International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 2; Issue 4; July 2017; Page No. 88-91

Phenolic mannich bases with antimicrobial potency

[*1] Abdel Karim M, [2] Talal S, [3] Khalid MS
[1] Faculty of Science, Sudan University of Science and Technology, Sudan
[2] Faculty of Pharmacy, Omdurman Islamic University, Sudan
[3] Faculty of Pharmacy, International University of Africa, Sudan

Abstract
Three Mannich bases: 1-(piperidinomethyl)-2-naphthol(I), 1-(porrylidinomethyl)-2-naphthol(II) and 1-(dimethylaminomethyl)-2-naphthol(III) were synthesized. They were identified by UV, IR, NMR and MS data. In diffusion bioassay, the synthesized bases were evaluated for their antimicrobial activity. Compound (I) showed good activity against *Escherichia coli* and *Pseudomonas aeruginosa* at a concentration of 200mgl/ml. Compound (II) exhibited very good activity against the Gram positive strains: *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 200mg/ml, while compound (III) showed good antifungal activity against *Aspergillus niger* at 200mg/ml.

Keywords: mannich bases, synthesis, antimicrobial activity

Introduction
Mannich bases could be synthesized by the condensation of an N-H amine with an aldehyede and an active hydrogen component. Secondary amines are usually employed since they do not afford multiproduct. Successful aminomethylation was achieved by employing formalin or benzaldehyde as an aldehyde component.
Mannich bases are known for their pharmacological potential. Some possess anticonvulsant activity [1, 2], others exhibit analgesic potency [3], while others act[4] as potential chemopreventive agents. Mannich bases with potent 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]. Mannich bases are considered as versatile intermediates in chemical and polymer chemistry [13, 14].

Materials
Analytical grade reagents (BDH) were used. The UV spectra were recorded on a Perkin-Elmer Lambda 2 UV-Visible spectrophotometer. Infra-red spectra were run on a Perkin-Elmer 1310 Infra-red spectrophotometer.[1]HNMR spectra were measured on EM-360 NMR spectrophotometer. Mass spectra were recorded on a Krates MS 80 RF mass spectrophotometer. The target molecules were evaluated for their antimicrobial potency against the following bacterial strains:

Table 1: Test organisms

Microorganism	Type	Source
Escherichia Coli	Gram –ve	TCC*25922
Bacillus subtilis	Gram +ve	CTC* 8236
Staphylococcus aureus	Gram +ve	TCC 25923
Pseudomonas aeroginosa	Gram –ve	NCTC6750
Aspergillus Niger	Fungus	ATCC9736
Candida albicans	Fungus	CTC10716

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

Methods
Synthesis of the Mannich base: 1- (piperidinomethyl) -2-naphthol (I)
Formalin (1.5g, 0.05 mol) was added dropwise to a mixture of 2-naphthol (7.2 g, 0.05 mol) and piperidine (4.3g, 0.05mol) 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- (pyrrolidinomethyl) -2-naphthol (II)
Formalin (1.5g, 0.05 mol) was added dropwise to a mixture of 2-naphthol (7.2 g, 0.05 mol) and pyrrolidine (3.5g, 0.05mol) 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- (dimethylaminomethyl) -2-naphthol (III)

Formalin (1.5g, 0.05 mol) was added dropwise to a mixture of 2-naphthol (7.2 g, 0.05 mol) and dimethylamine (2.3g, 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° C 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 suspensions 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 off with sterile normal saline, and the suspension was stored in the refrigerator until used.

