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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF NOVEL PYRIDINE DERIVATIVES

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ABSTRACT

A novel series of Imidazo[1,2-a]pyridine derivatives linked to secondary amines were synthesized from 2-(4-Fluorophenyl)-6-methylH- IMIDAZO[1,2-a]PYRIDINE. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Ampicillin and Greseofulvin. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

KEYWORDS: ***Imidazo[1,2-a]pyridine, Secondary amine, Antibacterial activity, Antifungal activity.***

INTRODUCTION

Imidazo[1,2-a] pyridines, a novel class of pharmaceutical compounds exhibit a broad range of biological activities. Besides, imidazo[1,2-a] pyridine scaffold is found in a number of marketed drug formulations, such as zolimidine (an antiulcer drug), zolpidem (ahypnotic drug), and alpidem (a nonsedative anxiolytic). As a result, numerous reports have described the structural modifications of this scaffold with the aim of developing novel therapeutic agents. It has long been known that imidazo [1, 2-a] pyridine derivatives exhibited diverse biological activities like antibacterial,[1,2] Antitumor,[3,4] Antiinflammatory,[5,6] antiviral,[7,8] anti malarial,[9] antifungal,[10, 11] antimicrobial,[12] antiprotozoal[13,14] and antitubercular[15] agents.

Considering the above observations and in connection to previous publications involving the synthesis of new biologically active heterocycles. Thus the efficient synthesis novel series of imidazo[1,2-a]pyridine derivatives linked to secondary amines compounds still represent highly pursued target.

EXPERIMENTAL

Material and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-

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MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

Synthesis of 2-(4-Fluorophenyl)-6-methyl*H*-imidazo[1,2-*a*]pyridine: A solution of 5-methylpyridin-2-amine (1.08 g, 0.01 mol) in methanol (10 ml) was added to 2-chloro-1-(4- fluorophenyl) ethanone (1.72 g, 0.01 mol) and the reaction mixture was refluxed with stirring for 6 hour in the presence of catalytic amount of triethylamine. After the completion of reaction, cool the content, the solid separated was filtered and dried *in vacuo*. Yield 68%,
M.P 192^{0}C, Anal. Calcd. For $C_{14}H_{11}FN_2$: Require: C, 74.32, H, 4.90, N, 12.38 %; Found: C, 74.30, H, 4.89, N, 12.35%. MS: *m/z* = 226.

General procedure for the preparation of 2-(4-Fluorophenyl)-6-methyl-3-(*N,N*- dialkylamine-4-ylmethyl)imidazo[1,2-*a*]pyridines (IP-1 TO IP-10): To a solution of 2-(4- fluorophenyl)-6-methyl*H*-imidazo[1,2-*a*]pyridine (2.26 g, 0.01 mol), formaldehyde (0.3 g,
0.01 mol) and different secondary amine (0.01 mol) in methanol (20 ml) was added and the reaction mixture was refluxed with stirring in the presence of 1-2 drop concentrated HCl. After completion of reaction, cool the reaction mass and add ice cold water and extracted with ethyl acetate. The organic layer was washed with water (2 × 10 ml) and dried with Na_2SO_4, solvent was removed *in vacuo* and the resulting crude product was pure by column chromatography to give the analytical pure compound. The physical constants of the product are recorded in Table-1.

2-(4-Fluorophenyl)-6-methyl-3-(morpholin-4-ylmethyl)imidazo[1,2-*a*]pyridine(IP-1) Purity by HPLC: 98 %; IR (KBr): 3114 (Ar, C-H str), 3009 (C-H str), 2956 (C-H str), 1657 (C=N str), 1576 (Ar, C=C str), 1460 (Ar, C=C str), 1345 (C-H str), 1214 (C-N str), 1158 (C- F), 841 (C-H, o.p. ban) cm^{-1}; ^{1}H NMR (400 MHz, CDCl3): δ ppm 2.29 (s, 3H, CH_3), 2.38-
2.40 (t, *J*=4.44 Hz, 4H, 2CH2), 3.58-3.61 (t, *J*=4.52 Hz, 4H, 2CH2), 3.81 (s, 2H, CH_2), 6.98-
7.01 (m, 1H, ArH), 7.03-7.08 (m, 2H, ArH), 7.45-7.47 (d, *J*=9.12 Hz, 1H, ArH), 7.68-7.72
(m, 2H, ArH), 8.06 (s, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 18.55, 51.94, 53.18, 66.97, 115.29, 115.50, 116.55, 121.68, 122.66, 127.93, 130.43, 130.51, 130.66, 130.69,
144.08, 144.18, 161.29, 163.74; MS: *m/z* = 326 $[M+1]^+$; Anal. Calcd for $C_{19}H_{20}FN_3O$: C,
70.13; H, 6.20; N, 12.91. Found: C, 69.83; H, 6.13; N, 12.76%.

