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SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL ANALGESIC AND ANTIINFLAMATORY ACTIVITY OF ACETOPHENONE DERIVATIVES

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ABSTRACT

A novel series of mannich bases of substituted acetophenone derivatives were synthesized. These compounds were identified on the basis of melting point range, R_f values, elemental analysis, IR and ^1H NMR spectral analysis. The compounds were evaluated for antimicrobial, analgesic and anti-inflammatory activities. All compounds exhibited significant to moderate biological activity.

Keywords: Mannich base, Acetophenone, antimicrobial, analgesic, anti-inflammatory activity.

INTRODUCTION

The acetophenone is an important lead compound in modern drug discovery. Literature review reveals that mannich bases of acetophenone derivatives exhibits diverse pharmacological activities like antimicrobial [1-2], analgesic [3], anti-inflammatory [4-5], anticonvulsant [6], antiviral [7] as well as antihypertensive activities [8], etc. Research in this area is still unexplored and is directed towards the synthesis of compounds with enhanced biological activity. Based on the above observation it is worthwhile to prepare newer novel mannich bases of acetophenone derivatives with enhanced biological activity.

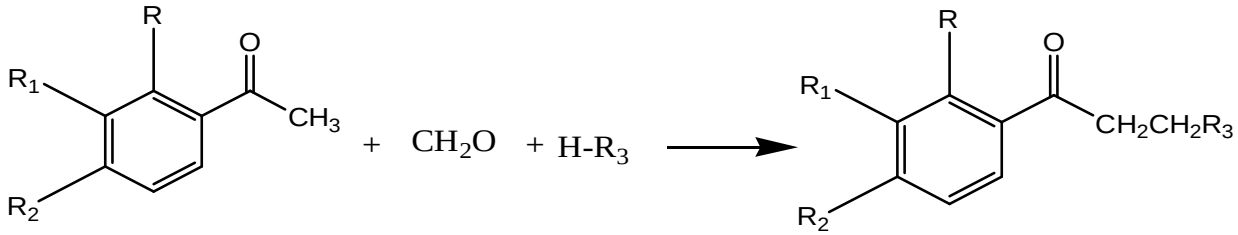
MATERIALS AND METHODS

All the chemicals procured from Central Drug House (P.) Ltd. and Rankem. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. Satisfactory C, H, N analysis was obtained for all the compounds on a Carlo Erba EA 1108 elemental analyzer. The IR spectra were recorded on Jasco FT/IR 5300 in KBr pellets and noted the absorption levels (cm^{-1}) were listed.^1H NMR spectra were run on JEOL GSX 400 NMR at 300 MHz in DMSO-d_6 as solvent and TMS as an internal standard. The Mass spectra were recorded on JEOL GC mate spectrometer.

GENERAL PROCEDURE FOR SYNTHESIS OF COMPOUNDS:

General synthesis of mannich bases of substituted acetophenone derivatives (a-g)

Substituted acetophenone (0.01mol) in 30 ml ethanol, different secondary amine (0.01ml) and formaldehyde solution (1ml) was refluxed for 3-4 hrs. On cooling the reaction mixture poured on crushed ice. The precipitate was filtered and recrystallised from ethanol. The purity of compound was established by single spot in TLC plate (silica gel). Solvent system used was benzene: chloroform: methanol (40:30:20).



SUBSTITUTED ACETOPHENONE

MANNICH BASE

ANTIMICROBIAL SCREENING [9]

Antimicrobial activity of the synthesized compounds was screened using the disc diffusion method against selected pathogens such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. The compounds were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Nutrient agar (anti-bacterial activity) and sabouraud dextrose agar medium (antifungal activity) was prepared and sterilized by an autoclave (121º C and 15 Ibs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with bacterial organisms in sterile nutrient agar medium at 45 ^{0}C, and fungal organism in sterile sabouraud's dextrose agar medium at 45^{0}C in aseptic condition. Sterile whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25,100 mg/disc was placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic discs of ciprofloxacin (100 µg /disc) and ketoconazole (100 µg /disc) were used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 h at 37 ± 1 ^{0}C for antibacterial activity and 48 h at 37±1 ^{0}C for antifungal activity. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around each disc.

ANALGESIC ACTIVITY [10]

All the compounds were screened for Analgesic activity in- vivo by Tail-flick method using Analgesiometer. Wistar albino mice of either sex (20-30g) in the groups of six animals each were selected by random sampling technique. Paracetamol at a dose level of 100 mg/kg is administered as a reference drug for comparison. The test compounds at dose level of 100 mg/kg are administered orally intragastric tube. The animals were held in position by a suitable restrained with the tail extending out and the tail (up to 5cm) was then dipped in a beaker of water maintained at 55±5°c. The time in seconds taken to withdraw the tail clearly out of the water was taken as the reaction time. The reading was recorded at 30, 60, 120 and 180 min. after administration of compounds. A cut off point of 10sec. was observed to prevent the tail damage.

