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SYNTHESIS OF FLAVANOID -DERIVED ANTIMICROBIAL MANNICH BASES

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ABSTRACT

One flavonoid(1) was synthesized via a general synthesis protocol. Three flavonoid-derived Mannich bases(2-4) were synthesized via the Mannich reaction of 2-hydroxyacetophenone with vanillin. The structures of the target molecules were elucidated by a combination of spectral tools (IR, UV, ^{1}HNMR, and MS). The synthesized compounds were screened for their antimicrobial activity. In cup plate agar diffusion bioassay, compound (2,3) showed activity against all test organisms at 200, 100 and 50mg/ml. The same trend was observed for compound (1). However, compounds (2) and (3) showed significant antimicrobial activity at 100 and 200mg/ml.

KEYWORDS: flavonoids, Mannich bases, Antimicrobial activity.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds that are widely distributed in the plant kingdom. They occur naturally as plant pigments in a broad range of fruits and vegetables as well as beverages.[1,2] Flavonoids are phenolics comprising 15 carbons, with two aromatic rings bound together by three carbon atoms that form an oxygenated heterocyclic ring (C_6-C_3-C_6). They are found throughout the plant kingdom and in particular in leaves and in the skin of fruits. Based on the variation of their heterocyclic ring, flavonoids are divided into different sub-classes: anthocyanidins, flavans, flavanones, flavones, flavonols, isoflavones, dihydroflavonols, flavan-3, 4-diols, coumarins, chalcones, dihydrochalcones and aurones. The basic C_6-C_3-C_6 flavonoid skeleton can have numerous substituents (e.g. hydroxyl, acetyl,

methoxy and methyl groups) and the majority of the flavonoids exist naturally as glycosides.[3,4]

Flavonoids have gained recent interest because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological effects including antimicrobial[5], cytotoxicity[6], anti-inflammatory[7] as well as antitumor activities.[8,9] Flavanones exhibit anti-oxidant, immunomodulatory and chemopreventive properties.[10] Flavanones with a hydroxyl functions at $C_{4'}$ and C_6 have shown significant cytotoxic and apoptotic effects against tumor cells, compared with other structurally related flavanones.[11] The hydroxylation at C_6 plays an important role in antioxidant activity of flavanones.[11]

MATERIALS AND METHODS

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}HNMR were recorded on EM-360 NMR Spectrophotometer. Mass spectra were measured on a Krates MS 80 RF Mass Spectrometer.

Methods

Synthesis of compound (1)

2-hydroxyacetophenone (2.72g, 20mmol) was added to vanilline (3.04g, 20mmol) in NaoH/ethanol 20% (20ml). The mixture was stirred for 24 hours at room temperature and left overnight. Removal of the solvent under reduced pressure gave the product.

Synthesis of compound (2)

Formalin(0.4g,5mmol) was added dropwise with stirring to a mixture of compound (1) (1.345g,5mmol) and piperidine (0.85g,5mmol) in absolute ethanol (10ml) at 0^0C. The mixture was then stirred for 1 hour and left overnight. Removal of the solvent under reduced pressure gave the product.

Synthesis of compound (3)

Formalin(0.4g,5mmol) was added dropwise with stirring to a mixture of compound (1) (1.345gm,5mmol) and N-methylpiperazine (0.86gm,5mmol) in absolute ethanol (10ml) at 0^0C. The mixture was then stirred for 1 hour and left overnight. Removal of the solvent under reduced pressure gave the product.

Synthesis of compound (4)

Formalin(0.4g,5mmol) was added dropwise with stirring to a mixture of compound (1) (1.345gm,5mmol) and dimethylamine (0.225gm,5mmol) in absolute ethanol (10ml) at 0^0C. The mixture was then stirred for 1 hour and left overnight.Removal of the solvent under reduced pressure gave the product.

