The Pharmaceutical and Chemical Journal, 2017, 4(6):107-113

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Synthesis and Antimicrobial Activity of Phenol-derived Mannich Bases

Abdel Karim M[1], Hala I[2], Khalid M[3]

[1]Sudan University of Science and Technology, Faculty of Science, Sudan
[2]Najran University, Faculty of Medicine
[3]Africa International University, Faculty of Pharmacy

Abstract Using p-acetamidophenol as an active hydrogen component, two Mannich bases: N,N-[(**5**-acatamido-2-hydroxy)benzyl]ethylamine(I) and N-[(5-acatamido-2-hydroxy)benzyl]ethylamine(II) were synthesized via a general synthesis protocol. .The structures of the target molecules were elucidated by a combination of spectral techniques (UV, IR, ^{1}H NMR and MS). The Mannich bases were evaluated for their antimicrobial activity against seven standard human pathogens. Compounds I and II showed excellent activity against *Escherichia coli* at 10 and 20mg/ml. Furthermore, compound II showed excellent activity against all test organisms at 20mg/ml. Both compounds exhibited significant antifungal activity at test concentrations.

Keywords Mannich bases, Synthesis, Antimicrobial activity

1. Introduction

The Mannich reaction is a three component condensation in which a compound containing an active hydrogen atom is allowed to react with an aldehyde and an NH-amine derivative. Secondary amines rather than primary amines are usually employed .The resulting product (Mannich Base) is an amine compound having the N atom linking the R substrate through a methylene moiety [1,2]. Mannich bases find diverse applications as antitubercular [3], antimalarial [4], vasorelaxing [5], anticancer [6] and analgesic drugs [7]. They are also used in the polymer industry as paints and surface active reagents [8]. Various 1,2,4-triazole derivatives have been reported to possess antibacterial, antifungal, anticancer, [9] antitubercular, [10] analgesic and anti-inflammatory properties[11]. Some phenolic Mannich bases were identified as IL -2 expression inhibitors in a T cell proliferation screening assay [12].
This study was aimed to the synthesis and evaluation of some Mannich bases for their antimicrobial activity.

2. Materials and Methods

2.1. Materials

Analytical grad reagents (BDH) were used. The UV spectra were recorded on a Perkin-Elmer Lambda 2 UV-Visible Spectrophotometer. Infra red spectra were measured on a Perkin-Elmer1310 Infra red Spectrophotometer. ^{1}H NMR spectra were recorded on EM-360 NMR Spectrophotometer. Mass spectra were measured on a Krates MS 80 RF Mass Spectrometer.

2.2. Methods

2.2.1. Synthesis protocols

Synthesis of the Mannich base: N,N-[(5-acatamido-2-hydroxy)benzyl]ethylamine(I)

Formalin (40 mmol) was added dropwise with stirring to a mixture of P-acetamidophenol (40 mmol) and ethylamine (20 mmol) in dioxane (15 ml) at 0 ºC. The mixture was then stirred for four hours at 0 ºC and left overnight. Removal of the solvent under reduced pressure gave the product.

Synthesis of the Mannich base: N-[(5-acatamido-2-hydroxy)benzyl]ethylamine(II)

Formalin (20 mmol) was added dropwise with stirring to a mixture of P-acetamidophenol (20 mmol) and ethylamine (20 mmol) in dioxane (15 ml) at 0 ºC. The mixture was then stirred for six hours at 0 ºC and left overnight. The solvent was removed under reduced pressure to afford the product.

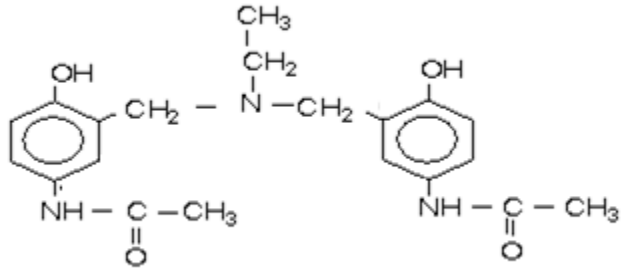
2.2.2. *In vitro* antimicrobial assay

The disc diffusion method was used to determine the antimicrobial activity of the oil. Fresh cultures of microorganisms grown for 24 h were used and diluted to 10^{-1} with sterile physiological saline solution (0.85% NaCl). 100 µl of test microorganisms containing 2.0×10^{6} colony forming units (CFU/ml) for bacteria were inoculated on the surface of Muller Hinton Agar plates. Sterile discs with a diameter of 6 mm were placed onto each agar plate containing microorganisms. Then the test solution was dropped onto discs under sterile conditions and incubated at 37 °C for 24 h. After incubation, the diameters of inhibition zones were measured in millimetres. All experiments were repeated three times. ampicillin, gentamycin and clotrimazole were used as positive controls, while DMSO was used as negative control.Control discs were tested on the same microorganisms under the same conditions.