ANTI-INFLAMMATORY ACTIVITY [11]

All the compounds were screened for anti-inflammatory activity in- vivo by carrageen induced rat hind paw oedema method using Plethysmograph. Albino rats of either sex weighing between 150-200 g were divided into eight groups of six animals The 1st group served as control and received the vehicle (saline) only. 2nd group of animals were treated with standard drug Aspirin (100 mg/kg). The animals of the other groups (3, 4, 5, 6, 7&8) were treated with calculated doses of synthesized derivatives. A mark was made on both the hind paws just below the tibio-tarsal joint. So that every time the paw could constant paw volume. After 30 min. of drug treatment and inflammation of induced in the left hind paw by injecting 0.1 ml of carrageen 1% solution in the sub planter region of all the animals. The paw volume is measured at 30, 60, 120 and 180 mins after the carrageen challenged.

The mean difference in initial paw volume and subsequent reading was noted and percentage inhibition of oedema was calculated using the formula.

Percentage inhibition = 100(1-Vt/Vc)

Where, Vt = represent oedema volume in test, Vc= represent oedema volume in control

RESULTS AND DISCUSSION

The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The structures of the synthesized compounds were supported by physical data (Table-1) and following spectral analysis.

Table-1: Physical data of Synthesized Compound

Compound	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)	R_f values
a	$C_{12}H_{16}BrNO_2$	286.16	228	65	0.67
b	$C_{13}H_{17}Cl_2NO$	274.19	225	70	0.50
c	$C_{11}H_{15}NO_2$	193.24	239	68	0.56
d	$C_{11}H_{14}ClNO$	211.69	210	72	0.60
e	$C_{11}H_{15}NO_3$	209.24	215	59	0.59
f	$C_{11}H_{13}Cl_2NO$	246.13	220	60	0.49
g	$C_{13}H_{19}NO_3$	237.29	238	78	0.65

The IR spectra of the compounds were done in a Jasco FT/IR5 300 spectrophotometer (ν_{max} cm^{-1}) by using KBr discs. The results of IR spectra were given in spectral detail heading which showed absorption bands for different groups.

The ^{1}HNMR spectra of the synthesized compounds were recorded on JEOL GSX 400 NMR spectrometer at 300 MHz using TMS as internal standard (chemical shifts in δ, ppm) and DMSO as the solvent. The results of the ^{1}HNMR spectra given under spectral detail heading showed that the numbers of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds.

The Mass spectra of the all the synthesized compounds were done on a JEOL GC mate spectrometer. The results presented in the spectral heading showed that the molecular mass of the synthesized compounds was nearer to the molecular mass of the expected compounds.

THE SPECTRAL DETAILS OF THE SYNTHESIZED COMPOUNDS:

1-(2-bromo-4-methoxyphenyl)-3-dimethylamino propan-1-one (a)

IR (KBr) ν_{max} :3060.6(Ar-H),3311.7(OH),2868.3(C-H),1675.0(C=O),1453.5(C=C),3418.7(C-Br); ^{1}H NMR(DMSO-d_6) δ:3.73(C-H,s),2.27(N-H,s),2.55(C-H,d),6.79(Ar-H,m);LC-MS: *m/z*286.16 (M^+); $C_{12}H_{16}BrNO_2$ (C:50.37,H:5.64,Br:27.92,N:4.89,O:11.18).

1-(2,4-dichlorophenyl)-3-diethylamino propan-1-one (b)

IR (KBr) ν_{max} :3060.6(Ar-H),2868.3(C-H),1675.0(C=O),1453.5(C=C),3411.7(C-Cl); ^{1}H NMR(DMSO-*d*$_6$) δ:3.72(C-H,s),2.27(N-H,s),2.58(C-H,d),6.78(Ar-H,m);LC-MS: *m/z*274.19 (M^+); $C_{13}H_{17}Cl_2NO$ (C:56.95,H:6.25,Cl:25.86,N:5.11,O:5.84).

1-(4-hydroxyphenyl)-3-dimethylamino propan-1-one (c)

IR (KBr) ν_{max} :3060.6(Ar-H),3311.7(OH),2869.3(C-H),1678.0(C=O),1454.5(C=C); ^{1}H NMR(DMSO-*d*$_6$) δ:2.25(C-H,s),2.25(N-H,s),2.55(C-H,d),6.79(Ar-H,m);LC-MS: *m/z*193.24 (M^+); $C_{11}H_{15}NO_2$ (C:68.37,H:7.82,N:7.25,O:16.56).

