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Synthesis and biological study of medicinally important Mannich bases derived from 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide

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Abstract—The paper describes synthesis and antibacterial study of biologically active Mannich bases of carboxamide derivative employing Mannich reaction of 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide with various sulfonamides/secondary amines .They were analysed by elemental analysis and characterized by UV, IR and ^{1}H NMR spectroscopic studies. The Mannich bases were screened for antibacterial activity against various gram-negative bacteria at various concentrations and were analysed statistically. The result has shown that the compounds are quite active against pathogens under study and were non-toxic. All the synthesized compounds were found to be low lethal as ascertained by LD_{50} test.

The carboxamide derivatives represented as 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide are antibiotics which form a group of natural products having notable antibacterial activity towards a broad range of pathogenic micro-organisms and are characterized by very low toxicity to the mammalian hosts of these pathogens. These properties together with the fact that the compounds are well absorbed and fully active, when administered orally, have made tetracycline one of the most useful families of chemotherapeutic agents.

There range of activity includes an inhibitory or a destructive effect on *rickettsiae*, large virus of the lymphogranuloma-pesittacosis group gram +ve and gram −ve cocci and bacilli. They are used for Rocky Mountain spotted fever, typhus, Q-fever, viral pneumonia and for some other viral and bacterial infections, such as, conjunctivitis, trachoma brucellosis and for the treatment of infections such as hemophilus influenza, urinary tract infections, chronic bronchitis, etc. It has also been used for cancer detection and bone studies.[1–4]

Sulfa drugs are building blocks of several types of Mannich bases.[5–10] The sulfonamide nucleus has well-known pharmacological properties: antibacterial,[11] anticancer,[12] antiinflammatory,[13] carbonic inhibitory,[14] analgesic[15] and insecticidal.[16] In view of the above and in continuation of our earlier study,[17] we report antibacterial activity of 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide Mannich bases and their comparative study with sulfonamides.

Various drugs obtained from Mannich reaction have proved to be more effective and less toxic than their parent compounds.[18] The versatile utility of the Mannich bases in polymers,[19] dispersants in lubricating oil[20] and in pharmaceutical chemistry[21] prompted us to prepare a series of amino methyl derivatives and evaluate their biological significance and toxicity. Statistics are used for final comparison.

In continuation of our earlier investigations, we report the synthesis of Mannich bases of 4-(dimethylamino)-1,4,4a, 5,5a, and 6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide.[22]

Keywords: 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide; sulfonamides; Mannich bases; Antibacterial activity; Toxicity and statistical analysis.
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Mannich bases of 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide were achieved in two steps, in the initial step we get Mannich bases from primary amines, that is, sulfonamides and finally Mannich bases from secondary amines are synthesized. Scheme 1 summarizes the procedures used to prepare the Mannich bases (4a–4f) from the carboxamide derivative. These newly synthesized compounds were characterized by elemental analysis, UV, IR, ^{1}H NMR spectral studies.[23]

The antimicrobial screening was performed using paper disc method on pathogenic strains of *Salmonella enteritidis* and *Pasturella multocida*. The Mannich bases were studied for their antibacterial property at concentrations of 20–40 mg ml^{-1} using methanol as solvent. The solvent did not exhibit any activity at the concentrations used.

