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STUDIES RELATED TO ANTIMICROBIAL ACTIVITY OF AMINOBENZYLATED MANNICH BASES OF UREA

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ABSTRACT

An elegant synthesis of newer aminobenzylated mannich bases (6a to 6j) is described in the present study. These were synthesized by reacting aromatic aldehydes, cyclic secondary amines and a reagent with active hydrogen atom such as Urea in the presence of hydrochloric acid. The constituents of the newly synthesized compounds have been established on the basis of their physical and spectral data. All the newly obtained compounds have been screened for *invitro* antimicrobial activity against bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtillis, Escherichia coli* and fungal strains *Candida albicans* and *Aspergillus niger.*

Keywords: Ciprofloxacin, Ketoconazole, Urea, Mannich bases.

INTRODUCTION

Mannich bases are well known for their use as polymer, resins, surface active agents [1], detergents additives [2], antioxidents [3]. They have broad range of biological activity including diuretic [4], antipsychotic [5], oxytocic [6], centrally acting muscle relaxant [7], anticancer [8], antimicrobial [9], antiviral [10], anthelmintic [11] etc., Amides are known to play a pivotal role in molecular recognition, being important compounds in supramolecular chemical anion sensors technology [12] and they are used as antimicrobial agents clinically [13].

To our knowledge mannich reaction using benzaldehyde have not been reported as yet except in our earlier studies [14]. Hence in view of the biological significance of mannich bases and amides, in the present study an attempt has been made to synthesize various aminobenzylated mannich bases by reacting heterocyclic compounds having N or O, with benzaldehyde / substituted benzaldehyde and amides such as urea as possible antibacterial and antifungal agents.

MATERIALS AND METHODS

The chemicals used were of commercial grade and are used without further purification. Melting points were taken in open capillary tube and are uncorrected. The progress of the reaction and purity of products were monitored by thin layer chromatography and spots were located by UV and in iodine chamber. The IR spectra are recorded by using SHIMADZU 8400 spectrophotometer using a thin film of potassium bromide pellets technique and frequencies are expressed in cm^{-1}. The ^1H NMR spectra were recorded on Brucker Advance 11400 NMR spectrometer in $CDCl_3$ with TMS as internal standard and the values are expressed in ppm (**Table 3**).

Procedure for the synthesis of aminobenzylated mannich bases of urea 6a to 6j: Ice cold secondary amines (0.1 mol) viz Morpholine (0.9 ml) / Piperidine (1.0 ml) / N-methyl piperazine (0.9 ml) / Indole (1.2 g) in ethanol were added drop wise to an ethanolic solution of urea with occasional stirring at

0-5^{0}C. A drop of hydrochloric acid was added to the above suspension and an ethanolic solution of benzaldehyde / substituted benzaldehyde was added in two portions with time interval of 30 minutes and stirred for about 24 hrs. The resulting mixture was allowed to stand at room temperature with occasional shaking for about three weeks[15]. The solid obtained was thus collected and recrystallised from chloroform (**Scheme 1**).

Antimicrobial Activity: All the synthesized compounds have been screened *in-vitro* for their antibacterial activity against gram positive bacteria *S. aureus* (ATCC 6632), *B. Subtillis* (ATCC 6633) and gram negative bacteria *E. coli* (ATCC 25923),*P. aeruginosa* (ATCC 25922) while antifungal activity against *C. albicans* (ATCC 10231) and *A. Niger* (ATCC 10232) by cup-plate agar diffusion method[16] using dimethyl formamide as solvent. After 24 and 72 hrs of incubation at 37±1^{0}C and 25±1^{0}C respectively, the antibacterial and antifungal activity was determined by measuring the zones of inhibition in mm. Standard antibacterial Ciprofloxacin and fungicide Ketoconazole were used under similar condition for comparison. Control test with solvents were performed for every assay but showed no inhibition of microbial growth (**Table 2**).

RESULTS AND DISCUSSION

The synthetic route for preparing the target compounds 6a to 6j in good yields were portrayed in **(Scheme:1).** One pot three component condensation of cyclic secondary amines, aromatic aldehydes and urea in hydrochloric acid gives the title compounds. The structures of the synthesized compounds were characterized by IR, ^{1}H NMR spectral studies **(Table 3)** and the physical data of 6a to 6j are presented in **(Table 1).**

Antibacterial studies of the title compounds revealed that compound 6f shown good activity against *B. substilis* (Zone of inhibition : 15mm), compounds 6b, 6f, 6h, 6i, against *E. coli* (12mm), compound 6f against *S. aureus* (17mm), compound 6j against *P. aureginosa* (16mm), whereas rest of the compounds have shown lesser activity than standard Ciprofloxacin. Antifungal studies of the compounds revealed that compound 6e had shown good activity against *A. niger* (12mm), the compound 6a against *C. albicans* (15mm) and the rest of the compounds shown lesser activity than standard Ketoconazole.