3. Results and Discussion

Two phenolic Mannich bases were synthesized via a general synthesis strategy involving the condensation of an active hydrogen component with primary amines in presence of formalin. The target molecules were then evaluated for their antimicrobial potential.

3.1. Mannich base: N,N-[(5-acatamido-2-hydroxy)benzyl]ethylamine(I)



V

The bis Mannich base I was synthesized by adding formalin(two equivalents) dropwise to a mixture of P-acetamidophenol(two equivalents) and ethylamine(one equivalent) in dioxane at 0 ^{o}C.

The UV spectrum (Fig.1) showed λ_{max} (MeOH) 249nm characteristic of a C=O absoption as extended chromophore. The IR spectrum (Fig.2) showed v(KBr) : 680, 804, 835(C-H, Ar.,bending), 1012 (CN), 1438, 1508, 1564 (C=C, Ar), 1654 (C=O), 2792 (C-H, aliphatic), 3161(N-H) and 3325cm^{-1}(OH). The Mass spectrum (Fig.3) gave m/z 371 for M^{+}.

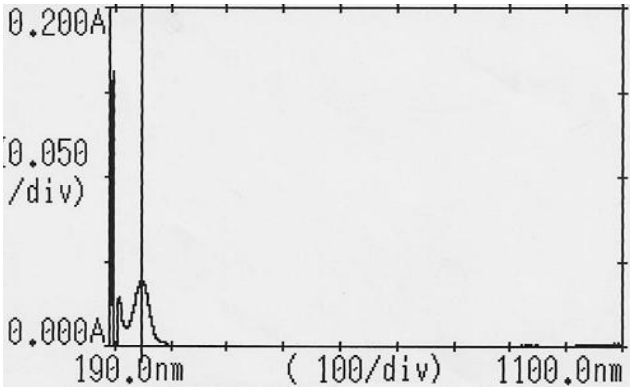


Figure 1: UV spectrum of compound 1

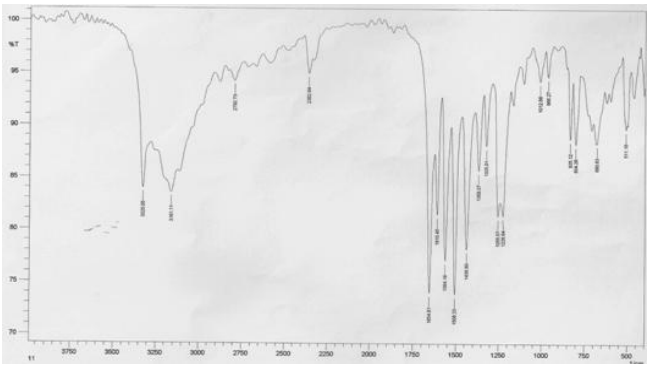


Figure 2: IR spectrum of compound 1

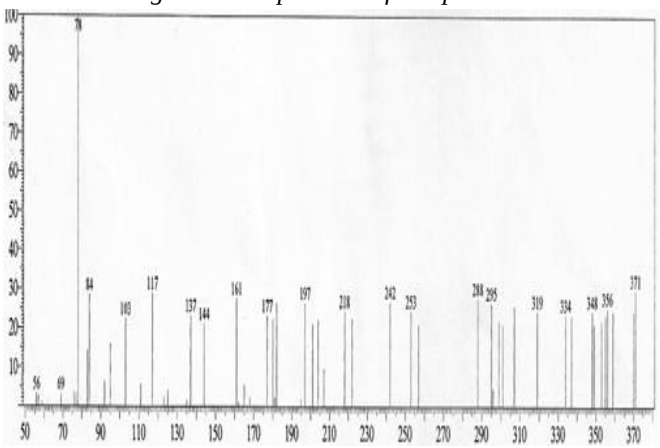


Figure 3: Mass spectrum of compound 1

The ^{1}H NMR spectrum (Fig.4) revealed the signals:

δ 1.70-2.01	multiplet	9H
δ 3.41	singlet	6H
δ 6.68	doublet	3H
δ 7.31	doublet	3H
δ 9.2	singlet	2H
δ 9.625	singlet	2H