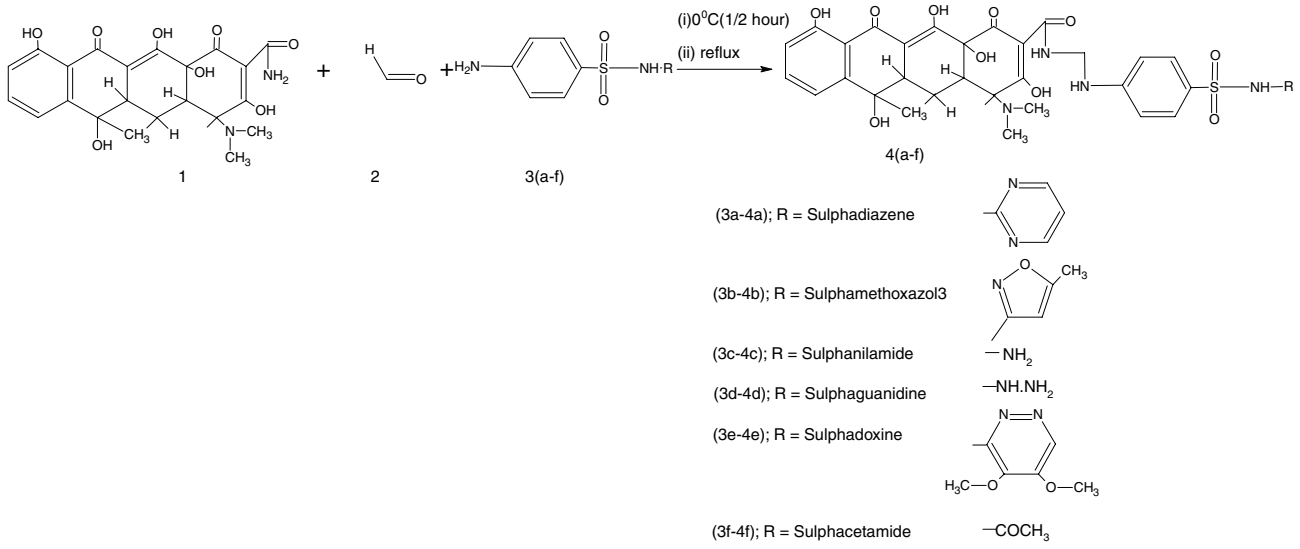
Table 1 reflects that Mannich bases 4a and 4b are statistically at par but these are significantly superior to compounds 4c, 4e and 4f against *S. enteritidis*.The highest zone of inhibition was recorded in concentration 40 mg/ml followed by the concentration 30 and 20 mg/ml.

In case of *P. multocida* the minimum zone of inhibition was recorded for Mannich base 4a, while the maximum was noted for 4c.The concentration 40 mg/ml was found to be significantly superior to all the concentrations. The results were statistically analysed.[24]

Further, Table 2 reveals that Mannich bases are statistically superior to their corresponding sulfonamides in exhibiting antibacterial activity. None of the sulfonamides had shown any activity at these concentrations. From the statistical data it is revealed that in case of *S. enteritidis*, Mannich bases 4a and 4c are significantly superior to sulfadiazene and sulfanilamide in inhibiting the growth of this pathogen. The rest sulfonamide fails to show any activity against *S. enteritidis*.

On comparing the antibacterial activity of Mannich bases to their corresponding sulfonamides against *P. multocida*, it indicates that 4c, 4d, 4e and 4f have shown zone of inhibition, thus proving superiority over sulfonamides.

The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *F* test.



Scheme 1. Synthesis of Mannich bases (4a–4f).

Table 1. Antibacterial screening of Mannich bases (Zone of inhibition in mm)

Compound	*Salmonella enteritidis* (concn in μg/ml)				*Pasturella multocida* (concn in μg/ml)			
	20	30	40	Av	20	30	40	Av
4a	20.00	20.86	21.20	20.68	20.40	20.80	21.86	21.02
4b	21.06	21.22	22.06	21.44	21.26	22.00	23.20	22.15
4c	14.68	15.02	16.00	15.23	28.36	28.60	29.08	28.68
4d	13.20	13.86	14.20	13.75	24.08	24.26	25.20	24.51
4e	15.00	15.20	15.46	15.22	22.15	23.30	24.40	23.28
4f	—	—	—	—	26.20	27.26	28.30	27.25
Av of concn	19.00	19.17	19.95		23.12	23.76	24.58	
		S.Ed.	CD at 5%			S.Ed.	CD at 5%	
Compound		0.093	0.201			0.083	0.176	
Concn		0.008	0017			0.039	0.079	
Inter-action		0.024	0.051			0.118	0.250	

Note: S.Ed., standard error of difference; CD, critical difference.

