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Synthesis and biological evaluation of some novel Mannich bases

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ABSTRACT

*A series of Mannich bases were prepared by the reaction of 7-methyl-2-(p-methyl phenyl)imidazo[1,2-a]pyridine (**2**) with secondary amines and p-formaldehyde in appropriate solvent. The newly synthesized compounds were characterized by elemental analysis, IR, ^1H NMR and mass spectra. All the synthesized compounds were tested for their antibacterial activities against Gram positive and Gram negative bacteria, and antifungal activities.*

Key Words: Imidazo[1,2-a]pyridine, mannich base, antimicrobial activity

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INTRODUCTION

Mannich base containing bridged N-atom exhibit diverse pharmacological action like antimicrobial[1], anticancer[2], antimalarial[3] and antiviral[4]. Hence it is pertinent to synthesize some novel mannich bases containing imidazo[1,2-a]pyridine moiety (Scheme-1). Imidazo[1,2-a]pyridines exhibit anti-inflammatory[5], antiulcer[6], antibacterial[7] properties. They have also been shown to be selective cyclin-dependant kinase inhibitors[8], GABA and benzodiazepine receptor agonists[9], and cardiotonic agents[10]. Drug formulation containing Imidazo[1,2-a]pyridine currently available on the market include alpidem (anxiolytic)[11], zolpidem (hypnotic)[12], zolimidine (antiulcer)[13] and olprinone (PDE-3 inhibitor)[14].

MATERIALS AND METHODS

Chemistry

General Procedures. All chemicals and reagents were obtained from Merck or BDH. The melting points are uncorrected and were taken in open capillaries. TLC analysis was carried out on silica gel-G pre-coated aluminum sheet (Merck) and detect under U.V. light. Infrared spectra were determined in KBr on a FT-IR- tensor spectrometer. ^1H NMR spectra were measured in BRUKER-300 MHz spectrometer using TMS as an internal standard and $CDCl_3$ as solvent. The physical characteristics of the synthesized compounds are listed in Table-1.

Preparation of 2-chloro-1-(p-methylphenyl)ethanone(1):
Chloroacetyl chloride(1.13 gm, 0.01m) was added to a solution of toluene(7 ml. 0.07m) and anhydrous $AlCl_3$(3.0 gm, 0.02m) at 0°C. The reaction mixture was stirred at 20°C for about 8 hrs. The product was separated from the reaction mixture by addition of con. HCl. The product was then extracted with ethyl acetate and the solid was wash with n-pentane. The progress of reaction was monitored by TLC.
Yield, 73%; M.P. 98°C.; (C_9H_9ClO; Calculated: C, 64.11; H, 5.38. Found: C, 64.03; H, 5.24).
TLC solvent system : Ethyl acetate : Hexane (2:8)

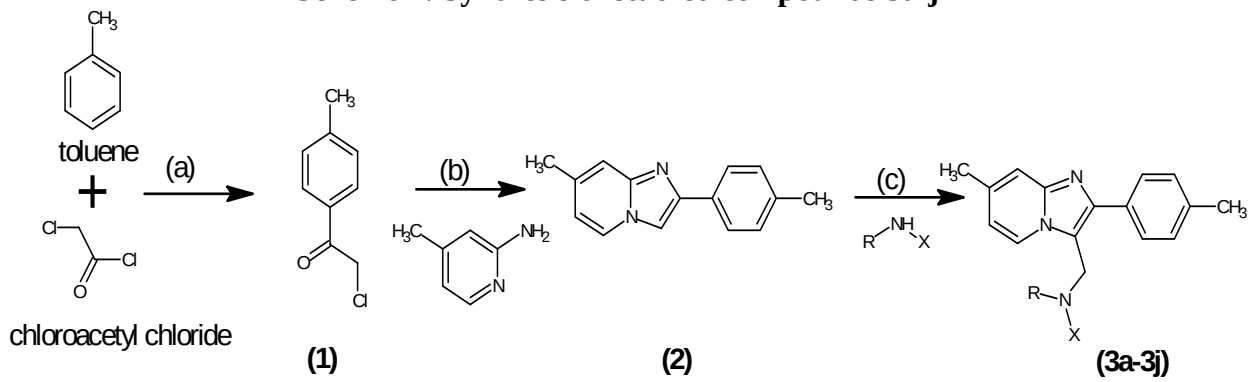
Preparation of 2-(p-methylphenyl)-7-methylimidazo[1,2-*a*]pyridine(2):
A mixture of 2-chloro-1-(p-methylphenyl)ethanone (1.89 gm, 0.01m) and 2-amino-4-methyl pyridine(1.08 gm, 0.01m) in DMF was refluxed at 140°C for about 6 hrs. Finally solid product was isolated and purified by methanol. The progress of reaction was monitored by TLC.
Yield 75%; M.P. 188°C.; ($C_{15}H_{14}N_2$; Calculated: C, 81.05; H, 6.35; N,12.60. Found: C, 80.91; H, 6.26; N, 12.48). TLC solvent system: Ethyl acetate : Hexane (4:6).

