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Synthesis and Evaluation of Some Mannich Bases of Oxytetracycline.

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ABSTRACT

New oxytetracycline Mannich bases were prepared and analytically and spectroscopically characterized. The antimicrobial screening tests by Well diffusion method revealed that some derivatives are biologically more active than the parent oxytetracycline and they are more active against *E. coli* and *K. pneumonia* bacteria than *A. niger* Fungus.

Keywords: Oxytetracycline, Mannich base, antimicrobial.

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INTRODUCTION

Mannich reaction [1] is a nucleophilic addition reaction which involves which involved the condensation of a compound with active hydrogen with an amine (primary or secondary) and formaldehyde. The schematic representation of general Mannich reaction is given in Scheme1.

Oxytetracycline is a broad-spectrum antibiotic, active against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections. The purpose of this work was to prepare new derivatives of oxytetracycline via Mannich reaction on position 2 (the amide) to yield a new derivative that could have better physical and microbiological properties.

The new derivatives were characterized using several techniques such as IR, NMR, elemental. The bioactivity of prepared compounds was studied against *E. coli, K. pneumoniae* and *A. niger* using Well Diffusion Method.

RESULTS AND DISCUSSION

The reaction schemes employed for the synthesis of the new target oxtetracycline Mannich bases **(1-4)** are illustrated in **Scheme 2** and **Scheme 3**.

Reaction of amide group of the oxytetracyline with iminium cation that was formed from the reaction of diethyamine, dipropylamine, morpholine and piperidine with formaldehyde in methanol afforded mannich bases **1-4** respectively.

Mannich bases **5** and **6** were prepared by the reaction of oxytetracycline with L-lysine hydrochloride in THF/water mixture (50:50). The two isomers **5** and **6** were separated using flash chromatography (silica gel, 5% MeOH/ether). The structures of compounds **1-6** were confirmed by spectral (IR, ^{1}H-NMR, ^{13}H-NMR) as well as elemental analysis.

ANTIMICROBIAL SCREENING

The new derivatives were subjected to antifungal and antibacterial screening tests against fungi as *A. niger* and bacteria like *E. coli* as well as *K. pneumonia*. The screening data are summarized in **Table 1**. The complexes are more active against bacteria than fungi. The MIZ values revealed that the two isomers of the lysino-oxytetracycline Mannich bases **5** and **6** exhibit antibacterial activity against *E. coli* (MZI = 40(114%), 39(111%) respectively which is more than that observed for antibacterial drug, Amoxicillin, MZI = 35, **Table 1**. Moreover, the other Mannich derivatives **1-4** have moderate antibacterial activity against *E. coli* with MIZ in the 26-31 mm and activity index ranged from 74% to 89%, in comparing to antibacterial drug, oxtetracycline. Moreover, the six derivatives showed less activity against *K. pneumonia* ranged from 68%-98%.

Table 1. Biological activities of the new derivatives against *A. niger, E. coli* and *K. Pneumonia*

No.	Inhibition zone (mm) and Activity Index (%)					
	A. niger	Activity Index (%)	E. coli	Activity Index (%)	K. Pneumonia	Activity Index (%)
DMSO	0	0%	0	0%	0	0%
Nystatin	25	100%	--	0%	--	--
Oxytetracycline	--	--	35	100%	44	100%
1	12	48%	29	83%	33	75%
2	13	52%	31	89%	30	68%
3	15	60%	26	74%	38	86%
4	17	68%	28	80%	35	79%
5	16	64%	40	114%	41	93%
6	18	72%	39	111%	43	98%

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Otherwise, the antifungal screening data reveled that all complexes have a weak activities against *A. niger* with MIZ values in the 12-18 mm ranges and activity index in the 48-72% ranges in comparing to antifungal drug Nystatin.

MATERIALS AND METHODS

General methods

All chemicals were obtained from Aldrich Chemical & Co. and used without purification.. melting point using Gallenkamp Melting point Apparatus Model MFB 595 and also analytical Thin Layer Chromatography. Thin Layer Chromatography was carried out using Silica Gel F_{254} precoated plate (Merck, Germany). IR spectra in KBr discs were recorded on a JASCO FT-IR 460 plus spectrophotometer.