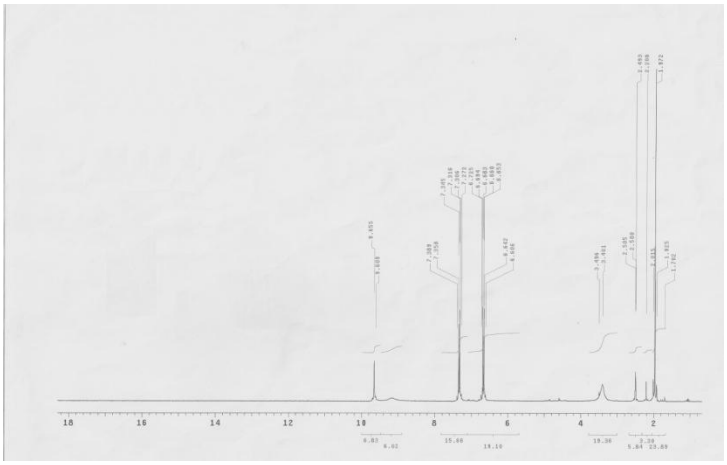
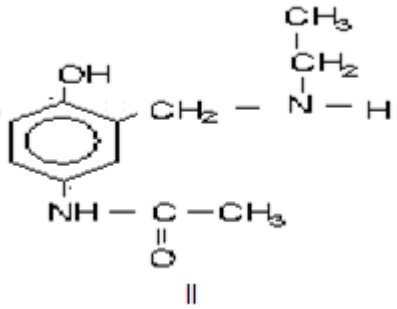


Figure 4: ^{1}H NMR spectrum of compound I

The multiplet at δ1.70-2.01(9H) was assigned for the two methylenes and one ethyl group, while the singlet at δ3.41 (6H) which overlaps DMSO residual water is due to two methyls in $-\overset{\overset{O}{\|}}{C}-CH_3$. The aromatic protons appeared as a double doublet at δ6.68 (3H) and δ7.31 (3H). The singlet at δ9.2 (2H) corresponds to two NH functions shifted downfield by electron-withdrawal influence of the neighboring carbonyl. The singlet at δ9.625 (2H) corresponds to two OH groups**.**

3.2. Mannich base: N-[(5-acatamido-2-hydroxy)benzyl]ethylamine(II)



The mono Mannich base II was synthesized by adding formalin (one equivalent) dropwise to mixture of P-acetamidophenol (one equivalent) and ethyl amine (one equivalent) in dioxane at 0°C. The UV spectrum (Fig.5) showed λ_{max}(MeOH) 249 nm which characteristic of the extended C=O chromophore .

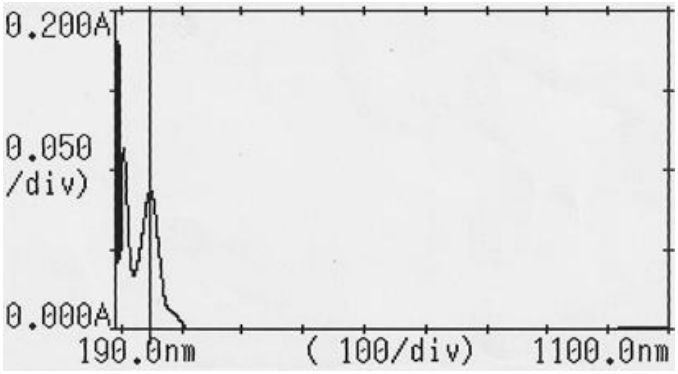


Figure 5: UV spectrum of compound II

The IR spectrum (Fig.6) showed v(KBr) : 682, 802,835 (C-H, Ar , bending) 1014(CN), 1438,1508,1562(C=C, Ar), 1654(C=O) 3136 (NH) and 3325cm^{-1}(OH). The Mass spectrum (Fig.7) gave m/z 208 for M^+.