Antimicrobial assay

The synthesized molecules were screened for antimicrobial activity against four bacterial strains: Gram-positive (*Staphylococus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and two fungal species **(***Aspergillus niger, Candida albicans*). The cup plate agar diffusion method was used.

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.[12]

Serial dilutions of the stock suspension were made in sterile normal saline solution and (0.02 ml) volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two 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 drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension

The fungal cultures were maintained on dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in (100ml) of sterile normal saline. Suspension were stored in the refrigerator until used.

Testing of antibacterial susceptibility

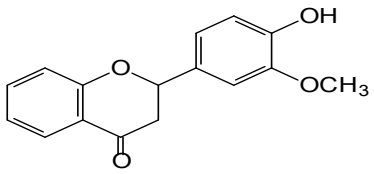
The disc diffusion bioassay was used to screen the antimicrobial activity of the target molecules and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.[12] Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of compound I. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured in duplicates and averaged.

Testing of antifungal susceptibility

The above mentioned method was adopted for antifungal activity, but instead of agar, Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

RESULTS AND DISCUSSION

Compound (1) was synthesized by adding 2-hydroxyacetophenone to 3- Vanillin in NaoH/Ethanol- 20% (20ml). The IR spectrum gave υ(KBr): 640,817, 877 (C-H, Ar., bending),1120 (C-O), 1434 (C=C, Ar.), 1685(C=O), 2981 (C-H,aliph.) and 3244 (OH stretching).

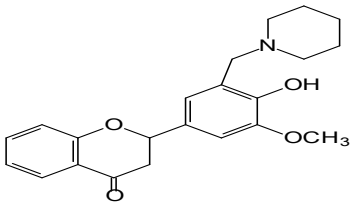


(1)

The ^{1}H NMR spectrum revealed the following signals : $\delta 1.3$ assigned for a methylene group; δ3.80 (s,3H) accounting for a methoxyl function.The resonances at δ6.9(d,3H) and δ7.9(m,4H) account for the aromatic protons.The mass spectrum gave m/z 271 corresponding to M^++1.

Compound (2)

Compound (2) was synthesized by adding formalin to a mixture of compound (1) and piperidine in absolute ethanol. The IR spectrum gave υ (KBr): 794, 817, 864(C-H, Ar., bending). 1159 (C-O), 1282 (C-N), 1448 (C=C, Ar.), 1637(C=O), 2937 (C-H,aliph.) and 3396 (OH stretching).

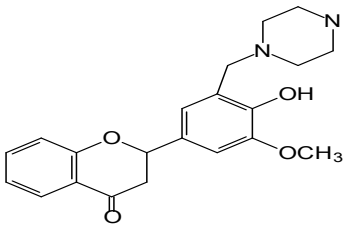


(2)

The ^{1}H NMR spectrum revealed the following signals: 1.40(s,2H) assigned for a methylene moiety; δ1.9 (t,6H) assigned for three methylenes; δ2.4 (t, 6H) accounting for the three methylenes(linked to N); δ3.2 (s,3H) attributed to a methoxyl function. The resonances at δ6.7(s,2H) and δ7.5(m,4H) account for the aromatic protons. The mass spectrum gave m/z 367 corresponding to M^+.

Compound (3)

Compound (3) was synthesized by adding formalin to a mixture of compound (1) and N-methylpiperazine in absolute ethanol. The IR spectrum of gave υ (KBr): 640,723,879 (C-H, Ar. bending), 1259 (C-O), 1363 (C-N),1433 (C=C, Ar.), 1666 (C=O), 2968 (C-H,aliph.) and 3390 (OH stretching).