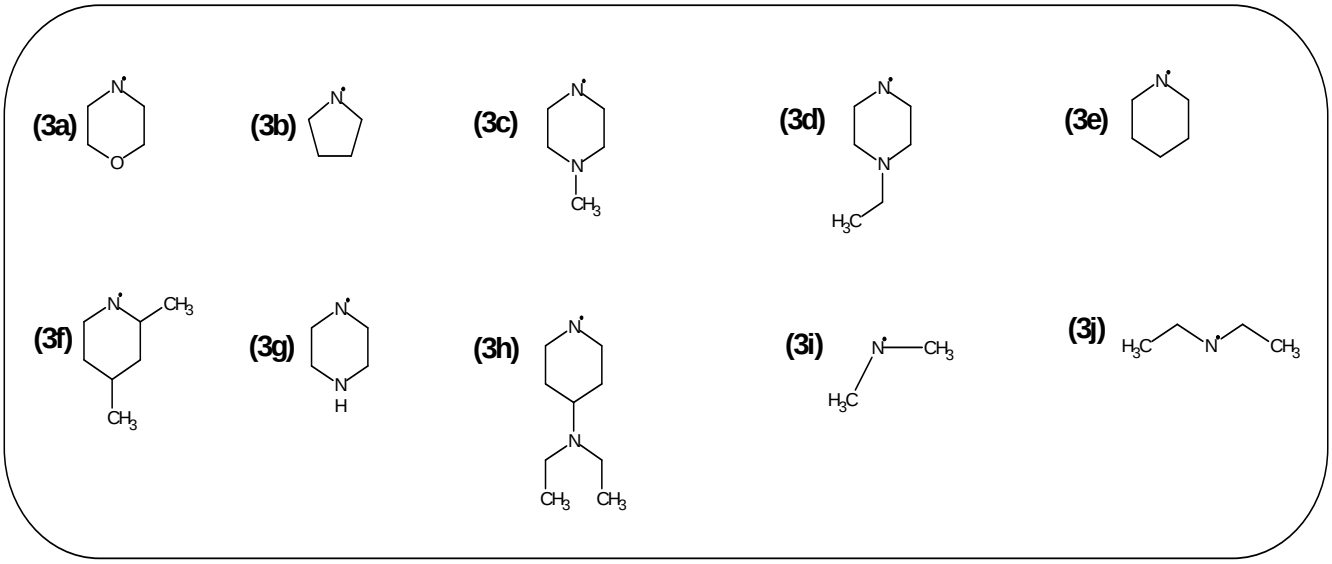
General preparation of *N*-{[7-methyl-2-(p-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]methyl}-*N*,*N*-diaryl/alkylamines (3a-j):
To a solution of 2-(p-methylphenyl)-7-methylimidazo[1,2-*a*]pyridine (1.70g, 0.01 mol), formaldehyde (0.3g, 0.01 mol) and the respective secondary amines (0.01 mol) in methanol (50 ml) was added and reflux for 8 hr. and left it overnight. The content was poured on to crushed ice. The product was isolated, dried and crystallized from hexane. The progress of reaction was monitored by TLC.

