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Synthesis of transition metal complexes of n-(piperazin-1-yl/morpholino ALKYL/ARYL) Benzamides as *anti*-inflammatory anti nociceptive and antibacterial agents

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ABSTRACT

A series of some solid transition metal complexes of some mannich bases, substituted N-(1-piperazino) benzamide with Ca (II), Cu (II) and Zn (II) complexes in the presence of ice cold condition. The newly synthesized compounds were characterized on the basis of elemental analysis, IR, 1H NMR and mass spectra. All the synthesized compounds were tested for their *anti*-inflammatory, anti nociceptive, antibacterial activities against Gram + and Gram – bacteria. The synthesized compounds were screened for inflammatory activity via standard approaches, and they have shown positive *anti* -inflammatory activity.

Introduction

Now a day certain organo metallic compounds have become an important tool for synthesizing various pharmaceutically active compounds and have turned out to be potential pharmacotherapeutic agents and possess antibacterial[1,2], anti-inflammatory[3,4], hypolipidemic[5], anti-oxidant[6] and anti-cancer[7] activities. Many transition elements have been extensively investigated with regard to their potential quality. However, several observations suggest that also other metal containing compounds might be suitable for the development of new chemotherapeutics[8]. Inspired from these above observations, the present work was planned to synthesize some solid transition metal complexes of some mannich bases, substituted N-(1-piperazino) benzamide with Ca(II), Cu(II) and Zn(II) complexes. The ligand system may co-ordinate with these metal ions in a bidentate manner through the oxygen atom of the carbonyl group and nitrogen atom of piperazine or oxygen atom of morpholine ring. Thus a series of titled compounds were synthesized and tested their antibacterial and antinociceptive activities.

Materials and methods:

Melting points were determined by open-ended capillary tube in the electrical melting point apparatus and were uncorrected and the purity of the compounds were checked by TLC using Merck grade aluminium foil GF_{254} plates of 0.25mm thickness and the spots were visually detected in an Iodine chamber. The structure of the synthesized compounds was elucidated by FT-IR (Perkin Elmer-1600 series) in KBr disc and ^{1}H NMR (Brucker 400 MHz) in DMSO-d_6. Elemental analysis determinations for final compounds were performed on Carlo Erba 108 and the analyses.

Experimental work

General procedure for synthesis of N-Mannich bases of N-(1-piperazin-1-yl / morpholino alkyl / aryl) benzamides Equimolar quantity (0.01 mol) of secondary amine such as piperazine, morpholine was separately mixed with benzamide and respective aldehydes such as acetaldehyde, benzaldehyde and N,N-dimethyl amino benzaldehyde and dissolved in methanol (30mL) and stirred well for 4 hrs in ice cold condition and kept at room temperature for about five days. The precipitate obtained was filtered and dried. It was washed with water followed by carbon tetra chloride to remove unreacted materials and recrystallized from methanol[9].

General procedure for synthesis of metal complexes of N-(1-piperazin-1-yl / morpholino alkyl / aryl) benzamides with Ca(II), Cu(II) and Zn(II)

N-Mannich base of N-(1-piperazin-1-yl / morpholino alkyl / aryl) benzamides being insoluble in water, all the complexes were prepared (M_1 – M_8) in non-aqueous medium. The respective mannich base was dissolved in choloroform and mixed with ethanolic solution of metal salt like $CaCl_2$, $CuCl_2$ and $ZnCl_2$ separately in 1:1 mole ratio. The reaction mixture was gently warmed on a water bath for ½ h. The resulting corresponding solid complex was filtered, washed with water and recrystallized from ethanol[10]. The physical and spectral data were tabulated in Table 1 and 2 respectively.

Biological evaluation

Anti-inflammatory activity

Male albino rats were used in experiments. The animals were kept in the groups (control, treated, standard) under constant temperature (25±1°C) and 12-h light/dark cycle. They had free access to standard mouse diet and tap water except during the experiment. On the day of the experiment, animals were transferred to individual cages randomly and allowed to acclimatize for 30 min before drug administration. Indomethacin and phenylbutazone were used as standard drugs. Newly synthesized compounds were dissolved in propylene glycol.

Paw oedema inhibition test was performed on albino rats by adopting the method[11]. Thirty min later, 0.2mL of 1%

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carrageenan suspension in0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw, and the paw volume was measured by a water plethysmometer socrel and then measured again 3 h later (Table 3). The mean increase of paw volume at each time interval was compared with that of control group at the same time intervals and percent inhibition values were calculated by the formula given below:

% anti-inflammatory activity = 1- (V_t=V_c) X 100

Where V_t and Vc are tested and control groups, respectively.