CONCLUSION

The compounds 6a to 6j were synthesized using Mannich reaction and evaluated for their possible antimicrobial activity. The compound 6f had showed better antibacterial activity against tested organisms and the compound 6g had showed good anti-fungal activity against *C. albicans.*

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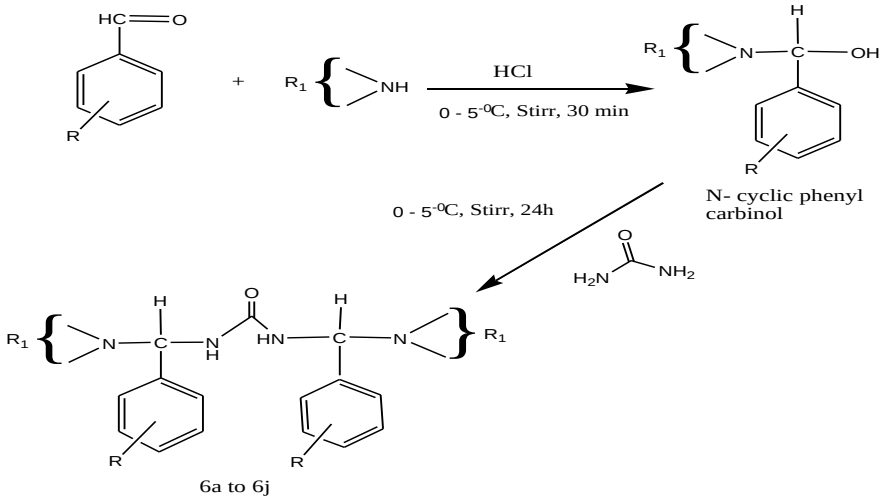
Scheme 1: Synthetic protocol for the synthesis of aminobenzylated mannich bases, 6a to 6j



Where,

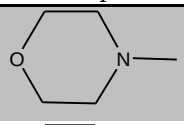
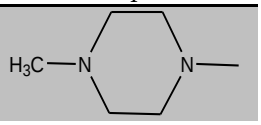
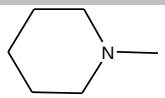
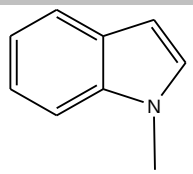
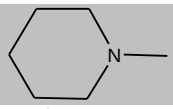
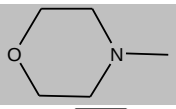
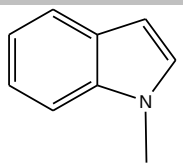
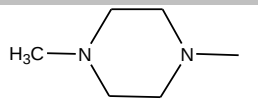
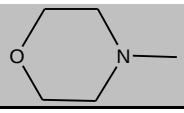
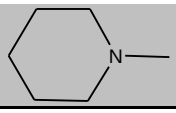
Compound	R	R_1	Compound	R	R_1
6a	4-OH		6f	4-OH-3-OCH_3	
6b	H		6g	4-$N(CH_3)_2$	
6c	4-$N(CH_3)_2$		6h	3-OH	
6d	4-OH		6i	4-OH	
6e	4-OH-3-OCH_3		6j	3,4-$(OCH_3)_2$	

Table 1: Physicochemical data of synthesized compounds 6a to 6j

Compound	Mol. formula	Mol. Weight	M.P (^{0}C)	% Yield	R_f value
6a	$C_{23}H_{30}N_4O_5$	442	228-229	55.2	0.80
6b	$C_{25}H_{34}N_4O$	406	159-161	42.0	0.77
6c	$C_{29}H_{48}N_6O$	492	176-178	22.5	0.68
6d	$C_{31}H_{26}N_4O_3$	502	151-153	28.0	0.58
6e	$C_{25}H_{34}N_4O_7$	502	179-181	19.5	0.50
6f	$C_{27}H_{40}N_6O_6$	528	230-232	31.0	0.70
6g	$C_{35}H_{36}N_6O$	556	183-185	30.0	0.72
6h	$C_{23}H_{30}N_4O_5$	442	238-240	35.0	0.45
6i	$C_{25}H_{36}N_6O_3$	468	102-104	38.0	0.58
6j	$C_{29}H_{42}N_4O_5$	526	191-194	50.0	0.32