***N*-Ethyl-*N*-{[2-(4-fluorophenyl)-6-methylimidazo[1,2-*a*]pyridin-3-yl]methyl}ethanamine (IP-2):** Purity by HPLC: 99 %; IR (KBr): 3072, 2980, 2862, 1603, 1554, 1434, 1456, 1119, 1035, 820 cm^{-1}; ^{1}H NMR (400 MHz, CDCl3): δ ppm 0.99-1.03 (t, *J*=7.08 Hz, 6H, CH_3), 2.29 (s, 3H, CH_3), 2.46-2.52 (q, *J*=7.2 Hz, 4H, CH_2), 3.98 (s, 2H, CH_2), 6.99-7.02 (m, 1H, ArH),
7.06-7.09 (m, 2H, ArH), 7.52-7.54 (d, *J*=10.56 Hz, 1H, ArH), 7.74-7.78 (m, 2H, ArH), 8.10 (s, 1H, ArH). ^{13}C NMR (100 MHz, CDCl3): δ ppm 13.11, 21.68, 50.93, 52.18, 115.50,
115.80, 116.75, 122.20, 123.30, 128.50, 130.80, 130.99, 131.06, 131.10, 144.48, 144.60,
161.80, 164.12; MS: *m/z* = 311 $[M]^+$; Anal. Calcd for $C_{19}H_{22}FN_3$: C, 73.28; H, 7.12; N,
13.49. Found: C, 72.53; H, 7.03; N, 13.33%.

2-(4-Fluorophenyl)-6-methyl-3-[(4-phenylpiperazin-1-yl)methyl]imidazo[1,2-*a*]pyridine (IP-3): IR (KBr): 3080, 2968, 2835, 1599, 1574, 1481, 1463, 1138, 1043, 850 cm^{-1}; MS: *m/z* = 400 $[M]^+$; Anal. Calcd for $C_{25}H_{25}FN_4$: C, 74.97; H, 6.29; N, 13.99. Found: C, 74.21; H, 6.07; N, 13.75%.

2-(4-Fluorophenyl)-6-methyl-3-(piperidin-1-ylmethyl)imidazo[1,2-*a*]pyridine (IP-4): IR (KBr): 3076, 2961, 2863, 1645, 1542, 1453, 1378, 1143, 1037, 832cm^{-1}; MS: *m/z* = 325
$[M+2]^+$; Anal. Calcd for $C_{20}H_{22}FN_3$: C, 74.28; H, 6.86; N, 12.99. Found: C, 74.05; H, 6.77;
N, 12.71%.

***N*-{[2-(4-Fluorophenyl)-6-methylimidazo[1,2-*a*]pyridin-3-yl]methyl}-*N*-isopropyl propan-2-amine (IP-5):** IR (KBr): 3084, 2967, 2857, 1605, 1559, 1462, 1376, 1134, 1054, 845 cm^{-1}; MS: *m/z* = 339 $[M]^+$; Anal. Calcd for $C_{21}H_{26}FN_3$: C, 74.30; H, 7.72; N, 12.38. Found: C, 73.17; H, 7.59; N, 12.26%.

2-(4-Fluorophenyl)-6-methyl-3-[(4-ethylpiperazin-1-yl)methyl]imidazo[1,2-*a*]pyridine (IP-6): IR (KBr): 3078, 2959, 2886, 1612, 1551, 1487, 1389, 1144, 1046, 848 cm^{-1}; MS: *m/z* = 353 $[M+1]^+$; Anal. Calcd for $C_{21}H_{25}FN_4$: C, 71.56; H, 7.15; N, 15.90. Found: C, 71.34; H, 7.01; N, 15.72%.

2-(4-Fluorophenyl)-6-methyl-3-[(4-methylpiperazin-1-yl)methyl]imidazo[1,2-*a*]pyridine (IP-7): IR (KBr): 3097, 2956, 2872, 1611, 1534, 1456, 1451, 1129, 1034, 865 cm^{-1}; MS: *m/z* = 339 $[M+1]^+$; Anal. Calcd for $C_{20}H_{23}FN_4$: C, 70.98; H, 6.85; N, 16.56. Found: C, 70.83; H, 6.74; N, 16.47%.

2-(4-Fluorophenyl)-6-methyl-3-(pyrrolidin-1-ylmethyl)imidazo[1,2-*a*]pyridine (IP-8): IR (KBr): 3082, 2954, 2868, 1614, 1559, 1446, 1471, 1139, 1043, 870cm^{-1}; MS: *m/z* = 309 $[M]^+$;
Anal. Calcd for $C_{19}H_{20}FN_3$: C, 73.76; H, 6.52; N, 13.58. Found: C, 72.32; H, 6.29; N,
13.45%.