Table 2. Comparative study of antibacterial activity of Mannich bases and their parent sulfonamides (Zone of inhibition in mm)

Compound	*Salmonella enteritidis* (concn in μg/ml)				*Pasturella multocida* (concn in μg/ml)			
	20	30	40	Av	20	30	40	Av
4a	20.00	20.86	21.20	20.68	20.40	20.80	21.86	21.02
a	10.00	15.00	20.00	15.00	28.00	30.00	39.00	29.33
4b	14.68	15.02	15.50	16.00	21.26	22.00	23.20	22.15
b	15.00	15.00	18.00	16.00	24.00	26.00	28.00	26.00
4c	15.00	15.20	15.46	15.22	28.36	28.60	29.08	28.68
c	10.00	12.00	16.00	12.66	22.00	25.00	28.00	25.00
4d	13.20	13.86	14.20	13.75	24.08	24.26	25.20	24.51
d	10.00	15.00	18.00	14.33	20.00	25.00	25.00	23.33
4e	15.00	15.20	15.46	15.22	22.15	23.30	24.40	23.28
e	—	—	—	—	22.00	24.00	25.00	23.66
4f	—	—	—	—	26.20	27.26	28.30	27.27
f	—	—	—	—	20.46	21.22	22.15	21.27
	S.Ed.	CD at 5%			S.Ed.	CD at 5%		
Mannich base and sulfonamides	0.329	0.706			0.220	0.456		
Concentration	0.054	0.111			0.027	0.056		
Interaction	0.154	0.331			0.096	0.200		

Note: S.Ed, standard error of difference; CD, critical difference.

If the values of the calculated F are higher than the table value of F at 5% level, the character under study is said to be significantly influenced by the treatment. The significant or non-significant difference due to each of the treatments was judged under each character using standard error of difference (S.Ed) and critical difference (CD) values. The S.Ed between two treatments was calculated using error mean sum of squares (EMS). The CD were computed by multiplying the S.Ed value with the t-table (at 5%) value for the error degree of freedom in order to judge the minimum difference in the means to qualify the treatment effects.

The Mannich bases were also screened for their toxicity by preliminary LD_{50} test. The test was performed on white mice weighing 25 g. Doses were given orally as well as intraperitoneally and mice were kept under observation for 72 h for each trial.[25] The Mannich bases showed no adverse toxic effect even at an oral dose of 1600 mg/kg of the body weight of mice. However, when dose was administered intraperitoneally, they proved to be lethal at the dose level of 1000 mg/kg of the body weight of mice.

In Conclusion the 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide (Mannich bases) appeared to be better and more potent antibacterial agents than the sulfonamides themselves. We, therefore, conclude that the Mannich bases could be used as constructive drug in preference to sulfonamides. Our findings will prove useful to those chemists, pharmacists and medicinal chemists who are interested in the synthesis of potential Mannich bases as drugs with minimum side effects and also having comparatively low cost. Understanding of Mannich reaction, use of Mannich base for large-scale production of chemicals and synthesis of new exotic materials are some of the intellectual challenges for the future generation of chemists.