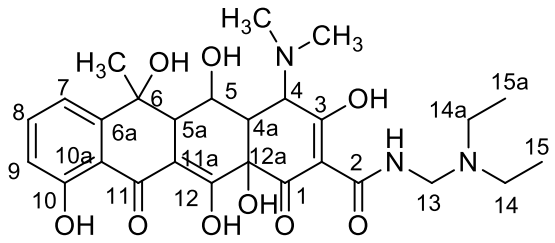
NMR spectroscopy was carried out using Brucker instrument model AVANCE II 600, Assignment of proton chemical shifts and the ^{13}C NMR is based on COSY, DEPT, HMQC and HMBC spectra and partly through the comparison with the reported values of similar compounds. Microanalyses, of the compounds were performed in the Micro analytical Laboratory in chemistry Department, the Hashemite University.

Synthesis

Synthesis of Mannich bases derived from secondary amines and oxytertacycline (1-4), General procedure:

Following the procedure of Rajesh and Bahekar [2] with some modifications, appropriate secondary amine (0.01 mole) as shown in Scheme 2, was gradually added to a solution of oxtetracycline (0.01 mole) in dried methanol (12 mL), followed by addition of formaldehyde solution (38 %, 0.8 mL, 0.015 mol). The reaction mixture was stirred for 1 h at room temperature and allowed to stand overnight at 0 °C. Then precipitate was filtered, dried and recrystallised using hot ethanol.

N-((Diethylamino)methyl)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo 1,4,4a,5,5a, 6, 11,12a-octahydrotetracene-2-carboxamide (1)



Yellow powder; yield: 83% at 25 °C, mp.154–156 °C. FTIR (KBr disc): (cm^{-1}): 3285, 3312 ν(N-H), 1713 ν(C=O), 1390 ν(CO).

^{1}H-NMR (600 MHz, δ/ppm, J/Hz, CD_3OD, 20°C): 1.01 (6H, t, j= 8.0, H-15, H-15a), 1.81 (3H, s, -CH_3), 2.60 (4H, q, j= 8.0, H-14&H-14a), 2.88 (1H, dd, j = 8.4, 0.7 Hz, H-5), 2.87 (1H, dd, j = 11.1 Hz, H-5a), 2.85 (1H, dd, J = 8.2, 0.7 Hz, H-4a), 2.96 (6H, br s, N-CH3), 3.21 (1H, dd, 8.3, 0.8 Hz, H-4), 3.71 (2H, t, H-13) 3.91 (1H, t, -NH), 4.33 (1H, s, -NH), 4.65 (1H, t, j= 8.0, H-18), 6.93 (1H, dd, j = 8.4, 0.7 Hz, H-8), 7.17 (1H, dd, j = 7.3, 0.7 Hz, H-7), 7.55 (1H, dd, j = 8.0, 0.8 Hz, H-9).

^{13}C-NMR (150 MHz, CD_3OD, 20°C) : 13.1 (C-15&C-15a), 24.2 (C-4a), 24.9 (-CH3), 28.0 (C-17) 31.4 (C-5a), 47.7 (C-14&C-14a), 52.2 (C-13), 55.1 (-NCH_3), 66.4 (C-5), 68.3 (C-6), 70.0 (C12a), 71.0 (C-4), 105.8 (C-3), 106.0 (C11a) 115.9 (C-2), 116.2 (C-7), 118.7 (C-8), 137.8 (C-9), 148.0 (C-6a), 163.8 (C-10), 173.4 (amide C=O), 174.2 (C-12), 196.0 (C-11), 210 (C-1).

Anal. Calcd for $C_{27}H_{35}N_3O_9$: C, 59.44; H, 6.47; N, 7.70. Found: C, 59.89; H, 6.43; N, 7.69.

