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Synthesis and characterization of some antimicrobial phenolic Mannich bases

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Abstract
Three Mannich bases: 2, 5-bis-morpholinomethyl hydroquinone (I), 2, 5-bis piperidino methylhydroquinone (II) and 1-(morpholinomethyl)-2-naphthol (III) were synthesized. They were identified by UV, IR, NMR and MS data. These bases were evaluated for their antimicrobial potential and promising activity was observed.

Keywords*:* Mannich bases, phenolic, antimicrobial activity

Introduction

In Mannich reaction a secondary or primary amine or ammonia condenses with an active hydrogen compound and an aldehyde to afford Mannich bases. Mannich bases are known for their biological potential. Some Mannich bases possess anticonvulsant activity [1, 2], others exhibit analgesic potency [3], while others act[4] as potential chemopreventive agents. Mannich bases with putative cytotoxic activity were reported [5, 6, 7]. Stephen *et.al* [8] claimed antimalarial activity for some aminomethylated phenols. Tomas *et.al* [9] described the antibacterial activity of some fused Mannich ketones. Afaf *et.al* [10]. reported some aminomethylated benzimidazoles with promising antimicrobial activity. The anticancer potential of some Mannich bases was outlined [11, 12].

Beside their biological properties, Mannich bases are industrially important compounds. They are employed as versatile intermediates in chemical and polymer chemistry [13, 14].

This study was designed to synthesize some phenolic Mannich bases and assessment of antimicrobial activity.

Materials and Methods

Materials

Analytical grade reagents (BDH) were used. The UV spectra were recorded on a Perkin-Elmer Lambda 2 UV-Visible Spectrophotometer. Infrared spectra were measured on a Perkin-Elmer1310 Infrared Spectrophotometer. ^{1}HNMR were recorded on EM-360 NMR Spectrophotometer. Mass spectra were measured on a Krates MS 80 RF Mass Spectrometer. To evaluate the synthesized compounds for antimicrobial activity the following test organisms were used:

Table 1

Micro organism	Type	Source
Escherichia Coli	G –ve	ATCC*25922
Bacillus subtilis	G +ve	NCTC* 8236
Staphylococcus aureus	G +ve	ATCC 25923
Pseudomonas aeroginosa	G –ve	NCTC6750
Aspergillus Niger	Fungus	ATCC9736
Candida albicans	Fungus	NCTC10716
Micro organism	Type	Source
Escherichia Coli	G –ve	ATCC*25922
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Aspergillus Niger	Fungus	ATCC9736
Candida albicans	Fungus	NCTC10716

*****NCTC: National Collection of type culture, Colindale England
*ATCC: - American type culture collection, Rockville, Maryland, USA.

Methods

Synthesis protocols

Synthesis of the Mannich base: 2, 5-bis Morpholinomethyl hydroquinone (I)

Ormalin (3g,0.1 mol) was added dropwise to a mixture of hydroquinone(5,5 g, 0.05 mol) and morpholine (8,7g, 0,1mol) in dioxane (25ml) at 0^0C. The mixture was then

stirred at 0^0 C for four hours, and left overnight. The solvent was removed under reduced pressure to afford the product.

Synthesis of the Mannich base: 2, 5-bis piperidinometh hydroquinone (II)

Formalin (3g,0.1 mol) was added dropwise to a mixture of hydroquinone (5,5 g, 0.05 mol) and piperidine (8.50, 0,1mol) in dioxane (25ml) at 0^0 C, the mixture was then stirred at 0^0 C for four hours, and left overnight. The solvent was removed under reduced pressure to give the product.

Synthesis of the Mannich base: 1-(morpholinomethl)-2-Naphthol (III)

Formalin (3g, 0.1 mol) was added dropwise to a mixture of hydroquinone (5, 5 g, 0.05 mol) and morpholine (8,7g, 0,1mol) in dioxane (25ml) at 0^0 C. The mixture was then stirred at 0^0 C for four hours, and left overnight. The solvent was removed under reduced pressure to give the product.