Biological evaluation
The newly synthesized compounds were evaluated for their antibacterial and antifungal activity by Broth Dilution Method. The Broth Dilution Method was performed using Muller-Hinton Broth (Hi-Media) medium. Suspension of each microorganism was prepared and applied to plates with serially diluted compounds (DMSO, solvent control) to be tested and incubated (approx. 20 h) at 37^{o}C. The Minimum Bactericidal Concentration (MBC) was considered to be the lowest concentration that was completely inhibited growth on agar plates. The bacteria strains *Escherichia coli* (MTCC-422), *Pseudomonas aeruginosa* (MTCC-441), *Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-443) were used for this study. Ampicillin, Chloramphenicol, Ciprofloxacin, & Norfloxacin used as the standard drug for evaluating antibacterial activity. The Minimal Bactericidal Concentration was measured in microgram/ml. and recorded in Table-2.

The compounds were evaluated for their antifungal activity using Broth Dilution Method with Saburoud's dextrose agar (Hi-Media). Suspension of each fungus were prepared and applied to agar plates with serially diluted compounds to be tested. The plates were incubated at 26^{o}C for 72 h and MIC's were determined. The fungus strains *Candida albicans* (MTCC-227), *Aspergillus niger* (MTCC-282) and *Aspergillus clavatus* (MTCC-1323) were used for this study. Greseofulvin & Nystatin were used as the standard drug for measuring Minimal Fungicidal Concentration (MFC). The Minimal Fungicidal Concentration is recorded in Table-3.

Scheme 1: Synthesis of studied compounds 3a-j





Reagents and condition: (a) Toluene, Anh. $AlCl_3$; (b) DMF, Reflux, 6-Hrs; (c) Methanol, Formaldehyde, 2nd Amine.

Table-1: Physical constants of synthesized compounds 3(a-j)

Sr. No.	R	Molecular Formula	M.W.	M.P. ºC	Rf* Value	Yield %	% of Nitrogen	
							Calcd.	Found.
3a	C_4H_8O-	$C_{20}H_{23}N_3O$	321.4	140	0.45	60	13.07	12.89
3b	C_4H_8-	$C_{20}H_{23}N_3$	305.4	123	0.55	63	13.76	13.40
3c	$C_5H_{11}N$-	$C_{21}H_{26}N_4$	334.4	136	0.40	65	16.75	16.13
3d	$C_6H_{13}N$-	$C_{22}H_{28}N_4$	386.8	146	0.60	64	16.08	15.88
3e	C_5H_{10}-	$C_{21}H_{25}N_3$	319.4	168	0.45	65	13.15	12.89
3f	C_7H_{14}-	$C_{23}H_{29}N_3$	347.4	109	0.40	62	12.09	11.86
3g	$C_4H_{11}N$-	$C_{20}H_{24}N_4$	320.4	131	0.50	61	17.48	17.16
3h	$C_9H_{19}N$-	$C_{25}H_{34}N_4$	390.5	143	0.30	57	14.35	14.19
3i	C_2H_7-	$C_{18}H_{21}N_3$	279.3	112	0.55	65	15.04	14.86
3j	C_6H_{10}-	$C_{20}H_{25}N_3$	307.4	121	0.50	66	13.67	13.39
Solvent System: Ethyl acetate: Hexane (8:2)								

Table-2 : Minimal Bactericidal Concentration (MBC) value of synthesized compounds 3(a-j)

Antibacterial Activity Table					
MINIMAL BACTERICIDAL CONCENTRATION (µg/ml)					
SR. NO.	CODE NO.	E. COLI	P.AERUGINOSA	S. AUREUS	S.PYOGENUS
		MTCC 443	MTCC 1688	MTCC 96	MTCC 442
01	3a	250	62.5	500	1000
02	3b	100	100	100	1000
03	3c	50	250	500	500
04	3d	500	500	250	500
05	3e	250	500	200	1000
06	3f	250	250	100	250
07	3g	200	100	500	250
08	3h	100	200	500	500
09	3i	500	200	250	500
10	3j	200	500	100	1000
11	Ampicillin	**100**	**100**	**250**	**100**
12	Chloramphenicol	**50**	**50**	**50**	**50**
13	Ciprofloxacin	**25**	**25**	**50**	**50**
14	Norfloxacin	**10**	**10**	**10**	**10**