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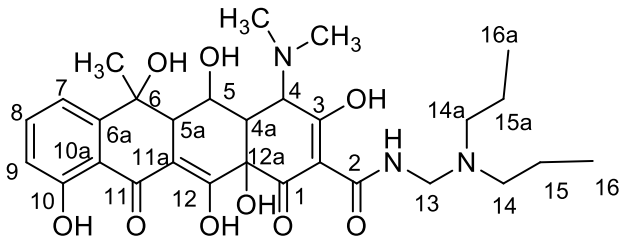
4-(Dimethylamino)-N-((dipropylamino)methyl)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5, 5a ,6, 11,12a-octahydrotetracene-2-carboxamide (2)



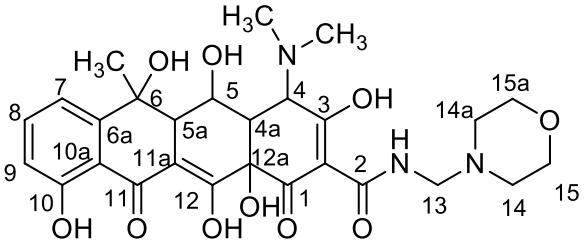
Yellow powder; yield: 73% at 25 °C, mp.134–136 °C. FTIR (KBr disc): (cm−1): 3280, 3315 ν(N-H), 1710 ν(C=O), 1391 ν(CO).

^{1}H-NMR (600 MHz, δ/ppm, J/Hz, CD_3OD, 20°C): 0.90 (6H, t, j= 8.0, H-16, H-16a), 1.51 (4H, m, H-15, H-15a), 1.80 (3H, s, -CH_3), 2.45 (4H, t, j= 8.0, H-14&H-14a), 2.87 (1H, dd, j = 8.4, 0.7 Hz, H-5), 2.83 (1H, dd, j = 11.1 Hz, H-5a), 2.82 (1H, dd, J = 8.2, 0.7 Hz, H-4a), 2.93 (6H, br s, N-CH3), 3.21 (1H, dd, 8.3, 0.8 Hz, H-4), 3.70 (2H, t, H-13) 3.91 (1H, t, -NH), 4.33 (1H, s, -NH), 4.66 (1H, t, j= 8.0, H-18), 6.90 (1H, dd, j = 8.4, 0.7 Hz, H-8), 7.14 (1H, dd, j = 7.3, 0.7 Hz, H-7), 7.54 (1H, dd, j = 8.0, 0.8 Hz, H-9).

^{13}C-NMR (150 MHz, CD3OD, 20°C) : 11.1 (C-16&C-16a), 20.1 (C-15&C-15a), 24.2 (C-4a), 24.9 (-CH_3), 31.2 (C-5a), 47.3 (C-14&C-14a), 52.1 (C-13), 55.1 (-NCH_3), 66.2 (C-5), 68.2 (C-6), 70.1 (C12a), 71.2 (C-4), 105.8 (C-3), 106.4 (C11a) 115.6 (C-2), 116.1 (C-7), 118.6 (C-8), 137.7 (C-9), 147.5 (C-6a), 163.6 (C-10), 173.5 (amide C=O), 174.4 (C-12), 196.2 (C-11), 210.1 (C-1).

Anal. Calcd for $C_{29}H_{39}N_3O_9$: C, 60.72; H, 6.85; N, 7.33. Found: C, 61.01; H, 6.73; N, 7.59.

4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-N-(morpholinomethyl)-1,11-dioxo-1,4,4a,5,5a,6, 11, 12a-octahydrotetracene-2-carboxamide (3)



Yellow powder; yield: 79%, mp.144–146°C. FTIR (KBr disc): (cm−1): 3277, 3311 ν(N-H), 1720 ν(C=O), 1381 ν(CO).

^{1}H-NMR (600 MHz, δ/ppm, J/Hz, CD_3OD, 20°C): 2.51 (4H, t, j= 7.8, H-14, H-14a), 1.81 (3H, s, -CH_3), 3.46 (4H, t, j= 7.8, H-15&H-15a), 2.88 (1H, dd, j = 8.4, 0.7 Hz, H-5), 2.87 (1H, dd, j = 11.1 Hz, H-5a), 2.83 (1H, dd, J = 8.2, 0.7 Hz, H-4a), 2.94 (6H, br s, N-CH3), 3.21 (1H, dd, 8.3, 0.8 Hz, H-4), 3.72 (2H, t, H-13) 3.91 (1H, t, -NH), 4.35 (1H, s, -NH), 4.66 (1H, t, j= 8.0, H-18), 6.90 (1H, dd, j = 8.3, 0.7 Hz, H-8), 7.16 (1H, dd, j = 7.3, 0.7 Hz, H-7), 7.52 (1H, dd, j = 8.0, 0.8 Hz, H-9).