Antimicrobial activity

Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37^0C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 108 -109 colony forming units per ml. The suspension was stored in the refrigerator at 4^0C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique [15]. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for 2 hours at room temperature for the drops to dry, and then incubated at 37 0C for 24 hours. After incubation the number of developed colonies in each plate was counted. The average number of colonies per drop (0.02ml) was multiplied by 50 to give the viable count of the stock suspension expressed as the number of colony forming units per ml of suspension (C.F.U. /ml). Each time a fresh stock suspension was prepared, all of the above experimental conditions were maintained constant so that suspension with very close viable counts would be obtained.

Preparation of fungal suspensions

Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25 ^{0}C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

***In vitro* testing of synthesized compounds for antimicrobial activity**

Testing for antibacterial activity

The cup-plate agar diffusion method [16] was adopted with some minor modifications. Six (ml) of the standardized bacterial stock suspension (108 -109 colony forming units/ml) were homogenously mixed with 600 ml of sterile molten nutrient agar which was maintained at 45^0 C in a water bath. Twenty (ml) aliquots of the inoculated nutrient agar were distributed into sterile Petri dishes and agar was left to settle. Each of these plates was divided into two halves. Two cups in each half (10mm in diameter) were cut using sterile corn borer (No.4). Each half was designed for one of the Mannich bases. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics.The agar discs were removed and alternate cups were filled with 0.1ml samples of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37^0C for 24hours. The above procedure was repeated for different concentrations of the Mannich bases and the standard antimicrobial chemotherapeutics.

Results and Discussion

The target Mannich bases were synthesized via a general synthesis protocol using dioxane as solvent at ice bath temperature. This procedure is considered better than that described by Chi *et al* [18]. Since it involves milder conditions, shorter reaction time and avoids the use of hazardous organic solvents such as benzene and *p*-xylene which are considered toxic to the environment. Furthemore it affords yields comparable to those reported by Chi. *et.al* [18].

Compound (I) was synthesized by reacting hydroquinone with two equivalents of morpholine and formaldehyde. After the usual work up a Mannich base was obtained, m.p. 191°C (43%) **(**Lit.188°-191°C (45%)**.** The IR spectrum of compound (I) gave **ν** (KBr) 758(C-H,Ar. bending), (1200,C-O), (1459, 1563, C=C,Ar.) and 3282cm^{-1} (OH). The UV spectrum gave λ_{max} (MeOH) 205, 225nm which is a characteristic pattern of enolic chromophores (210 and 270nm) [17].

The ^{1}H NMR spectrum showed a signal at δ 2.30 ppm (s**,** 12H) characteristic of six methylenes linked to nitrogen in piperidine moiety, while the signal at δ 3.45 ppm (s, 8H) was assigned for other protons of the piperidine moiety. The signal centered at δ 6.5 ppm (2H) was assigned for the aromatic protons.

The above cumulative data limits the structure of compound (I) to the following:

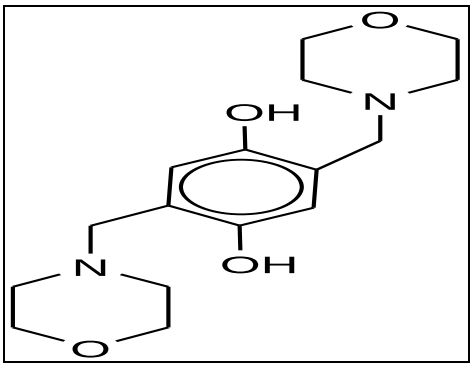

Fig 1: 2, 5-bis (morpholinomethyl) hydroquinone

Compound (II) was synthesized by reaction of hydroquinone with two equivalents of piperidine and formaldehyde. The IR spectrum of (II) gave **ν** (KBr) 772 (C-H,Ar. bending),

1209(C-O), 1494, 1580(C=C,Ar.) and 3426cm^{-1} (OH).The UV spectrum gave the λ_{max} (MeOH) 230nm which is consistent with the absorption pattern of the enolic chromophores [17].

The ^{1}H NMR spectrum revealed a resonance at δ 1.50 ppm (s, 20H) assigned for the protons of piperidine moiety, while the resonance at δ 2.30 ppm (s, 4H) is characteristic of the protons in (-CH_2-N-). The resonance at δ 3.4 (s, 8H) was assigned for four methylenes linked to N in the piperidine moiety, while the signal centered at δ 6.5 ppm (2H) accounts for the aromatic protons.