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21. General procedure for the preparation of Mannich base of 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamide (0.01 mol) was dissolved in 20 ml methanol. This was followed by equimolar addition of sulfonamide (0.01 mol) in small installments with constant stirring at efficient ice cooling. The reaction mixture was cooled well. Then 2.5 ml of formaldehyde solution (37%) was added slowly with continuous stirring. The mixture was kept as such for half an hour after adjusting the pH of reacting mixture to 3.5–4 with hydrochloric acid. After half an hour, the resulting reacting mixture was placed on a water bath for refluxing. The reflux time varied with different sulfonamides used. The mixture was then kept at 0 °C for 4 days, when the desired product formed. The Mannich base formed was recrystallized with dry distilled methanol/ethanol/DMF.
22. Physical and spectral characteristics of compound (4a–4f):
Compound (4a)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamido methyl sulfadiazine, $C_{33}H_{34}N_6O_{10}S$, mp 159–160 °C, C(%)—56.02 (55.09), H(%)—4.68 (4.81), N(%)—11.60 (11.89), UV 220 (S=O), 252 (Ar Ring), 260 (sulfonamide moiety), 363 (diketone-moiety), IR (KBr) 3441ν_{as} (NH) in sec amide, 3327 ν_{NH} of SO_2NH, 3055 ν (=C–H) of aromatic ring, 2941 ν_{as} C–H in CH_2, 2731 ν >CH_2N<, 1838 ν (C=O), 1330, ν_{as} S=O, 1149, ν (C–H) in disubstituted benzene, 838 out of plane C–H in disubstituted aromatic ring, ^{1}H NMR 2.90 (d, 2H, J = 7.5, CH_2); 5.60 (s, 1H, NH); 6.50–7.90 (m, ArH); 7.20 (s, 1H, CONH); 11.70 (s, 1H, SO_2NH); 1.80 (s, 1H, C_6Me_2); 2.67 (s, 1H, Nme_2); 2.20 (s, 1H, C_{4a}); 2.40 (s, 1H, C_{5a}); 2.30 (s, 1H, C_5); 3.09 (s, 1H, C_4).
Compound (4b)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamido methylsulfamethoxazole, $C_{33}H_{35}N_5O_{11}S$, mp −148 to 150 °C, C(%)—55.80 (55.85), H(%)—4.90 (4.93), N(%)—9.80 (9.87), UV 222 (S=O), 254 (Ar Ring), 262(sulfonamide moiety), 366 (diketone-moiety), IR (KBr) 3440 ν_{as} (NH) in sec amide, 3320 ν_{NH} of SO_2NH, 3040 ν (=C–H) of aromatic ring, 2920 ν_{as} C–H in CH_2, 2730 ν >CH_2N<, 1820 ν (C=O), 1340, ν_{as} S=O, 1120, ν (C–H) in disubstituted benzene, 850 out of plane C–H in disubstituted aromatic ring, ^{1}H NMR 2.80 (d, 2H, J = 7.5, CH_2); 5.80 (s, 1H, NH); 6.60–7.10 (m, ArH); 7.20 (s, 1H, CONH); 11.80 (s, 1H, SO_2NH); 1.70 (s, 1H, C_6Me_2); 2.60 (s, 1H, Nme_2); 2.20 (s, 1H, C_{4a}); 2.50 (s, 1H, C_{5a}); 2.30 (s, 1H, C_5); 3.10 (s, 1H, C_4).