^{13}C-NMR (150 MHz, CD_3OD, 20°C) : 24.1 (C-4a), 24.6 (-CH_3), 31.3 (C-5a), 53.7 (C-14&C-14a), 66.1 (C-15&C-15a), 52.1 (C-13), 55.0 (-NCH3), 66.3 (C-5), 68.2 (C-6), 70.2 (C12a), 71.4 (C-4), 105.8 (C-3), 106.0 (C11a) 115.9 (C-2), 116.2 (C-7), 118.7 (C-8), 137.8 (C-9), 148.0 (C-6a), 163.5 (C-10), 173.2 (amide C=O), 174.1 (C-12), 195.6 (C-11), 210.2 (C-1).

Anal. Calcd for $C_{27}H_{33}N_3O_{10}$: C, C, 57.95; H, 5.94; N, 7.51. Found: C, 58.01; H, 6.03; N, 7.59.

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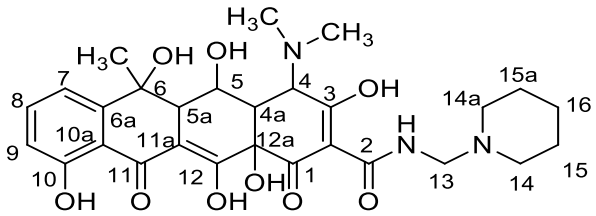
4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-N-(piperidin-1-ylmethyl)-1,4,4a,5, 5a, 6, 11,12a-octahydrotetracene-2-carboxamide (4)



Yellow powder; yield: 73% at 25 °C, mp.164–166 °C. FTIR (KBr disc): (cm−1): 3271, 3310 ν(N-H), 17240 ν(C=O), 1385 ν(CO).

^{1}H-NMR (600 MHz, δ/ppm, J/Hz, CD_3OD, 20°C): 0.93 (6H, t, j= 8.0, H-16), 1.54 (4H, m, H-15, H-15a), 1.83 (3H, s, -CH_3), 2.46 (4H, t, j= 8.0, H-14&H-14a), 2.88 (1H, dd, j = 8.4, 0.7 Hz, H-5), 2.87 (1H, dd, j = 11.1 Hz, H-5a), 2.81 (1H, dd, J = 8.2, 0.7 Hz, H-4a), 2.92 (6H, br s, N-CH_3), 3.22 (1H, dd, 8.3, 0.8 Hz, H-4), 3.75 (2H, t, H-13) 3.90 (1H, t, -NH), 4.35 (1H, s, -NH), 4.65 (1H, t, j= 8.0, H-18), 6.95 (1H, dd, j = 8.4, 0.7 Hz, H-8), 7.18 (1H, dd, j = 7.3, 0.7 Hz, H-7), 7.52 (1H, dd, j = 8.0, 0.8 Hz, H-9).

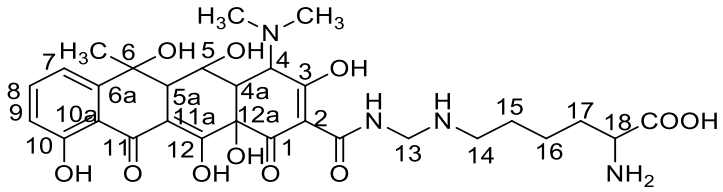
^{13}C-NMR (150 MHz, CD_3OD, 20°C): 11.5 (C-16), 20.0 (C-15&C-15a), 24.1 (C-4a), 24.5 (-CH_3), 31.4 (C-5a), 47.7 (C-14&C-14a), 52.2 (C-13), 55.1 (-NCH3), 65.4 (C-5), 68.3 (C-6), 70.0 (C12a), 71.4 (C-4), 105.8 (C-3), 106.0 (C11a) 115.6 (C-2), 116.2 (C-7), 118.7 (C-8), 137.8 (C-9), 148.0 (C-6a), 163.8 (C-10), 173.4 (amide C=O), 174.2 (C-12), 196.5 (C-11), 211.1 (C-1).