***In vitro* testing of synthesized compounds for antimicrobial 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 cork borer (No.4). Each half was designed for a test solution. 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 test solution 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

Three Mannich bases of heteroaromatic ring systems were synthesized by a general synthesis protocol using dioxane as solvent at ice bath temperature. This procedure is considered better than that described by Chi *et al.* [17]**,** since it involves milder conditions and shorter reaction time. Furthemore it affords yields comparable to those reported by Chi.*et al.* [17]**.**

Compound (I) was synthesized by reaction of 2-naphthol with piperidine and formaldehyde. After the usual workup, compound I, m.p 95°C (87%) was isolated. The IR spectrum gave **ν** (KBr) 767 (C-H, Ar. bending), 925(O-H out of plane bending), 1235(C-O), 1442, 1476, 1598(C=C, Ar.), 3452cm^{-1} (OH). The UV spectrum gave λ_{max} (MeOH) (235, 280 nm) which is a characteristic pattern of bicyclic phenolic systems [18]. The ^{1}H NMR spectrum revealed a pattern characteristic of the target molecule. The signal at δ 1.5 (m, 6H) is characteristic of three methylene protons in the piperidine moiety, while the signal at δ 3.5 ppm (s, 4H) accounts for two methylenes in the piperidine moiety shifted downfield by the electron-withdrawal effect of nitrogen. The resonance at δ 2.40 ppm (m, 2H) was assigned for the benzylic protons. The signals at δ 7.04 (d, 1H), δ 7.75 (4H), δ 7.89 ppm (d,1H) account for the aromatic protons. The mass spectrum gave m/z 242 for (M^+ +1).

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

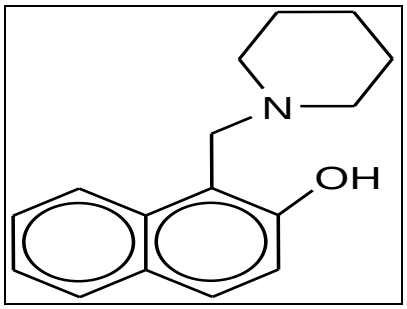

Fig 1: 1-(Piperidinolmethyl)-2-naphthol

Compound (II) was synthesized by reaction of 2-naphthol with pyrrolidine and formaldehyde at ice bath temperature. After the usual work- up a Mannich base (II), m.p 98°C (65%) was isolated. The IR spectrum gave **ν** (KBr) 754 (C-H,Ar.), 943 (O-H out of plane bending), 1051(C-O), 1426 1593, 1617, (C=C, Ar.), 3430cm^{-1}(O-H). The UV spectrum showed λ_{max} (MeOH) (220, 280 nm) which is consistent with the absorption pattern of the bicyclic phenolic systems. The ^{1}H NMR spectrum revealed a signal at δ 1.63 ppm (m, 4H) assigned for two methylenes protons of the pyrrolidine moiety. The signal at δ 2.8 ppm (s, 4H) is characteristic of other methylenes of the piperidine moiety being shifted downfield by electron-withdrawal effect of nitrogen.

The resonance at δ 2.40 ppm (m, 2H) accounts for the benzylic protons. The signals at δ 7.08 ppm (1H), δ 7.5(4H) and δ 8.1 ppm (1H) are characteristic of the aromatic protons. In the electron beam the ion m/z 227, corresponding to the molecular ion, was recorded

The above cumulative data suggests the following structure for compound (II):

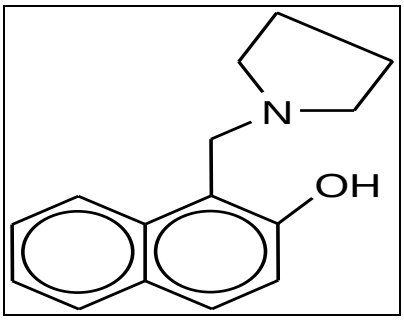

Fig 2: 1-(Pyrrolidinolmethyl)-2-naphthol

Compound (III) was synthesized via the condensation of 2-naphthol with dimethylamine and formaldehyde at ice bath temperature. The IR spectrum of gave **ν** (KBr) 741 (C-H,Ar.,bending), 1264(C-O), 1461,1597(C=C,Ar.) and 3430cm^{-1}(OH). The UV spectrum showed λ_{max} (MeOH) 225, 280 nm. ^{1}H NMR spectrum showed a signal at δ 3.9 ppm (s, 8H) characteristic of two methyls and one four methylene shifted downfild due to elecyton-withdrawal effect of nigtrogen..The signals at δ 7.08, 7.63 (m, 4H) and δ 8.19 ppm (d, 1H) was assigned. The mass spectrum gave m/z202 for (M^+ +1). An important fragment appeared at at *m/z* 143.9 due to the cleavage of the bond between the aromatic ring and the benzylic carbon giving rise to [$C_{10}H_7O$]. Thus compound (III) is a mono Mannich base of the following structure:

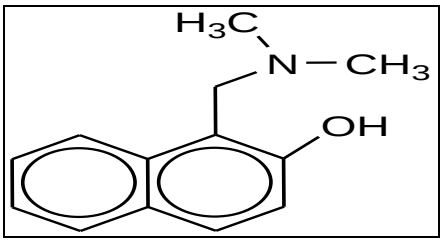

Fig 3: 1-(N N-dimethylamino methyl)-2-naphthol

Antimicrobial Activity

The diffusion bioassay was used to assess the antimicrobial activity of the target molecules. The results are displayed in Table (2). Results were interpreted as follows: (<9mm: inative; 9-12mm: partially active; 13-18mm: active; > 18mm:very active).Tables (3) and (4) represent the antibacterial and antifungal activities of standard drugs respectively.

Compound (I) showed good activity against *E.Coli* and *P. aeroginosa* at a concentration of 200mgl/ml. Compound (II) showed very good activity against the Gram positive strains: *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 200mg/ml.Compound (III) showed activity good antifungal activity against *Aspergillus niger* at 200mg/ml (Tables 2).

Table 2: Antimicrobial activity of Mannich bases

Comound	Concn. (mg/ml)	Ec.	Ps.	Bs.	Sa.	An.	Ca.
Compound I	200	15	14	-	10	-	-
	100	8	10	-	-	-	-
	50	-	-	-	-	-	-
Compound II	200	-	13	16	16	-	-
	100	-	8	-	-	12	8
	50	-	-	-	-	-	-
Compound III	200	-	8	-	-	15	12
	100	-	-	-	-	12	8
	50	-	-	-	-	-	-

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

Table 3: Antibacterial activity of standard chemotherapeutic agents

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

Table 4: Antifungal activity of standard chemotherapeutic agents

Drugs	Concentrations (mg/ml)	M. D. I. Z (mm)	
		A.niger	***C.albicans***
Nystatine	50	17	28
	25	13	22
	12.5	8	19
Clotrimazol	30	22	38
	15	17	31
	7.5	16	29

References

1. Mutle DA, Vnsal C. Hacettepe University Journal of the Faculty of Pharmacy. 2007; 27(1):1.
2. Ozan R, Zuhal O, Unsal C, Butent G, Abdullah AB. Argnei-Forch, Drug Res. 2005; 55(8):431.

3. Nabil M, *et al*. Journal of Islamic Acedemy of Science. 1993; 6(2):99.
4. Robert M, *et al*. Eur. J Med. Chem. 2006; 41:263.
5. Ina-Gull H, *et al.* pharmaceutica Acta Helvetiae. 2000; 74:393.
6. Ebru M, Halise IG, Cuvit K. Molecules. 2007; 12:2579.
7. Jontathan R, *et al*. Eur. J Med. Chem. 2002; 37:35.
8. Stephen J, Judith J, Leslie M. J Med. Chem. 1987; 30:906.
9. Tomas L, *et al.* Eur. J Med. Chem. 2002; 37:803.
10. Afaf H, Fahmy HH, Ali A. *Molecules.* 2000; 5:1429.
11. Janathan R, *et al*. J Med. Chem. 1998; 41:4012.
12. Dimmock, JR, Kamar P. Curr. Med. Med. 1997; 4:1.
13. Aura AM, Martin-Lopez P, O'Leary KA, Williamson G, Oksman-Caldentey KM, Poutanen K. *et al*. European journal of nutrition. 2005; 3:133.
14. Dimmolic JR, *et al.* J Med Chem. 1983; 18:249