Table 2: Antimicrobial activity of synthesized compounds 6a to 6j

Compounds	Bacterial strains				Fungal strains	
	B. Subtilis	*E. coli*	*S. aureus*	*P. aeruginosa*	*A. niger*	*C. albicans*
6a	09	11	10	08	10	15
6b	11	12	09	08	07	08
6c	09	10	11	13	05	06
6d	14	12	13	11	11	10
6e	12	11	14	13	12	09
6f	15	12	17	11	09	07
6g	11	09	10	10	11	13
6h	12	12	11	09	10	12
6i	13	12	14	13	10	11
6j	09	09	13	16	04	04
Ciprofloxacin	20	23	29	33	-	-
Ketoconazole	-	-	-	-	24	29

Table 3: Spectral data of compounds 6a to 6j

Compounds	IR (KBr, Vmax, cm^{-1})	^1H NMR ($CDCl_3$, δppm)
6a: 1, 3-bis-(4-hydroxy-1-morpholin-1 yl)benzylurea.	3427 (NH), 1629 (amide C=O), 1146 (C-N-C of Morpholine), 3073 (CH-Ar), 2869 (CH-aliphatic), 1562 (C-C Ar)	10.5 (s, 2H, Ar-OH), 9.8-9.9 (s, 2H, CONH), 6.8-7.8 (m, 8H, Ar-H), 4.4-4.6 (s, 2H, Ar-CH), 3.0-4.0 (k, 8H, _OCH_2Morpholine), 1.0-2.2 (t, 8H, NCH_2Morpholine).
6b: 1, 3-bis – (phenyl (piperidin-1-yl) methyl) urea	3438 (NH), 1585 (amide C=O), 1035 (C-N-C of Piperidine), 2929 (CH-Ar), 1238 (CH-aliphatic), 1157 (C-C Ar)	6.8-7.7 (m, 10H, Ar-H), 5.5(s, 2H, CONH), 4.6-4.8 (s, 2H, Ar-CH), 3.8-4.0(d, 4H, -CH_2 Piperidine), 3.0-3.4(t, 16H, -CH_2 α + CH_2β Piperidine)
6c: 1,3-bis – [(4-dimethylaminophenyl)piperidin-1-yl)methyl] urea	3430 (NH), 1665 (amide C=O), 1030 (C-N-C of Piperidine), 3031 (CH-Ar), 1238 (CH-aliphatic)	6.8-7.7 (m, 8H, Ar-H), 5.5(s, 2H, CONH), 4.6-4.8 (s, 2H, Ar-CH), 3.8-4.0(d, 4H, -CH_2 Piperidine), 3.0-3.4(t, 20H, -CH_2 α + CH_2β Piperidine), 1.9-2.2(s, 12H, Ar-N(CH_3)$_2$)
6d: 1, 3-bis – [(4-hydroxyphenyl) (indolin-1-yl) methyl] urea.	3396 (NH), 1660 (amide C=O), 1160 (C-N-C of Indole), 3036(CH-Ar), 1342 (CH-aliphatic), 1608 (C-C Ar)	9.7-9.8 (s, 2H, Ar-OH), 6.5-7.2 (m, 20H, Ar-H), 5.5 (s, 2H, CONH), 4.9 (s, 2H, Ar-CH)
6e: 1, 3-bis – [(4-hydroxy-3-methoxyphenyl) (Morpholine -1-yl) methyl] urea.	3428 (NH), 1645 (amide C=O), 1145 (C-N-C of Morpholine), 3042 (CH-Ar), 2862 (CH-aliphatic), 1562 (C-C Ar)	11.3-11.4 (s, 2H, Ar-OH), 8.7 (s, 2H, CONH), 6.8-7.5 (m, 6H, Ar-H), 6.3 (s, 2H, Ar-CH), 1.0-2.0 (t, 22H, -OCH_2 + NCH_2 Morpholine + Ar-OCH_3)
6f: 1,3-bis – [(4-hydroxy-3-methoxyphenyl)(4-methylpiperizin-1-yl)methyl] urea	3428 (NH), 1645 (amide C=O), 1089 (C-N-C of piperazine), 3042 (CH-Ar), 2862 (CH-aliphatic), 1562 (C-C Ar)	9.7-9.6 (s, 2H, Ar-OH), 6.8-7.3 (m, 6H, Ar-H), 8.9-9.0 (s, 2H, CONH), 5.6-6.0 (s, 2H, Ar-CH), 4.9-5.0 (s, 6H, -NCH_3), 1.4-4.0 (t, 22H, -NCH_2 α + NCH_2β piperizine + Ar-OCH_3)
6g: 1,3-bis – [(4-dimethylaminophenyl)(indolin-1-yl)methyl] urea	3396 (NH), 1660 (amide C=O), 1160 (C-N-C of Indole), 3036 (CH-Ar), 1342 (CH-aliphatic), 1608 (C-C Ar)	9.7-9.8 (s, 2H, Ar-OH), 6.5-7.2 (m, 20H, Ar-H), 5.5 (s, 2H, CONH), 4.9 (s, 2H, Ar-CH), 1.9-2.2(s, 10H, Ar-N(CH_3)$_2$)
6h: 1,3-bis – [(3-hydroxyphenyl) (morpholin-1-yl)methyl] urea	3169 (NH), 3024 (CH-Ar), 2910 (CH-aliphatic), 1616 (amide C=O), 1599 (C-C Ar), 1146 (C-N-C of Morpholine), 1159 (C-O str Ar-OH)	10.1 (s, 2H, Ar-OH), 8.7 (s, 2H, CONH), 6.8-7.5 (m, 8H, Ar-H), 6.3 (s, 2H, Ar-CH), 1.0-2.0 (t, 16H, -OCH_2 + NCH_2 Morpholine)
6i: 1,3-bis – [(4-hydroxy phenyl) (N-methyl piperazin-1-yl)methyl] urea	3416 (NH), 3161 (CH-Ar), 2750 (CH-aliphatic), 1608 (amide C=O), 1579 (C-C Ar), 1163 (C-O str Ar-OH) 1089 (C-N-C of N-methylpiperizine),	10.5 (s, 2H, Ar-OH), 9.8-9.9 (s, 2H, CONH), 6.8-7.8 (m, 8H, Ar-H), 4.9-5.0 (s, 6H, -NCH_3), 4.4-4.6 (s, 2H, Ar-CH), 3.0-4.0 (t, 8H, NCH_2piperizine), 1.0-2.2 (t, 8H, NCH_2 piperizine).
6j: 1,3-bis – [(3,4-dimethoxy phenyl) (piperadin-1-yl)methyl] urea	3392 (NH), 1647 (amide C=O), 1147 (C-N-C of Piperidine), 2847, 1286 (CH-aliphatic), 1028 (C-C Ar)	9.7-9.6 (s, 2H, Ar-OH), 6.8-7.3 (m, 10H, Ar-H), 8.9-9.0 (s, 2H, CONH), 5.6-6.0 (s, 2H, Ar-CH), 3.8-4.0(d, 4H, -CH_2 Piperidine),3.4-4.0 (t, 22H, -NCH_2 α + NCH_3β piperidine + Ar-OCH_3)