Anal. Calcd for $C_{28}H_{35}N_3O_9$: C, 60.31; H, 6.33; N, 7.54; Found: C, 60.41; H, 6.73; N, 7.49.

Synthesis of tetracycline Mannich bases (5-6) using L-lysine hydrochloride, General procedure:

Similar procedure to that used before in preparation compounds **1-4** was performed but the solvent was THF/water as showen in Scheme 3.

2-Amino-6-(((4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamido)methyl)amino)hexanoic acid (5).



Orange crystals; yield: 83% at 25 °C, mp.144–147 °C. FTIR (KBr disc): (cm^{-1}): 3289, 3310 ν(N-H), 1710 ν(C=O), 1391 ν(CO).

^{1}H-NMR (600 MHz, δ/ppm, J/Hz, CD_3OD, 20°C): 1.55 (4H, m, 2-CH2, H-14&H-17), 1.81 (3H, s, -CH3), 1.90 (4H, m, 2-CH2, H-15&H-16), 2.88 (1H, dd, j = 8.4, 0.7 Hz, H-5), 2.87 (1H, dd, j = 11.1 Hz, H-5a), 2.85 (1H, dd, J = 8.2, 0.7 Hz, H-4a), 2.96 (6H, br s, N-CH3), 3.21 (1H, dd, 8.3, 0.8 Hz, H-4), 3.71 (2H, t, H-13) 3.91 (1H, t, -NH), 4.33 (1H, s, -NH), 4.65 (1H, t, j= 8.0, H-18), 6.93 (1H, dd, j = 8.4, 0.7 Hz, H-8), 7.17 (1H, dd, j = 7.3, 0.7 Hz, H-7), 7.55 (1H, dd, j = 8.0, 0.8 Hz, H-9).

^{13}C-NMR (150 MHz, CD_3OD, 20°C) : 23.1 (C-16), 24.2 (C-4a), 24,9 (-CH_3), 28.0 (C-17) 31.4 (C-5a), 40.5 (C-14), 30.7 (C-15), 52.2 (C-13), 55.1 (-NCH3), 66.4 (C-5), 68.3 (C-6), 70.0 (C12a), 71.0 (C-4), 105.8 (C-3), 106.0 (C11a) 115.9 (C-2), 116.2 (C-7), 118.7 (C-8), 137.8 (C-9), 148.0 (C-6a), 163.8 (C-10), 173.4 (amide C=O), 174.2 (C-12), 175.4 (COOH) 196.0 (C-11), 210 (C-1).

Anal. Calcd for $C_{29}H_{38}N_4O_{11}$: C, 56.30; H, 6.19; N, 9.06; Found: C, 56.29; H, 6.13; N, 9.09.

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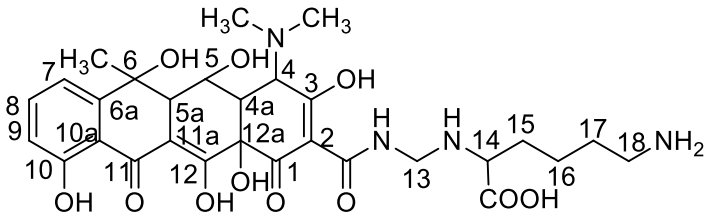
6-Amino-2-(((4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamido)methyl)amino)hexanoic acid (6)



Orange crystals; yield: 90% at 25 °C, mp.164–166 °C. FTIR (KBr disc): (cm^{-1}): 3290, 3315 ν(N-H), 1706 ν(C=O), 1381 ν(CO).