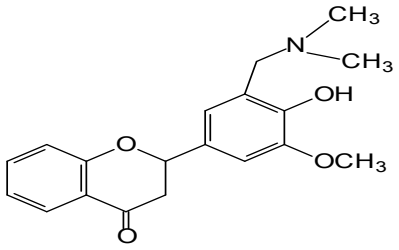


(3)

The ^{1}H NMR spectrum revealed the following signals: δ1.1 (s,4H) assigned for two methylenes; δ1.5 (d,6H) accounting for three methylenes; δ3.2 (s,3H) attributed to a methoxyl function.The aromatic protons appeared at δ6.7(s,2H) and δ7.5(m,4H). The mass spectrum gave m/z 368 corresponding to M^++1.

Compound (4)

Compound (4) was synthesized by adding formalin to a mixture of compound (1) and dimethylamine in absolute ethanol. The IR spectrum gave υ(KBr) : 640, 723,865 (C-H, Ar. bending), 1226 (C-O) , 1361 (C-N),1456 (C=C, Ar.), 1654 (C=O), 2972 (C-H,aliph.) and 3419 (OH stretching).



(4)

The ^{1}H NMR spectrum revealed the following signals : δ0.9 (s,6H) assigned for two methyl groups ; δ1.5 (d,2H) assigned for a methylene moiety. The signal at δ1.9 (t,1H) accounts for a methine moiety while the resonance at δ3.50 was attributed to a methoxyl function. The resonances at δ6.7(s,2H) and δ7.8(m,4H) account for the aromatic protons.The mass spectrum gave m/z 327 corresponding to M^+.

Antimicrobial activity

The synthesized compounds were screened for their antimicrobial activity at different concentration (50mg/ml, 100mg/ml and 200mg/ml) against standard human pathogens: Gram +ve bacteria: *Staphylococcus aureus* and *Bacillus subtilis*, Gram -ve: *Pseudomonas aeruginosa*, *Echerichia coli* and the fungal species *Aspergillus niger* and *Candida albicans*. The mean diameters of inhibition zone (MDIZ) and the minimum inhibitory concentration (MIC) produced by compounds (1-4) on standard microorganisms are presented in Table (1). The results were interpreted in commonly used terms: < 9 mm considered inactive; 9-12 mm partially active; 13-18 mm active and more than 18 mm very active. The antibacterial and antifungal activities of standard chemotherapeutic agents are displayed in Tables (2) and (3) respectively.

In disc diffusion, compound (1) showed activity against all test organisms at 200,100 and 50mg/ml. The same trend was observed in (3 and 4). However, compounds (2) and (3) showed significant antimicrobial activity at 100 and 200mg/ml. Further optimization of these leads is underway.

Table 1: Antimicrobial activity of synthesized compounds against standard organisms.

Sample	Conc. mg/ml	E.c	P.a	B.s	S.a	C.a	A.n
Compound(1)	200	15	15	15	16	15	14
	100	14	13	14	14	14	13
	50	14	13	14	14	14	13
Compound(2)	200	17	17	18	18	14	18
	100	17	16	18	18	13	17
	50	16	16	17	17	13	15
Compound(3)	200	21	20	18	23	20	20
	100	18	18	15	20	17	18
	50	17	16	15	17	15	16
Compound(4)	200	15	15	15	14	17	18
	100	14	13	14	13	15	14
	50	13	12	12	12	13	13

Antibacterial activity of standard chemotherapeutic agents against standard bacteria:M.D.I.Z (mm)

Drug	Conc. mg/ml	B.s.	S.a.	E.c.	P.a.	S.t
Ampicillin	40	15	30	-	-	-
	20	14	25	-	-	-
	10	11	15	-	-	-
Gentamycin	40	25	19	22	21	22
	20	22	18	18	15	17
	10	17	14	15	12	14

Antifungal activity of standard chemotherapeutic agents against standard fungi.

Drug	Conc. mg/ml	A.n	C.a
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- S.a*: Staphylococcus aureus*
- E.c*: Escherichia coli*
- P.a: *Pseudomonas aeruginosa*
- A.n: *Aspergillus niger*
- C.a: *Candida albicans*
- S.t: *Salmonella typhi*
- *B.a: Bacillus subtilis*

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