Antinociceptive activity:

Healthy swiss strain albino mice of either sex (25 – 30 g) were used for this study. The animals were maintained in the departmental animal house under standard laboratory conditions for one week before and during the experiments. They were fed with standard pellet diet and water was allowed to have *ad libitum*. All animals were handled according to the guidance for investigation of experimental pain in conscious animals[12]. The experimental protocol was approved by the Institutional Animal Ethical Committee (CPCSEA and IAEC No. AKCP/A5 (c) /111).

Swiss strain albino mice of either sex weighing 25 – 30 g were used for this study. All newly synthesized titled compounds were tested for anti-nociceptive activity by Thermal method (Eddy's Hot Plate Method) suggested by Eddy and Leimbach[13]. The animals were divided into ten groups (n = 6) and group I served as control and received vehicle. Group II received the standard drug, pentazocine (5 mg/kg, b.wt, i.p.)[14]. Group III - X received the test compounds (50 mg/kg b.wt., i.p.). The reaction time was noted for all groups on Eddy's hot plate before and after treatment of standard drug and synthesized compounds at 30, 60, 120 and 240 mts. The results were well compared with standard drug and recorded in Table 4.

Statistical Analysis

All the data are expressed as mean ± SEM. The values obtained for the above parameters in extracts were compared with control group using One-Way ANOVA followed by Dunnett's test[15]. The values of $P < 0.01$ and $P < 0.001$ were considered to indicate a significant difference between the groups.

Test microorganisms

Gram positive organisms such as *Bacillus subtilis, Proteus vulgaris* and Gram negative organisms such as *Escherichia coli, Pseudomonas auregenosa* were used for this study. All the bacterial cultures were procured from microbiology lab, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu, India.

Antibacterial activity

The antibacterial activity was performed by cup-plate method[16]. The respective bacterial culture was spread (swabbed) into the nutrient agar plates for uniform distribution of colonies. Using a sterile cork borer, 6 mm wide well was made on each agar plates. All the synthesized compounds (100 mcg/mL), a solvent control (2% v/v Tween 80) and the standard antibacterial drug (10 mcg/mL) were added into each wells using a sterile micropipette. The plates were incubated for 24 h at 37°C. After incubation, the zone of inhibition was measured and the values were recorded in Table 5. All the experiments were done in triplicate.

Ulcerogenic activity

An ulcerogenic liability of newly synthesized compounds was checked by the method [17] Albino rats were fasted for 24 h prior to drug administration. All animals were sacrificed 8 h after drug treatment, and then their stomachs and small intestines were microscopically amined to assess the incidence of hyperaemia, shedding of epithelium, petechial and frank haemorrhages and erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

Acute toxicity study

Approximate lethal dose (ALD50) of compounds was determined in albino mice. The test compounds were given orally at different dose levels in groups of 10 animals. After 24 h of drug administration, percent mortality in each group was observed and from the data obtained ALD50 was calculated[18].

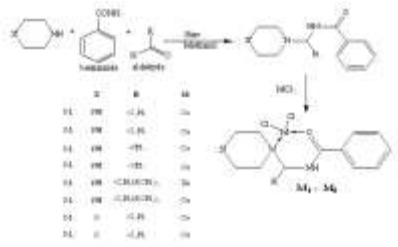
Results and discussion:

A series of novel transition metal complexes M_1-M_8 were synthesized as potential anti-nociceptive and antibacterial agents as depicted in Scheme 1. The compounds having secondary amine such as piperazine, morpholine was separately mixed with benzamide and dissolved in the respective aldehyde in presence of methanol lead the corresponding mannich bases were the starting materials for the titled compounds. Respective mannich a base in chloroform was mixed with ethanolic solution of respective metal salts lead the titled compounds (M_1-M_8). The melting points and Rf value by TLC indicated the formation of new chemical entity. The structure of the synthesized compounds was established by IR and ^{1}H-NMR spectral data as well as elemental analyses data.

The compounds were evaluated for *invivo* anti-inflammatory activity by carrageenan induced paw edema method in a dose of 50 mg/ml. M_2 and M_8 shows significant anti inflammatory activity.