1-(4- chlorophenyl)-3-dimethylamino propan-1-one (d)

IR (KBr) ν_{max} :3061.6(Ar-H),2869.3(C-H),1675.0(C=O),1454.5(C=C),3412.7(C-Cl); ^{1}H NMR(DMSO *d*$_6$) δ:3.72(C-H,s),2.25(N-H,s),2.56(C-H,d),6.78(Ar-H,m);LC-MS: *m/z*211.69 (M^+);$C_{11}H_{14}ClNO$(C:62.41,H:6.67,Cl:16.75,N:6.62,O:7.56).

1-(2,6-dihydroxyphenyl)-3-dimethylamino propan-1-one (e)

IR (KBr) ν_{max} :3060.6(Ar-H),3312.7(OH),2869.3(C-H),1678.0(C=O),1454.5(C=C); ^{1}H NMR(DMSO-*d*$_6$) δ:2.25(C-H,s),2.25(N-H,s),2.55(C-H,d),6.79(Ar-H,m);LC-MS: *m/z*209.24 (M^+); $C_{11}H_{15}NO_3$ (C:63.14,H:7.23,N:6.69,O:22.94).

1-(2,4-dichlorophenyl)-3-dimethylamino propan-1-one (f)

IR (KBr) ν_{max} :3062.6(Ar-H), 2866.3(C-H),1675.0(C=O),1452.5(C=C),3411.7(C-Cl); ^{1}H NMR(DMSO-*d*$_6$) δ:3.70(C-H,s),2.24(N-H,s),2.56(C-H,d),6.77(Ar-H,m);LC-MS: *m/z*246.13 (M^+); $C_{11}H_{13}Cl_2NO$ (C:53.58,H:5.32,Cl:28.80,N:5.59,O:6.50).

1-(2,4-dihydroxyphenyl)-3-diethylamino propan-1-one (g)

IR (KBr) ν_{max} :3062.6(Ar-H),3322.7(OH),2870.3(C-H),1678.0(C=O),1455.5(C=C); ^{1}H NMR(DMSO-*d*$_6$) δ:2.26(C-H,s),2.26(N-H,s),2.54(C-H,d),6.78(Ar-H,m);LC-MS: *m/z*237.29 (M^+); $C_{13}H_{19}NO_3$ (C:65.80,H:8.07,N:5.90,O:20.23).

The Antimicrobial activities of all synthesized compounds were screened by disc diffusion method. For all seven compounds minimum inhibitory concentration was determined using standard Ciprofloxacin and Ketoconazole. All the compounds showed significant inhibitory activity against the microbes with the 100µg/ml which produces 100% inhibition against the microorganism. The results were tabulated in table 2 were given as zone of inhibition and MIC.Results showed that the compounds were having a very good antimicrobial activity.

The synthesized compounds were screened for their analgesic activity. The results were tabulated in table 3. All the compounds showed good analgesic activity. Out of all the synthesized compounds a, d, e, f and g showed good analgesic activity.

The synthesized compounds were screened for their anti-inflammatory activity. The results were tabulated in table 4. All the compounds showed near about good anti-inflammatory activity. Out of all the synthesized compounds a, d, e and g showed good anti-inflammatory activity. The SEM values were calculated by one-way ANOVA method followed by Dunnet multiple comparison tests using a computer program.

Table-2: Antimicrobial activity of Synthesized Compound

Compound	Con. (µg/ml)	Antibacterial activity [Zone of inhibition (mm)]				Antifungal activity [Zone of inhibition (mm)]	
		B.subtilis	***E.coli***	***S.aureus***	***P.auregenosa***	***C.albicans***	***A.niger***
a	100	18	19	16	17	21	17
b	100	19	20	18	16	21	18
c	100	11	15	14	13	17	14
d	100	19	20	21	19	22	18
e	100	18	17	16	19	20	16
f	100	20	21	22	21	21	16
g	100	12	11	13	13	14	15
Ciprofloxacin	100	21	25	24	20	-	-
Ketoconazole	100	-	-	-	-	25	18
DMSO(control)	-	-	-	-	-	-	-