Compound (4c)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamido methyl sulfanilamide, $C_{29}H_{32}N_4O_{10}S$, mp −169 to 170 °C, C(%)—55.40(55.41), H(%)—5.08 (5.09), N(%)—8.90 (8.91), UV 220 (S=O), 252 (Ar Ring), 261(sulfonamide moiety), 363 (diketone-moiety), IR (KBr) 3445 ν_{as} (NH) in sec amide, 3350 ν_{NH} of SO_2NH, 3050 ν (=C–H) of aromatic ring, 2945 ν_{as} C–H in CH_2, 2735 ν >CH_2N<, 1810 ν (C=O), 1345, ν_{as} S=O, 1110, ν (C–H) in disubstituted benzene, 850 out of plane C–H in disubstituted aromatic ring, ^{1}H NMR 2.49 (d, 2H, J = 7.5, CH_2); 5.60 (s, 1H, NH); 6.50–7.90 (m, ArH); 7.10 (s, 1H, CONH); 11.20 (s, 1H, SO_2NH); 1.60 (s, 1H, C_6Me_2); 2.62 (s, 1H, Nme_2); 2.10 (s, 1H, C_{4a}); 2.40 (s, 1H, C_{5a}); 2.30 (s, 1H, C_5); 3.20 (s, 1H, C_4).
Compound (4d)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamido methylsulfaguanidine, $C_{30}H_{34}N_6O_{10}S$, mp −185 to 186 °C, C(%)—53.60 (53.73), H(%)—5.02 (5.07), N(%)—12.50 (12.53), UV 218 (S=O), 255 (Ar Ring), 264(sulfonamide moiety), 366 (diketone-moiety), IR (KBr) 3446 ν_{as} (NH) in sec amide, 3335 ν_{NH} of SO_2NH, 3045 ν (=C–H) of aromatic ring, 2900 ν_{as} C–H in CH_2, 2730 ν >CH_2N<, 1810 ν (C=O), 1340, ν_{as} S=O, 1120, ν(C–H) in disubstituted benzene, 830 out of plane C–H in disubstituted aromatic ring, ^{1}H NMR 2.60 (d, 2H, J = 7.5, CH_2); 5.90 (s, 1H, NH); 6.50–7.80 (m, ArH); 7.20 (s, 1H, CONH); 11.10 (s, 1H, SO_2NH); 1.70 (s, 1H, C_6Me_2); 2.70 (s, 1H, Nme_2); 2.20 (s, 1H, C_{4a}); 2.30 (s, 1H, C_{5a}); 2.40 (s, 1H, C_5); 3.10 (s, 1H, C_4).
Compound (4e)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamido methyl sulfadoxine, $C_{35}H_{38}N_6O_{12}S$, mp −236 to 238 °C, C(%)—54.60 (54.83), H(%)—4.96 (4.96), N(%)—10.96 (10.96), UV 220 (S=O), 252 (Ar Ring), 260 (sulfonamide moiety), 364 (diketone-moiety), IR (KBr) 3440 ν_{as} (NH) in sec amide, 3350 ν_{NH} of SO_2NH, 3060ν (=C–H) of aromatic ring, 2930 ν_{as} C–H in CH_2, 2730 ν >CH_2N<, 1805 ν (C=O), 1330, ν_{as} S=O, 1115, ν (C–H) in disubstituted benzene, 825 out of plane C–H in disubstituted aromatic ring, ^{1}H NMR 2.80 (d, 2H, J = 7.5, CH_2); 5.80 (s, 1H, NH); 6.60–7.10 (m, ArH); 7.10 (s, 1H, CONH); 11.20 (s, 1H, SO_2NH); 1.80 (s, 1H, C_6Me_2); 2.60 (s, 1H, Nme_2); 2.10 (s, 1H, C_{4a}); 2.30 (s, 1H, C_{5a}); 2.40 (s, 1H, C_5); 3.00 (s, 1H, C_4).
Compound (4f)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a pentahydroxy naphthacene carboxamido methyl sulfacetamide, $C_{31}H_{34}N_4O_{11}S$, mp −218 to 219 °C, C(%)—55.42 (55.52), H(%)—5.01 (5.07), N(%)—8.30 (8.32), UV 220 (S=O), 252 (Ar Ring), 260 (sulfonamide moiety), 366 (diketone-moiety), IR (KBr) 3442 ν_{as} (NH) in sec amide, 3355 ν_{NH} of SO_2NH, 3050 ν (=C–H) of aromatic ring, 2940 ν_{as} C–H in CH_2, 2740 ν >CH_2N<, 1820 ν (C=O), 1335, ν_{as} S=O, 1112, ν (C–H) in disubstituted benzene, 830 out of plane C–H in disubstituted aromatic ring, ^{1}H NMR 2.90 (d, 2H, J = 7.5, CH_2); 5.90 (s, 1H, NH); 6.70–7.20 (m, ArH); 7.30 (s, 1H, CONH); 11.60 (s, 1H, SO_2NH); 1.80 (s, 1H, C_6Me_2); 2.70 (s, 1H, Nme_2); 2.10 (s, 1H, C_{4a}); 2.40 (s, 1H, C_{5a}); 2.60 (s, 1H, C_5); 3.00 (s, 1H, C_4).
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