Antinociceptive activity was evaluated for all the synthesized transition metal complexes by Eddy's hot plate method in mice. The results showed that compounds M_1, M_2, M_3, M_4, M_5 and M_6 exhibited highly significant anti-nociceptive activity whereas compounds M_7 and M_8 leads decrease in activity at a dose of 50 mg/kg. Also all the compounds were tested for their antibacterial susceptibility using cup plate method. Compounds M_1, M_2, M_3, M_4, M_5 and M_6 showed moderate antibacterial activity against the tested bacteria at a concentration of 100mcg/mL. Compounds, M_7 and M_8 exhibited mild antibacterial activity. All the results were well compared with the standard drugs. This result depicted that the replacement of piperazine with morpholine decreases the activity.

Scheme



Conclusion:

Different novel transition metal complexes were synthesized from mannich bases and characterized. All the spectral data accused the structure of the synthesized compounds. Anti-nociceptive and antibacterial activities were evaluated for all the newly synthesized compounds. The results revealed that the importance of piperazine moiety in the complex for the above tested activities. The present study concluded that the experimental procedures make this methodology a better modesty for the synthesis of novel transition metal complexes with mannich bases for possible anti-

nociceptive and antibacterial activities and lead to intensive research and worthwhile contribution in medicinal chemistry.

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Table 1 Physical data of newly synthesized metal complexes

Comp. code	Molecular formula	M.W.	m.p. (^{0}C)	R_f value	% yield	Elemental analysis % calculated (% found)				
						C	H	N	O	Cl
M_1	$C_{18}H_{21}CaCl_2N_3O$	406	106	0.64	65	53.20 (53.12)	5.21 (5.14)	10.34 (10.26)	3.94 (3.74)	17.45 (17.32)
M_2	$C_{18}H_{21}CuCl_2N_3O$	429	114	0.78	68	50.30 (50.14)	4.92 (4.88)	9.78 (9.64)	3.72 (3.64)	16.50 (16.42)
M_3	$C_{13}H_{19}CaCl_2N_3O$	344	96	0.82	76	45.35 (45.22)	5.56 (5.44)	12.20 (12.12)	4.65 (4.60)	20.59 (20.46)
M_4	$C_{13}H_{19}CuCl_2N_3O$	367	84	0.68	71	42.46 (42.32)	5.21 (5.16)	11.43 (11.34)	4.35 (4.26)	19.28 (19.18)
M_5	$C_{20}H_{26}ZnCl_2N_4O$	474	102	0.74	62	50.60 (50.48)	5.52 (5.46)	11.80 (11.72)	3.37 (3.30)	14.94 (14.82)
M_6	$C_{20}H_{26}CuCl_2N_4O$	472	76	0.92	64	50.80 (50.72)	5.54 (5.48)	11.85 (11.72)	3.38 (3.32)	14.99 (14.88)
M_7	$C_{18}H_{20}CaCl_2N_2O_2$	407	92	0.88	66	53.07 (52.86)	4.95 (4.86)	6.88 (6.76)	7.86 (7.78)	17.41 (17.32)
M_8	$C_{18}H_{20}CuCl_2N_2O_2$	430	112	0.76	72	50.18 (50.12)	4.68 (4.62)	6.50 (6.46)	7.43 (7.36)	16.46 (16.36)