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2-(4-Fluorophenyl)-6-methyl-3-[(2-methylpiperidin-1-yl)methyl]imidazo[1,2-*a*]pyridine (IP-9): IR (KBr): 3046, 2964, 2834, 1621, 1558, 1448, 1356, 1126, 1048, 837 cm^{-1}; MS: *m/z* = 337 $[M]^+$; Anal. Calcd for $C_{21}H_{24}FN_3$: C, 74.75; H, 7.17; N, 12.45. Found: C, 74.37; H, 7.05; N, 12.36%.

2-(4-Fluorophenyl)-6-methyl-3-[(4-methylpiperidin-1-yl)methyl]imidazo[1,2-*a*]pyridine (IP-10): IR (KBr): 3089, 2956, 2885, 1625, 1545, 1446, 1426, 1146, 1023, 842 cm^{-1}; MS: *m/z* = 337 $[M]^+$; Anal. Calcd for $C_{21}H_{24}FN_3$: C, 74.75; H, 7.17; N, 12.45. Found: C, 74.36; H, 7.09; N, 12.31%.

Scheme 1: Synthesis of novel series of imidazo[1,2-a]pyridine derivatives

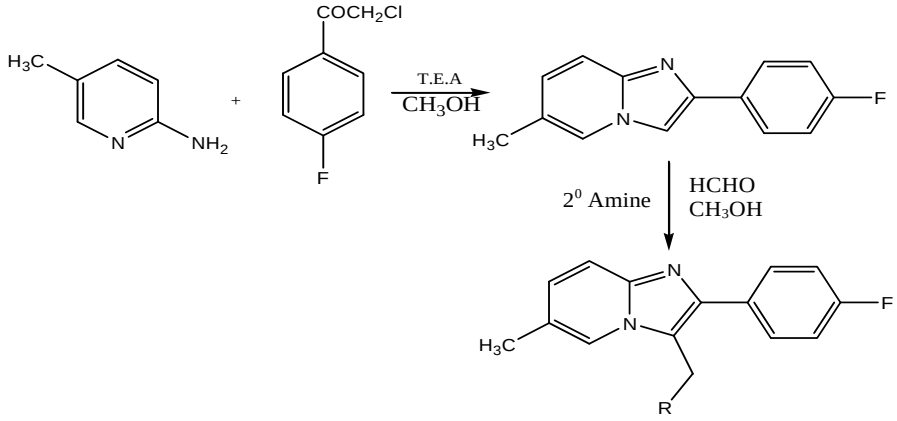

Table-1: Physical constant of synthesized novel series of imidazo[1,2-a]pyridine derivatives linked to secondary amines

Compound	Substitution (Ar)	M.F	M.W	M.P (^{0}C)
IP-1	Morpholine	$C_{19}H_{20}FN_3O$	325.28	183-185
IP-2	Diethylamine	$C_{19}H_{22}FN_3$	311.39	147-148
IP-3	1-Phenylpiperazine	$C_{25}H_{25}FN_4$	400.49	192-194
IP-4	Piperidine	$C_{20}H_{22}FN_3$	323.40	198-199
IP-5	1-Methylpiperazine	$C_{21}H_{26}FN_3$	339.44	124-126
IP-6	1-Ethylpiperazine	$C_{21}H_{25}FN_4$	352.44	223-224
IP-7	Diisopropylamine	$C_{20}H_{23}FN_4$	338.42	173-175
IP-8	Pyrrolidine	$C_{19}H_{20}FN_3$	309.38	144-145
IP-9	2-Methylpiperidine	$C_{21}H_{24}FN_3$	337.43	153-155
IP-10	4-Methylpiperidine	$C_{21}H_{24}FN_3$:	337.43	134-136

BIOLOGICAL EVALUATION

Preparation of Culture Media

Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 13gm of dehydrated powder (HI-media) in 100ml of distilled water. Saubouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture

Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^{0}C.

Standardization of Stock Culture

Stock cultures were placed in the incubator (37^{0}C for bacteria and 24^{0}C for fungi) and shaken well. One ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10^{-1} to 10^{-10}. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15×10^8 cfu/ml.

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Preparation of Working Stock Culture

Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 10^5cfu/ml. This was then used for further *in vitro* screening.

Preparation of Drug Dilutions

Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Antimicrobial Screening

Synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger*(MTCC 282).

Determination of MIC

The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 25^0C respectively for 24 hours for bacteriae and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC).The MIC values of the test compounds are recorded in Table-2.