The above cumulative data limits the structure of (II) to the following:

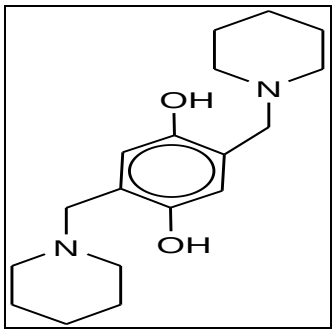

Fig 2: 2, 5-bis (piperidinomethyl) hydroquinone

Compound (III) was synthesized by reaction of 2-naphthol with morpholine and formaldehyde. After the usual work- up a Mannich base was obtained, m.p. 112°C (Lit.111-113°C) **(**92%) [18], The IR spectrum gave **ν** (KBr) 740 (C-H,Ar., bending), 939(O-H out of plane bending), 1230(C-O), 1458, 1477, 1591(C=C, Ar.) and 3422cm^{-1}(OH). The UV spectrum gave λ_{max}(MeOH) 235, 280 nm which is consistent with the absorption pattern of bicyclic phenolic systems.

The **^{1}**H NMR spectrum gave a signal at δ 3.5 ppm (10H) was assigned for the protons of the morpholine moiety and the methylene bridge. The signal at δ 7.07 ppm (d, 2H) is characteristic of C_6 - and C_7- protons while the resonances at δ 7.29 (d, 1H), δ7.4(d,1H), δ 7.71 (m, 1H) and δ 8.0(d,1H) ppm account for C_3-, C_4-, C_5 – and C_8 - protons respectively. The above cumulative data suggests the following structure for III:

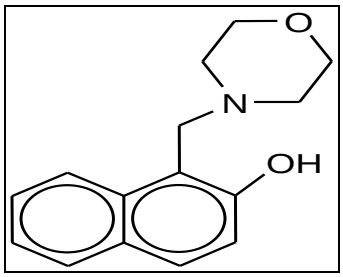

Fig 3: 1-(Morpholinylmethyl)-2-naphthol

Antimicrobial activity

The target Mannich bases were screened for antimicrobial activity against standard human pathogens. The average of the diameters of the growth inhibition zones are shown in Table (1). The results were interpreted in commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active). Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Compound (I) showed very good activity against *Pseudomonas aeruginosa* and excellent activity against *Aspergillus niger* (Table 2) at a concentration of 200mg/ml. However at a concentration of 100mg/ml it showed significant active only against *Aspergillus nige.*

Compound (II) revealed excellent activity against *Pseudomonas aeruginosa* at 200mg/ml. It also showed significant activity against the same organism at at a concentrations of 100 and 50mg/ml.

Compound (III) was inactive against all tested organisms at all test concentrations.

Table 2: Antimicrobial activity of synthesized Mannich bases

Comp.	Concn. (mg/ml	Ec.	Ps.	Bs.	S.a	An.	Ca.
Comp.I	200	-	16	-	1	23	-
	100	-	12	-	9	17	-
	50	-	8	-	-	10	-
Comp. II	200	-	21	10	13	18	16
	100	-	15	-	8	10	8
	50	-	15	-	-	-	-
Comp. III	200	-	-	-	-	-	-
	100	-	-	-	8	-	-

B.s: bacillus subtilis
S.a: Staphylococcus aureus
E.c: Escherichia coli
Ps.: Pseudomonas aeruginosa
A.n: Aspergillus niger
C.a: Candida albicans
MDIZ: Mean diameter of growth inhibition zone (mm). Average of two replicates.

Table 3: Antibacterial activity of standard chemotherapeutic agents

Drug	Conc (mg/ml)	M.D.I.Z (mm) B.s	S.a	E.c	P.s
Ampicilline	40	15	30	_	_
	20	14	26	_	_
	10	11	16	_	_
	20	22	19	19	15

Table 4: Antifungal activity of standard chemotherapeutic agent

Drugs	Conc. (mg/ml)	M.D. I. Z (mm)	
		A.niger	*C. albicans*
Nystatine	50	17	28
	25	13	22
	12.5	8	19

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