Table 2 Spectral data of newly synthesized metal complexes

Comp. code	IR (KBr disc) υ_{max} (cm^{-1})	^{1}H NMR (DMSO-d_6) δ (ppm)
M_1	3328 (NH), 3095 (Ar.CH), 2960 (CH), 1585 (Ar.C=C), 1221(C-N), 709 (sub.Ar)	2.40 (s, NH), 2.99 (s, CH_2), 3.36 (s, CH_2), 5.96 (d, CH), 7.04-7.78 (m, ArH)
M_2	3308 (NH), 3090 (Ar.CH), 2962 (CH), 1581 (Ar.C=C), 1222(C-N), 712 (sub.Ar)	2.20 (s, NH), 2.64(s, CH_2), 3.32 (s, CH_2), 5.99 (d, CH), 7.14-7.86 (m, ArH)
M_3	3314 (NH), 3084 (Ar.CH), 2956 (CH), 1578 (Ar.C=C), 1491 (CH_3)1218, (C-N), 698 (sub.Ar),	1.70 (s, CH_3), 2.22 (s, NH), 2.76 (s, CH_2), 3.62 (s, CH_2), 4.98 (d, CH), 7.04-7.32(m, ArH)
M_4	3336 (NH), 3092 (Ar.CH), 2966 (CH), 1598 (Ar.C=C), 1456 (CH_3), 1226 (C-N), 706 (sub.Ar)	1.72 (s, CH_3), 2.40 (s, NH), 2.99 (s, CH_2), 3.36 (s, CH_2), 4.86 (d, CH), 7.14-7.44 (m, ArH)
M_5	3330 (NH), 3096 (Ar.CH), 2962 (CH), 1590 (Ar.C=C), 1452 (CH_3), 1224 (C-N), 847 (di.sub.Ar)	2.34 (s, NH), 2.85 (s, CH_3), 2.76 (s, CH_2), 3.52 (s, CH_2), 5.96 (d, CH), 6.74-7.54 (m, ArH)
M_6	3326 (NH), 3086 (Ar.CH), 2954 (CH), 1586 (Ar.C=C), 1448 (CH_3), 1222 (C-N), 842 (di.sub.Ar)	2.22 (s, NH), 2.86 (s, CH_3), 2.92 (s, CH_2), 3.64 (s, CH_2), 5.98 (d, CH), 6.38-7.66 (m, ArH)
M_7	3316 (NH), 3090 (Ar.CH), 2956 (CH), 1582 (Ar.C=C), 1218 (C-N), 714 (sub.Ar)	2.14 (s, NH), 3.42 (s, CH_2), 3.84 (s, CH_2), 5.82 (d, CH), 7.02-7.48 (m, ArH)
M_8	3310 (NH), 3084 (Ar.CH), 2968 (CH), 1586 (Ar.C=C), 1224(C-N), 716 (sub.Ar)	2.12 (s, NH), 3.22 (s, CH_2), 3.86 (s, CH_2), 5.96 (d, CH), 7.04-7.86 (m, ArH)

Table 3 Anti-Inflammatory and ulcerogenic activity of synthesized compounds M1-M8

Compound Code no	Anti-Inflammatory activity		Ulcerogenic activity		ALD_{50} mg/kg p.o
	Dose (mg/kg)p.o	% edema inhibitor relation	Dose	% of animal with ulcer	
M_1	50	67	200	30	>500
M_2	50	72	200	10	>500
M_3	50	58	200	40	>500
M_4	50	52	200	10	>500
M_5	50	61	200	20	>500
M_6	50	44	200	30	>500
M_7	50	52	200	40	>500
M_8	50	79	200	10	>500
Indomethacin	50	91	----	----	----

Table 4 Anti-nociceptive activity of newly synthesized metal complexes

Treatment	Basel Reaction Time (sec) (Mean ± SEM) before Treatment	Reaction Time (sec) after administration (Mean ± SEM)			
		30 min	60 min	120 min	240 min
Control	3.32±0.20	3.37±0.16	3.24±0.22	3.46±0.26	3.42±0.56
Standard	3.92±0.22	10.54±0.38**	12.56±0.36**	13.62±0.18**	14.78±0.62**
M_1	3.24±0.48	5.96±0.28**	7.04±0.32**	8.98±0.26**	10.20±0.46**
M_2	3.44±0.42	6.48±0.22**	7.26±0.66**	9.44±0.32**	10.76±0.56**
M_3	3.82±0.36	6.62±0.22**	9.78±0.26**	10.82±0.32**	11.96±0.32**
M_4	3.46±0.28	7.74±0.42**	12.14±0.28**	13.66±0.28**	14.14±0.44**
M_5	2.38±0.64	4.72±0.26*	6.76±0.22*	7.96±0.36*	9.96±0.24**
M_6	3.62±0.42	4.98±0.32*	6.78±0.26*	9.16±0.44**	10.88±0.46**
M_7	3.42±0.26	4.46±0.24*	5.32±0.26*	5.62±0.32*	6.42±0.78*
M_8	3.46±0.34	4.12±0.42*	4.66±0.72*	5.28±0.46*	5.76±0.94*

$^{**}P < 0.001$ and $^{*}P < 0.01$ statistically (Mean ± SEM) significant from control group (n=6)

Table 5 Anti-bacterial activity of newly synthesized metal complexes

Compound code	Diameter of Zone of inhibition (mm)			
	B.Subtilis	*P. vulgaris*	*E. coli*	*P. aerogenes*
M_1	16	16	17	18
M_2	9	11	12	13
M_3	10	9	11	12
M_4	15	16	17	18
M_5	14	13	15	16
M_6	13	12	14	15
M_7	8	9	8	8
M_8	10	8	8	9
Ofloxacin	19	19	20	21