REFERENCES

1. Karil RE, Lee RJ. US patent, US 4384138, 1983.
2. Otto FP. US patent, US 3649229, 1972.
3. Horodysky AG, Kaminski JM. US patent, US 4394278, 1983.
4. LeeJM, Plattner JJ, Ours CW,Horrom BW, Smital JR, Martin JC, Pernet AG, Bunnell PR, El Masryz SE, Dodge PW. J Med Chem, 1984; 27: 1579.
5. Scott MK, Martin GE, Distefano DL, Fedde CL, Kukla MJ, Barrett DL,Baldy WJ, Elgin RJ, Kesslick JM, Mathiasen JR, Strank RP, Vaguht JL. J Med Chem, 1992; 35: 552-8.
6. Cohen A, Hall RA, Heath-Brown B, Parker MW, Rees AH. British J Pharmacol Chemother, 1957; 12: 194.
7. Shiozawa A, Narita K, Izumi G, Kurashige S, Sakitama K, Ishikawa M. Eur J Med Chem, 1995; 30: 85.
8. Dimmock JR, Kumar P. Curr Med Chem, 1997; 4:1.
9. Shah TB, GuptaA, Choudhari VS, Patel H, Patel VC. Ind J Chem, 2010; 49(B): 578-86.
10. Edwards ML, Ritter HW, Stemerick DM, Stewart KT. J Med Chem, 1983; 26: 431.
11. Chaluvaraju KC, Bhat KI. J Young Pharmacist, 2011; 3(3): 243-45.
12. Beer PD, Drew MGB, Hesek D, Jagessar R. J Chem Soc Chem Commun, 1995; 1187.
13. Raymond CJ, Davendra RP. New York Sci J, 2008;1(1);22-6.
14. Chaluvaraju KC, Bhat KI. J Ind Council Chem, 2009;26(1):90-3.
15. Chaluvaraju KC, Ishwar Bhat K. Asian J Chem, 2008;20(6):4335-38.
16. Barry AL. Antimicrobial susceptibility test; Priucioles and Practices, Lea and Febiger, Philaderphia. 1976; 236.