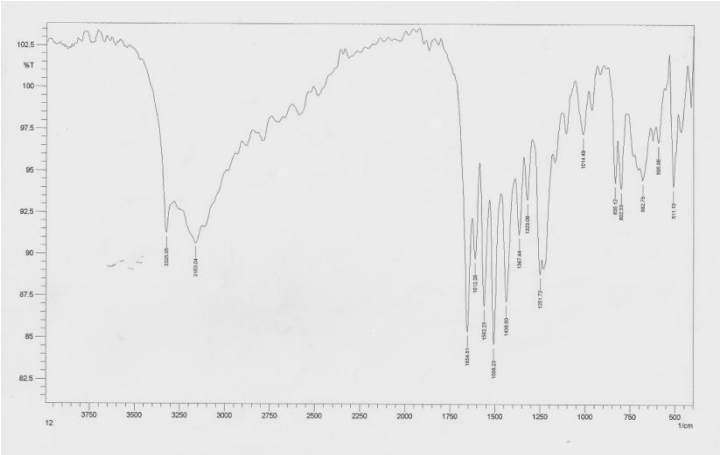


Figure 6: IR spectrum of compound II

The [1]HNMR spectrum (fig. 8) revealed the following signals:

δ 0.80-1.99	multiplet	7H
δ2.10	singlet	3H
δ 7.33	doublet	3H
δ9.61	singlet	2H

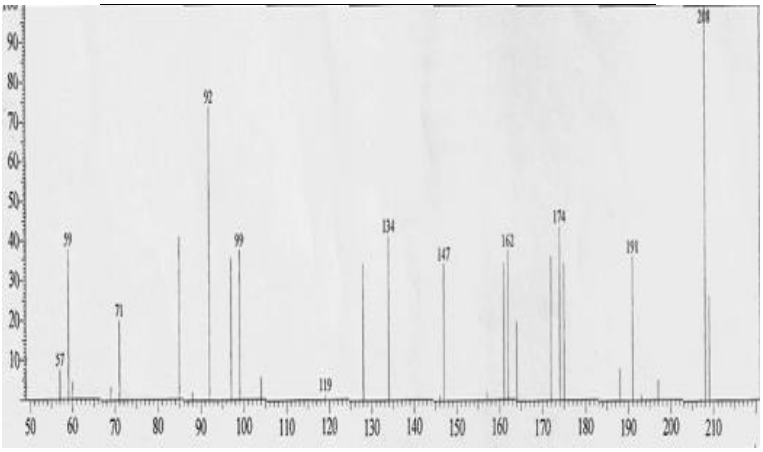


Figure 7: Mass spectrum of compound II

The multiplet at δ0.80-1.99(7H) was assigned for the ethyl and the methylene of the Mannich side chain. The singlet at δ 2.10 (3H) corresponds to the methyl group in ($-\overset{\overset{O}{\|}}{C}-CH_3$).The aromatic protons appeared as a double at δ7.33 (3H), while the singlet at δ 9.61 (2H) corresponds to the N-H group being shifted downfield by the electron-withdrawal effect of the neighboring carbonyl function.

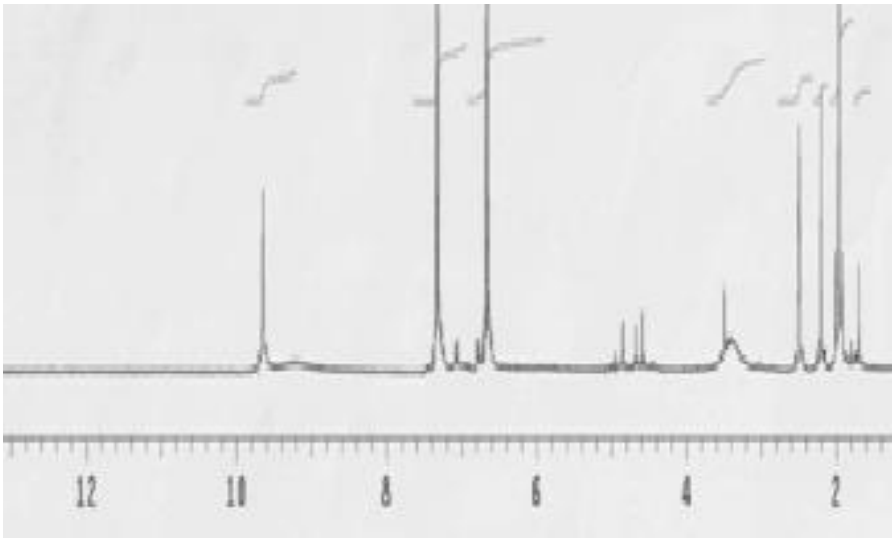


Figure 8: [1]H NMR spectrum of compound II

3.3. Antimicrobial activity

The targeted series of ketonic and phenolic Mannich adducts were evaluated for their antimicrobial potential against seven standard pathogenic microbes : *Salmonella typhi*, *Staphylococcus aureus* (*Sa.*), *Escherichia coli* (*Ec.*), *Pseudomonas aeruginosa* (*Pa.*), *proteus vulgaris* (*Pv.*),*Aspergillus Niger(An.) and Candida albicans (Ca)*-Table1. Mean diameter or growth inhibition zones (MDIZ) is expressed in (mm) - average or two replicates, Inhibition zone:

Table 1: Test organisms

S. No.	Micro organism	Type	Source
1	*Salmonella typhi*	G-ve	ATCC 27933
2	*Staphylococcus aureus*	G+ve	ATCC** 25923
3	*Pseudomonas aeroginosa*	G-ve	NCTC 6750
4	*Escherichia coli*	G-ve	ATCC 25922
5	*proteus vulgaris*	G-ve	ATCC 25925
6	*Aspergillus Niger*	fungi	ATCC 9736
7	*Candida albicans*	fungi	NCTC 10716

* NCTC. National collection of type culture, Colindale. England

** ATCC. American type culture collection, Maryland, USA

Compounds I and II showed excellent activity against *Escherichia coli* at 10 and 20mg/ml. Furthermore, compound II showed excellent activity against all test organisms at 20mg/ml. Both compounds exhibited significant antifungal activity at test concentrations.

Table 2: Antimicrobial activity of synthesized Mannich bases

Comp.	Conc (mg/ml)	Ec.	Sa.	Pv.	Pa.	St.	Ca.	An.
Comp. I	10	20	12	12	-	-	18	18
	20	20	17	17	13	14	20	20
Comp. II	10	20	20	15	13	-	29	27
	20	25	27	20	20	25	30	30

Table 3: Antibacterial activity of standard drugs

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4: Antifungal activity of standard drug

Drug	Conc. (mg/ml)	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa*: Staphylococcus aureus*

Ec*: Escherichia coli*

Pa: *Pseudomonas aeruginosa*

An: *Aspergillus niger*

Ca: *Candida albicans*

St: *Salmonella typhi*

References

1. Blicke, F. (1942). "Organic Reaction." John Wiley and Sons ,New York Vol.10, P305.
2. Ina-Gull, H. (2000). Cytotoxic activities of mono and bis Mannich bases derived from acetophenone against Renca and Jurkat cell. *pharmaceutica Acta Helvetiae, 74*, 393.

3. Joshi, S., Khosla, N., & Tiwari, P. (2004). In vitro study of some medicinally important Mannich bases derived from antitubercular agent. *Bioorganic & medicinal chemistry*, *12*(3), 571-576.
4. Lopes, F., Capela, R., Gonçaves, J. O., Horton, P. N., Hursthouse, M. B., Iley, J., ... & Moreira, R. (2004). Amidomethylation of amodiaquine: antimalarial N-Mannich base derivatives. *Tetrahedron letters*, *45*(41), 7663-7666.
5. Ferlin, M. G., Chiarelotto, G., Antonucci, F., Caparrotta, L., & Froldi, G. (2002). Mannich bases of 3H-pyrrolo [3, 2-f] quinoline having vasorelaxing activity. *European journal of medicinal chemistry*, *37*(5), 427-434.
6. Holla, B. S., Veerendra, B., Shivananda, M. K., & Poojary, B. (2003). Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1, 2, 4-triazoles. *European Journal of Medicinal Chemistry*, *38*(7), 759-767.
7. Malinka, W., Świątek, P., Filipek, B., Sapa, J., Jezierska, A., & Koll, A. (2005). Synthesis, analgesic activity and computational study of new isothiazolopyridines of Mannich base type. *Il Farmaco*, *60*(11), 961-968.
8. Tramontini, M., Agioline, L., & Ghedeni, N. (1988). *Polymers*, 9,771.
9. Holla, B. S., Rao, B. S., Sarojini, B. K., Akberali, P. M., & Kumari, N. S. (2006). Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents. *European journal of medicinal chemistry*, *41*(5), 657-663.
10. Walczak, K., Gondela, A., & Suwiński, J. (2004). Synthesis and anti-tuberculosis activity of N-aryl-C-nitroazoles. *European journal of medicinal chemistry*, *39*(10), 849-853.
11. Amir, M., & Shikha, K. (2004). Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2, 6-dichloroanilino) phenyl] acetic acid derivatives. *European Journal of Medicinal Chemistry*, *39*(6), 535-545.
12. Geng, B., Fleming, P. R., Umlauf, S., Lin, A., & Pallai, P. V. (2002). The Synthesis and Selective IL-2 Inhibitory Activity of Bis Piperazine–Phenol Mannich Adducts. *Bioorganic & medicinal chemistry letters*, *12*(5), 775-778.