Table-3 : Minimal Fungicidal Concentration (MFC) value of synthesized compounds 3(a-j)

Antifungal Activity Table				
MINIMAL FUNGICIDAL CONCENTRATION (µg/ml)				
SR. NO.	CODE NO.	C. ALBICANS	A. NIGER	A. CLAVATUS
		MTCC 227	MTCC 282	MTCC 1323
01	3a	200	>1000	>1000
02	3b	100	>1000	>1000
03	3c	500	>1000	>1000
04	3d	200	500	500
05	3e	250	200	250
06	3f	100	500	500
07	3g	100	1000	>1000
08	3h	200	500	500
09	3i	500	500	250
10	3j	500	>1000	>1000
11	Nystatin	**100**	**100**	**100**
12	Greseofulvin	**500**	**100**	**100**

Table-4: Spectral data of synthesized compounds 3(a-j)

Sr. No	No	IR data cm^{-1}	NMR data δppm	Mass m^{+1}
1	3a	3044, 2962, 2852, 1605, 1555, 1495, 1460, 1378, 1231, 1197, 1105, 831	2.26 (s, 3H, Ar-CH_3), 2.38 (s, 3H, Py-CH_3), 2.47 (t, 4H, Morp), 3.66 (t, 4H, Morp), 3.93 (s, 2H, CH_2), 7.05–8.15 (m, 7H, Ar-H)	322
2	3b	3060, 2958, 2860, 1610, 1562, 1494, 1451, 1361, 1249, 1190, 1108, 833	2.29 (s, 3H, Ar-CH_3), 2.42 (s, 3H, Py-CH_3), 3.10 (m, 8H, Pyrol) 3.91 (s, 2H, CH_2), 6.99–8.10 (m, 7H, Ar-H)	306
3	3c	3053, 2961, 2853, 1608, 1572, 1484, 1461, 1373, 1261, 1182, 1112, 830	2.21 (s, 3H, Ar-CH_3), 2.34 (s, 3H, Py-CH_3), 2.40 (s, 3H, N-CH_3), 3.42 (t, 4H, Pipe), 3.71 (t, 4H, Pipe), 4.02 (s, 2H, CH_2), 7.00–8.21 (m, 7H, Ar-H)	335
4	3d	3048, 2950, 2856, 1614, 1565, 1485, 1459, 1371, 1250, 1178, 1106, 836	1.54 (t, 3H, -CH_3), 2.23 (s, 3H, Ar-CH_3), 2.32 (s, 3H, Py-CH_3), 3.02 (q, 2H, -CH_2), 3.44 (t, 4H, Pipe), 3.73 (t, 4H, Pipe), 4.10 (s, 2H, CH_2), 7.06–8.31 (m, 7H, Ar-H)	387
5	3e	3041, 2951, 2863, 1619, 1562, 1493, 1460, 1364, 1248, 1180, 1116, 841	2.26 (s, 3H, Ar-CH_3), 2.31 (s, 3H, Py-CH_3), 3.13 (m, 10H, Piperidine) 3.85 (s, 2H, CH_2), 6.80–8.03 (m, 7H, Ar-H)	320
6	3f	3040, 2978, 2843, 1620, 1532, 1484, 1461, 1341, 1239, 1187, 1110, 846	2.03 (d, 6H, -CH_3), 2.29 (s, 3H, Ar-CH_3), 2.39 (s, 3H, Py-CH_3), 3.63 (m, 6H, Piperidine), 4.10 (s, 2H, CH_2), 4.51 (m, 1H, -CH), 4.93 (m, 1H, -CH) , 7.06–8.31 (m, 7H, Ar-H)	348
7	3g	3043, 2953, 2861, 1618, 1568, 1476, 1451, 1364, 1251, 1173, 1102, 828	2.26 (s, 3H, Ar-CH_3), 2.35 (s, 3H, Py-CH_3), 2.48 (m, 8H, Piperazine), 3.86 (s, 2H, CH_2), 6.88–7.95 (m, 7H, Ar-H)	321
8	3h	3050, 2947, 2853, 1614, 1553, 1485, 1445, 1352, 1230, 1181, 1109, 824	1.62 (t, 6H, -CH_3), 2.23 (s, 3H, Ar-CH_3), 2.36 (s, 3H, Py-CH_3), 3.10 (q, 4H, -CH_2), 3.58 (m, 8H, Piperidine), 4.10 (s, 2H, CH_2),), 5.08 (m, 1H, -CH), 6.45–7.83 (m, 7H, Ar-H)	391
9	3i	3044, 2970, 2862, 1617, 1581, 1493, 1470, 1364, 1250, 1191, 1101, 849	2.18 (s, 3H, Ar-CH_3), 2.21 (s, 3H, Py-CH_3), 2.32 (s, 6H, -CH_3), 3.88 (s, 2H, CH_2), 7.00–8.13 (m, 7H, Ar-H)	280
10	3j	3062, 2971, 2870, 1607, 1561, 1473, 1454, 1362, 1246, 1165, 1109, 833	1.48 (t, 6H, -CH_3), 2.21 (s, 3H, Ar-CH_3), 2.39 (s, 3H, Py-CH_3), 3.11 (q, 4H, -CH_2), 4.18 (s, 2H, CH_2), 6.10–7.91 (m, 7H, Ar-H)	308

