefinic), 1672 (C=N, Pyridine), 722 (-C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 5.353-5.358 (d, 4H, hydroxyl protons), 6.838-6.847 (d, 4H, aromatic protons), 7.221-7.227 (d, 2H, aromatic protons), 7.562-7.565 (d, 2H, aromatic protons), 7.682-7.687 (d, 4H, aromatic protons), 8.215-8.364 (m, 8H, aromatic protons), 8.876 (s, 2H, -CH-proton). LC-MS: Molecular ion (M^+) & (M+2) peak at m/z 612.09 & 614.13. Analysis calculated for $C_{37}H_{26}ClN_3O_4$; C (72.61/72.73), H (4.28/4.37), N (6.87/6.94).

4,4'-(4-Chloro Phenyl Pyridine-2,6-diyl) bis (N-2,4-dihydroxy Benzylidene Aniline) (6o): mp (°C): 252-254. IR (KBr) cm^{-1}: 3439 (O-H, Phenolic), 3115 (CH=CH, Aromatic), 3085 (CH=N, olefinic), 1682 (C=N, Pyridine), 743 (-C-Cl). ^{1}HNMR (400MHz, $CDCl_3$) [δ, ppm]: 5.363-5.368 (d, 4H, hydroxyl protons), 6.433-6.446 (d, 2H, aromatic protons), 7.537-7.552 (m, 4H, aromatic protons), 7.662-7.680 (m, 6H, aromatic protons), 8.112-8.304 (m, 8H, aromatic protons), 8.867 (s, 2H, -CH-proton). LC-MS: Molecular ion (M^+) & (M+2) peak at m/z 612.13 & 614.07. Analysis Calculated for $C_{37}H_{26}ClN_3O_4$; C (72.61/72.71), H (4.28/4.35), N (6.87/6.96).

Scheme



R= H, 4-CN, 4-Cl; R'= H, 4-CN, 4-Cl, 2,3-OH, 2,4-OH

Antimicrobial activity [17]

Antibacterial studies

The antibacterial activity of 4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) derivatives (6a-o) was screened against four different strains of bacteria (Two Gram-Positive Bacteria: *Bacillus subtilis* and *Streptococcus Pneumonia* and two Gram-Negative Bacteria: *Escherichia coli* and *Proteus vulgaris*) by agar diffusion method, the zone of inhibition in mm were showed in Table 2.

Antifungal studies

The antifungal activity of 4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) derivatives (6a-o) was screened against two strains of fungi such as Saccharomyces cerevisiae and Candida albicans by agar diffusion method and the zone of inhibition in mm were showed in Table 2.

Table 2: Antimicrobial activity of 4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) derivatives (6a-o).

Compound	Zone of Inhibition (at µg/ml: mm)																	
	B. subtilis			*S. pneumonia*			*E. coli*			*P. Vulgaris*			*S. cerevisiae*			*C. albicans*		
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
6a	09	11	13	10	12	13	10	12	14	11	13	14	08	11	13	07	10	12
6b	10	12	14	11	13	14	10	13	15	10	12	14	11	13	15	10	12	15
6c	12	14	16	14	16	18	13	15	18	12	14	16	13	15	18	14	17	19
6d	09	11	12	08	10	12	07	09	12	07	09	10	10	12	14	11	13	15
6e	07	09	11	06	08	10	07	10	11	06	08	10	09	11	13	10	12	15
6f	06	09	13	08	11	13	08	10	13	10	12	14	13	15	17	14	16	18
6g	11	13	16	12	14	16	13	15	17	11	13	15	15	18	20	16	19	22
6h	15	17	20	16	18	20	14	17	20	16	18	20	18	20	22	20	22	24
6i	07	09	11	06	08	11	07	10	12	05	08	11	15	17	19	14	16	18
6j	05	07	10	07	09	11	06	08	11	08	10	12	13	15	18	12	15	18
6k	11	13	15	10	12	14	12	14	16	10	12	15	05	07	09	04	06	08
6l	13	15	17	14	17	19	13	16	19	12	15	17	08	10	12	07	09	11
6m	17	20	22	16	18	21	18	20	22	15	18	21	11	14	16	10	13	15
6n	08	10	12	06	08	10	07	09	12	07	10	12	05	08	10	07	09	11
6o	07	09	12	08	10	11	08	10	12	08	11	13	05	07	09	07	09	11
AMP	23	25	27	21	23	26	22	25	28	20	23	26	-	-	-	-	-	-
KTZ	-	-	-	-	-	-	-	-	-	-	-	-	21	23	26	24	26	28

AMP: Ampicillin, KTZ: Ketoconazole.

RESULTS AND DISCUSSION

Chemistry

Target compounds, 6a-o were synthesized following the reaction sequence outlined in Scheme 1. By the condensation reaction of 4-Substituted-Phenyl-2, 6-bis (4-Amino Phenyl) Pyridine derivatives (5a-c) with Substituted Aromatic Aldehydes dissolved in minimum volume of Ethanol, and then 2-3 drops of Concentrated Sulphuric acid are added to the reaction mixture. The structure of the products, 6a-o was established by Physico-chemical and spectroscopic analysis. The IR spectra of 6a-o showed bands at 3050-3150 cm^{-1} (C-H aromatic), 2220-2210 cm-1 (CN), 1640-1600 cm-1 (C=N) and 1400-540 cm^{-1} (C-Cl). The ^{1}HNMR spectra of these compounds gave further support for the Substituted Phenyl Pyridine structure, since they showed a doublet at δ 5.201-5.605 ppm attributed to the Hydroxy Protons and multiplet observed at δ 6.312-7.813 ppm attributed to Aromatic Phenolic Substituted Pyridine nucleus and also the multiplet peak observed at δ 8.211-8.347 ppm Pyridinyl Aromatic proton, a singlet observed at δ 8.612 ppm of (-CH-) methylene proton. Other aromatic proton signals were appeared at δ 6.0-8.0 ppm. The mass spectra showed the corresponding molecular ion peak $[M]^{+}$ and $[M]^{2+}$ as a compound and their isotopes and the fragmentation patterns was characteristic of respective Pyridines.

Antimicrobial activity

All fifteen derivatives (6a–o) were evaluated for their *in vitro* antimicrobial activity, the *in vitro* antimicrobial activity of all the synthesized compounds was carried out by agar diffusion method. The results were showed in Table 2. From the results, the data reveals that amongst all the synthesized compounds (6a-o), compounds 6m and 6h were exhibited good activity against Gram positive bacteria (*Bacillus subtilis & Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli & Proteus vulgaris*) when compared to standard antibiotic ampicillin, which was statistically significant. Antifungal study revealed that compounds 6m, 6h, 6l, 6c, 6g, 6k and 6b shows more potent as compared to standard fungicidal Ketoconazole against *Saccharomyces cerevisiae & Candida albicans*.

CONCLUSION

4,4'-(4-Substituted Phenyl Pyridine-2,6-diyl) bis (N-Substituted Benzylidene Aniline) derivatives (6a-o) were successfully synthesized by an efficient

condensation reaction of 4-Substituted Phenyl-2, 6-bis (4-Amino Phenyl) Pyridine derivatives (5a-c), Substituted Aromatic Aldehydes. The synthesized compounds were confirmed by spectral analysis. Further, the synthesized compounds were subjected to antimicrobial activity. From the results, the compound 6m exhibited significant Antimicrobial activity with reference to standard drugs.

Acknowledgements

One of the author very thankful to The Director "Synapse Life Sciences", Warangal and the Management and Director, Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal for providing necessary facilities to carry out research work.

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