Table: 3- Analgesic activity of Synthesized Compound

Compound	Dose (mg/ml)	Reaction time in seconds (Mean± Sem),				(%) Activity
		30 Min	1Hr	2 Hr	3 Hr	
a	100	3.23±0.11*	5.23±0.32**	6.23±0.33**	7.06±0.20**	37.03
b	100	2.23±0.11	3.63±0.20*	4.53±0.11**	5.73±0.20**	19.25
c	100	2.56±0.11	3.43±0.33*	3.63±0.11*	5.56±0.22**	11.01
d	100	2.83±0.30	5.06±0.37**	6.73±0.11**	7.0±0.20**	33.25
e	100	3.32±0.11*	5.4±0.11**	6.00±0.11**	7.06±0.20**	40.10
f	100	3.06±0.30*	6.0±0.36**	7.0±0.30**	7.40±0.32**	36.61
g	100	3.12±0.25	5.136±0.33**	6.15±0.22**	7.33±0.33**	39.05
Paracetamol	100	3.4±0.33**	5.32±0.21**	7.15±0.29**	7.82±0.30**	41.03
Control (CMC)	100	2.15±0.16	2.32±0.21	2.56±0.21	2.72±0.72	6

n= 6 animals in each group, Significance level: ** p< 0.01, *p< 0.05 as compared with the respective control

Table:4-Anti-Inflammatory activity

Compound	Dose (mg/ml)	Paw volume in ml(Mean± Sem)				(%) Inhibition
		30 Min	1Hr	2 Hr	3 Hr	
a	100	0.91 ± 0.02*	0.89 ± 0.01**	0.84 ± 0.01**	0.77 ±0.01**	21.31
b	100	1.00 ± 0.02	0.96 ± 0.01	0.93 ± 0.02*	0.92 ± 0.01*	15.37
c	100	1.00 ± 0.01	0.93 ± 0.01*	0.92 ± 0.01*	0.91 ± 0.01*	14.51
d	100	0.92±0.01**	0.90 ± 0.01**	0.82 ± 0.01**	0.75 ±0.01**	21,16
e	100	0.93 ± 0.01*	0.91 ± 0.02**	0.83 ± 0.01**	0.80 ±0.02**	22.36
f	100	0.92 ± 0.01*	0.92 ± 0.01**	0.83 ± 0.01**	0.78 ±0.01**	19.68
g	100	0.89±0.01**	0.91 ± 0.01**	0.81 ± 0.01**	0.77 ±0.01**	23.90
Aspirin	100	0.88±0.01**	0.87 ± 0.01**	0.78 ± 0.01**	0.68 ±0.01**	30
Control (CMC)	100	1.02 ± 0.01	1.06 ± 0.02	1.01 ± 0.01	0.99 ± 0.01	5

n= 6 animals in each group, Significance level: ** p< 0.01, *p< 0.05 as compared with the respective control

CONCLUSION

The research work was oriented towards the finding of newer mannich bases of acetophenone derivatives with antimicrobial, analgesic and anti-inflammatory activities. The different substituted mannich bases of acetophenone derivatives were synthesized. All synthesized compounds showed very good biological activity against previously reported mannich bases of acetophenone derivatives.

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REFERENCES

1. Muthumani P, Subha MCS and Chowdoji Rao K. A facial synthesis and antimicrobial activity of mannich bases of substituted aminophenol and acetophenone. E-Journal of Chemistry. 2010;7(3):745-50.
2. Gul M, Souad S and Amal R. Synthesis and preliminary biological activity of the effect of acetophenone derived mono mannich bases, Letters in Drug Design & Discovery. 2010;3: 27-30.
3. Aleksandra B, Banerjee M and Samantray A. Synthesis, analgesic, anti-inflammatory activity of mannich bases of acetophenone. Tropical Journal of Pharmaceutical Research. 2008; 7(2): 961-968.
4. Ilango K, Manikandan RS and Ramalakshmi N. Synthesis and anti-inflammatory activity of some amino phenol derivatives of Gallic Acid, E-Journal of Chemistry. 2009; 6(1):13-18.
5. Masafumi K, Jie M and Zhuang Q. Three-component one-pot synthesis of 1,4 dihydropyrano [2,3-c] substituted acetophenone in aqueous media, Synthetic communications. 2004; 34(24) : 4557–4563.
6. Snezana M, Mandhane, Aavula KK and Rajamannar T. Timed pentylenetetrazol infusion test: A comparative analysis with S.C.PTZ and MES models of anticonvulsant screening in mice, E-Journal of Chemistry. 2007; 5: 112-134.
7. Tewari AK and Mishra A. Synthsis and antiviral activities of N-substituted-2-substituted aminophenol derivatives. Indian Journal of Chemistry. 2006; 45B: 489-493.
8. Mirjana and Voronina T. Synthsis of anti hypertensive drugs by mannich bases, Journal of Pharm Chem. 2004; 38(9):467-79.
9. Kokare CR. Antimicrobial screening. Pharm. Microbio. ‘Exp.Tech. 2005;1:50-59.

10. Kulkarni SK. Screening of analgesic and anti-inflammatory activity. Handbook Exp. Pharm. 2009; 3:123-126.
11. Kulkarni SK. Screening of analgesic and anti-inflammatory activity. Handbook Exp. Pharm. 2009; 3:126-128.