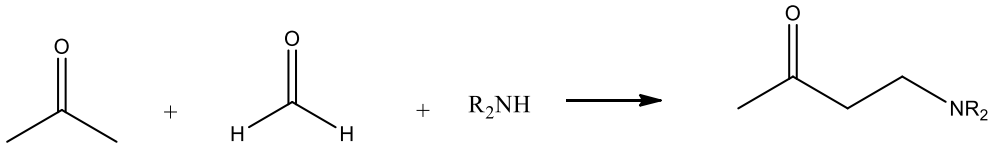
^{1}H-NMR (600 MHz, δ/ppm, J/Hz, CD3OD, 20°C): 1.55 (4H, m, 2-CH2), 1.79 (2H, m, H-13), 1.91 (4H, m, 2-CH2), 1.81 (3H, s, -CH3), 1.93 (1H, s, H-14) 2.88 (1H, dd, j = 8.4, 0.7 Hz, H-5), 2.87 (1H, dd, j = 11.1 Hz, H-5a), 2.85 (1H, d, J = 8.2 Hz, H-4a), 2.98 (6H, br s, N-CH3), 3.73 (1H, t, 8.3, 2.8 Hz, H-5), 3.90 (1H, t, -NH), 4.35 (1H, s, -NH), 6.93 (1H, dd, j = 8.4, 0.7 Hz, H-8), 7.17 (1H, dd, j = 7.3, 0.7 Hz, H-7), 7.54 (1H, t, j = 8.0 Hz, H-9).

^{13}C-NMR (150 MHz, CD3OD, 20°C) δ: C 19.2 (C-4a), 23.1 (-CH3), 23.1 (C-13) 24.8 (C-5a), 40.5 (C-18), 28.0 (C-16) 40.3 (C-18), 47.9 (C-13), 55.2 (-NCH3), 58.3 (C-6), 66.3 (C-14) 66.4 (C-5), 68.3 (C-12a), 71.0 (C-4), 105.8 (C-3), 106.0 (C-11a) 115.5 (C-2), 116.2 (C-7), 118.7 (C-8), 137.8 (C-9), 148.9 (C-6a), 163.8 (C-10), 173.2 (amide C=O), 174.2 (COOH), 196.0 (C-11), 219 (C-1).

Anal. Calcd for $C_{29}H_{38}N_4O_{11}$: C, 56.30; H, 6.19; N, 9.06; Found: C, 56.35; H, 6.23; N, 9.19.

Biological activity

In vitro antibacterial and antifungal assays were performed by well diffusion method in the Lab of microbiology, Biology Department, Faculty of Science, The Hashemite University [58-61]. Both positive (nystatin for fungi, oxtetracyline for bacteria) and negative (solvent, DMSO) controls were used in the technique. The complexes and ligand were tested against fungi such as *Aspergillus niger (A. niger)* and bacteria like *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumonia)* cultured on Czapek Dox´s agar and nutrient agar as medium respectively. In a typical procedure, a well was made on the agar medium inoculated with the fungi or bacteria. The well was filled with the test solution (20 mg\ mL) using a micropipette and the plate was incubated at 28 and 37 °C respectively for 72 h. During this period, the test solution diffused and the growth of the inoculated fungi or bacteria was affected. The inhibition zone (MIZ) developed on the plate was measured. Each test was carried out for three times to minimize the error. The activity index for the complexes was calculated by following formula.