Table-2: Antimicrobial activity of novel series of imidazo[1,2-a]pyridine derivatives linked to secondary amines

Compound	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*
IP-1	100	100	250	250	200	1000
IP-2	100	200	100	500	250	500
IP-3	250	250	250	100	500	1000
IP-4	200	250	125	500	1000	250
IP-5	250	200	500	500	500	1000
IP-6	100	62.5	100	200	500	200
IP-7	200	500	62.5	250	250	500
IP-8	500	250	500	200	200	200
IP-9	200	500	200	100	500	200
IP-10	500	200	250	250	500	250
Ampicillin	250	100	100	100	NT	NT
Greseofulvin	NT	NT	NT	NT	500	100

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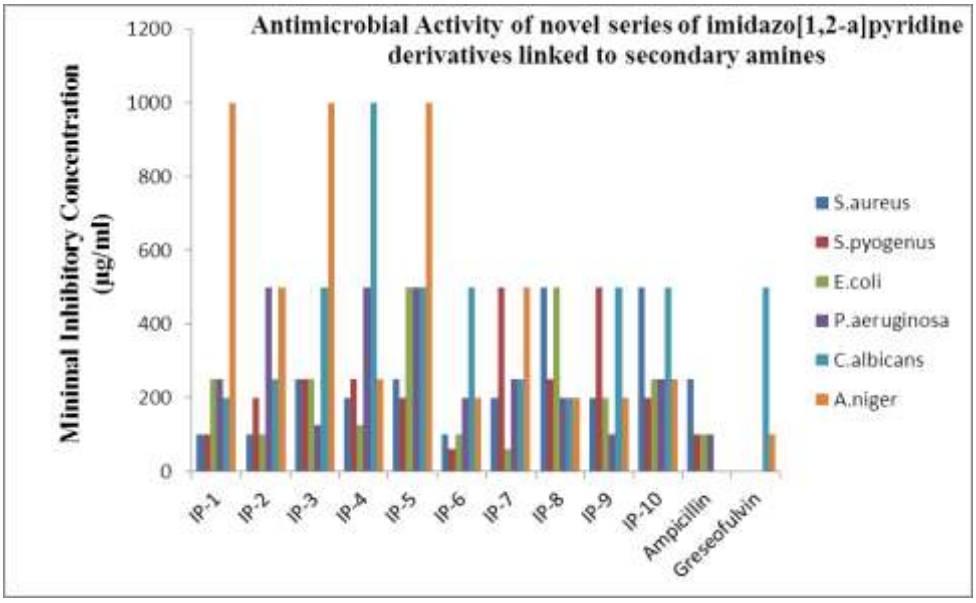

Fig 1: Antimicrobial activity of novel series of imidazo[1,2-a]pyridine derivatives linked to secondary amines

RESULTS AND DISCUSSION

A solution of 5-methylpyridin-2-amine to 2-chloro-1-(4-fluorophenyl)ethanone in presence of methanol and refluxed with stirring for 6 hour in the presence of catalytic amount of triethylamine gives 2-(4-Fluorophenyl)-6-methyl*H*-imidazo[1,2-*a*]pyridine. The solution of 2-(4-Fluorophenyl)-6-methyl*H*-imidazo[1,2-*a*]pyridine and formaldehyde with different secondary amine in methanol and refluxed in the presence of concentrated HCl, extracted with ethyl acetate forms the titled compounds.

The data recorded in Table 2 indicated that compounds IP-1, IP-2 and IP-6 are more potent towards the *Staphylococcus aureus*. Compounds IP-4, IP-7 and IP-9 are moderately potent towards the *Staphylococcus aureus*. Compound IP-6 is more potent towards the *Streptococcus pyogenes*. Compounds IP-1 is moderately potent towards the *Streptococcus pyogenes*. Compound IP-7 is more potent towards the *Escherichia coli*. Compounds IP-2 and IP-6 are moderately potent towards the *Escherichia coli*. Compounds IP-2 and IP-6 are moderately potent towards the *Pseudomonas aeruginosa*. All these compounds are compared with the standard reference (Ampicillin) for their antibacterial activities. Compounds IP-1, IP-2, IP-7 and IP-8 are more potent towards the *Candida albicans*. Remaining compounds except IP-4 are moderately potent.

CONCLUSION

In this study, novel series of imidazo[1,2-a]pyridine derivatives linked to secondary amines were synthesized and evaluated for their antimicrobial activities. Results revealed that the compounds exhibited significant *in-vitro* activity. Compounds **IP-1, IP-2** and **IP-6** are more potent against all the bacterial strains. Compounds **IP-1** and **IP-8** are more potent against all the fungal strains. Remaining compounds also showed moderate to weak antimicrobial activities. The study would be a fruitful matrix for the development of novel series of imidazo[1,2-a]pyridine derivatives linked to secondary amines for further bio-evaluation.

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