RESULTS AND DICUSSION

In the present study, 2-chloro-1-(p-methylphenyl)ethanone **1** was prepared by condensation of toluene and chloroacetyl chloride. 7-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine **2** was synthesized by the reactions of 2-chloro-1-(p-methylphenyl)ethanone **1** with 2-amino-4-methylpyridine. The treatment of compound **2** with formaldehyde and different secondary amines in methanol resulted in the formation of *N*-{[7-methyl-2-(p-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]methyl}-*N*,*N*-diaryl/alkylamines **3(a-j)**. After the Mannich reactions, ^{1}HNMR spectra of mannich bases **3(a-j)** show peak at around δppm 4.05(s, 2H), for CH_2 group, which confirms the formation of Mannich bases. Compounds **1** showed peak at around δppm 2.35 for methyl group, while due to cyclisation, compound **2** showed two separate peaks at around δppm 2.38 and δppm 2.41 for methyl groups. The FTIR spectra of compound **2** show absorption bands at around 1580 cm^{-1} and 1168 cm^{-1} which confirm the presence of C=N, C-N groups, while compounds **3** shows absorption bands at around 2860 cm^{-1}, 1590 cm^{-1} and 1176 cm^{-1} which confirm the presence of CH_2, C=N, C-N function groups, respectively in Mannich bases. The spectral results of substituted Mannich bases **3a-j** are given in Table-4.

The Minimal Bactericidal Concentration (MBC) values of antimicrobial testing reveals that the compound 3b(100µg/ml) showed similar MBC value against E. Coli and P. Aeruginosa as compared to Ampicillin, while the values of MBC against S. Aureus is very low as compare to standard drug Ampicillin. The MBC value of compound 3a(62.5µg/ml) is comparable to Chloramphenicol against P. Aeruginosa. The compounds 3b(100µg/ml), 3f(100µg/ml) and 3j(100µg/ml) show very low MBC values against S. Aureus in comparison to the MBC value of Ampicillin.

Compounds 3b(100µg/ml), 3f(100µg/ml) and 3g(100µg/ml) show equivalent Minimal Fungicidal Concentration (MFC) values with reference to Nystatin against the C. Albicans. Further these MFC values are remarkably less then that of Greseofulvin. The MFC values of the all newly synthesized compounds against A. Niger and A. Clavatus are more than that of the standard drugs.

CONCLUSION

It can be concluded from the MBC values that the pyorrolidine substituted Mannich base show equivalent activity with that of Ampicillin and 1-methylpiperazine substituted Mannich base gives similar activity to that of Chloramphenicol against E. Coli. The low MBC value of pyrrolidine, 2,4-dimethylpiperidine, piperidine and diethyl substituted compounds of the series indicate very good activity as compared to Ampicillin against S. Aureus. All the Mannich bases show excellent or moderate activity against C. Albicans as compared to the standard drugs.

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