Scheme 1. General Mannich reaction

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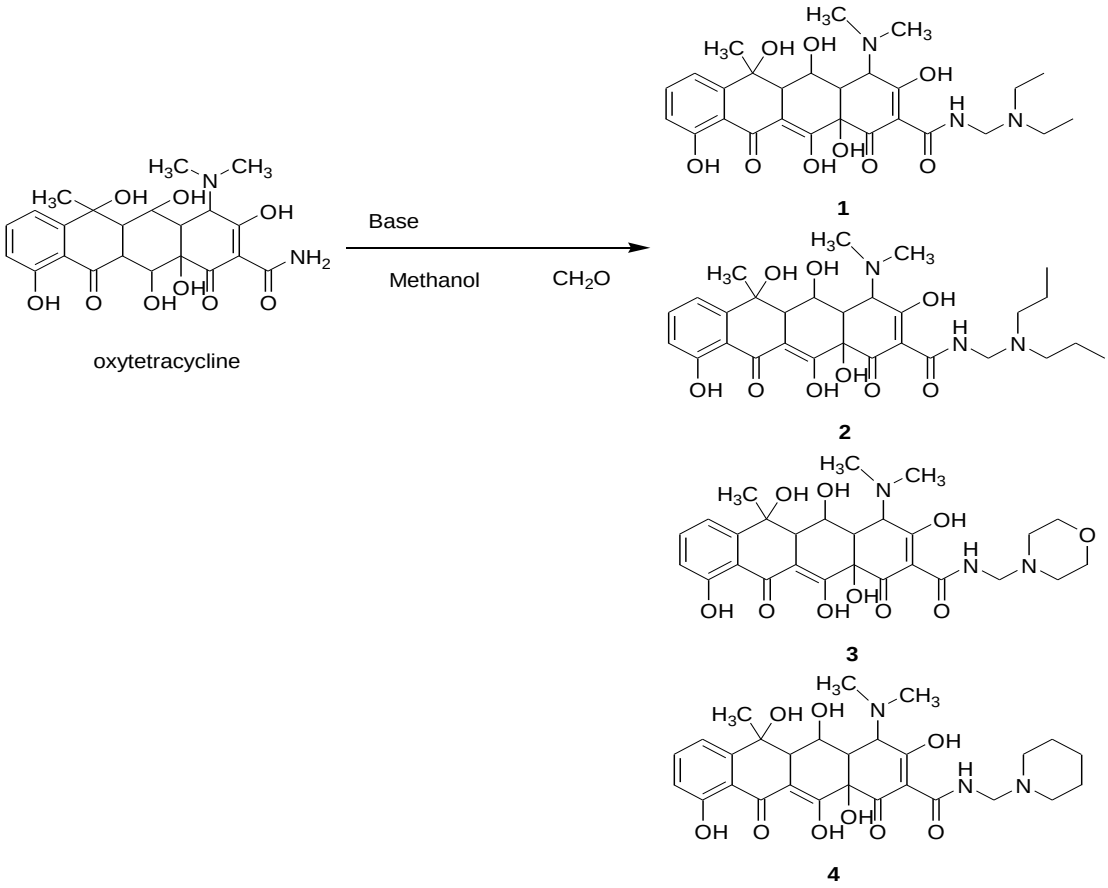
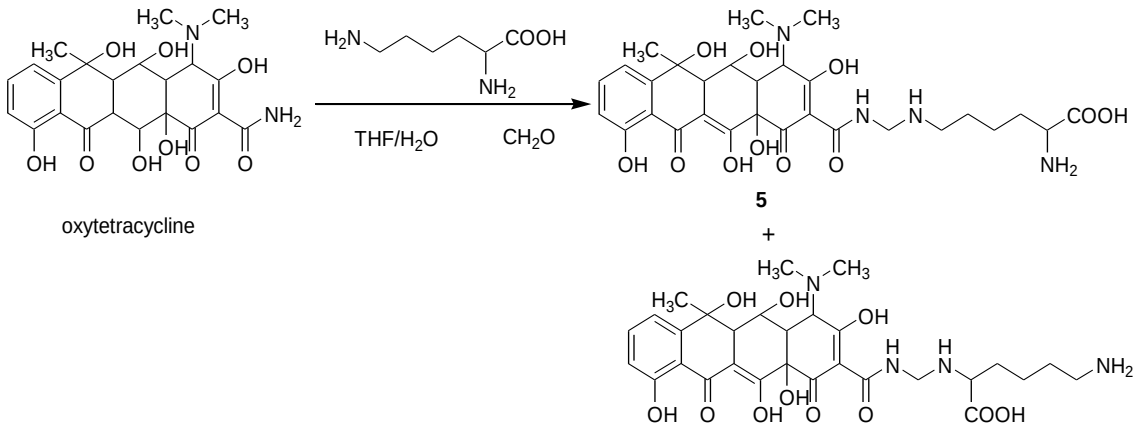


Scheme 2. General method for preparation of Mannich bases 1-4



Scheme 3. Preparation of Mannich bases 5-6

CONCLUSIONS

In this study we have reported the characterization of synthetic Mannich bases **1-6**. The in vitro antimicrobial activity of synthetic Mannich bases were investigated. Antimicrobial screening tests showed that the new compounds have exhibit higher antibacterial activities than antifungal activities. However, the compounds showed mild to good active against fungi than bacteria. The data revealed that the compounds **5** and **6** exhibit antibacterial activity against *E.* more than that observed for antibacterial drug, oxytetracycline.

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