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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF MANNICH BASES OF NICOTINAMIDE WITH DICLOFENAC AND MEFENAMIC ACID

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ABSTRACT

Mannich bases of Nicotinamide with diclofenac and mefenamic acid were synthesized and studied for their anti-inflammatory activity. Nicotinamide on reaction with secondary amine, i.e., diclofenac and mefenamic acid in presence of formaldehyde and hydrochloric acid furnished the title compounds. The Mannich bases were characterized by spectral and analytical techniques. These were evaluated for their anti-inflammatory activity by carrageenan induced rat paw edema method and exhibited significant activity.

Keywords: Anti-inflammatory, Mannich bases, nicotinamide, diclofenac, mefenamic acid.

INTRODUCTION

Nicotinamide, also known as niacinamide, nicotinic acid amide or pyridine-3-carboxamide is a component of vitamin B_3. It is involved in a wide range of biological processes via its major metabolite NAD (nicotinamide adenine dinucleotide), including the production of energy, the synthesis of fatty acids, cholesterol and other steroids, signal transduction and the maintenance of the integrity of the genome. Nicotinamide has been reported as antidiabetic [1], antioxidant [2], anti-inflammatory [3] and anti-carcinogenic [4]. It has putative activity against osteoarthritis [5] and granuloma annulare [6]. It has also been found as inhibitor of Poly (ADP-Ribose) Polymerase [7, 8] or PARP-inhibitor. Poly (ADP-ribose) polymerase-1 [also known as poly (ADP-ribose) synthetase and poly (ADP-ribose) transferase] is a nuclear enzyme. The role of PARP has been implicated in the pathogenesis of stroke [9, 10], myocardial ischemia [11, 12], diabetes [13, 14], osteoarthritis [15, 16] and various other forms of inflammation. Therefore, inhibition of PARP by pharmacological agents may prove useful for the therapy of these diseases.

Diclofenac [2-((2, 6-dichlorophenyl) amino) benzeneacetic acid] is as an anti-inflammatory, analgesic and antipyretic agent and has been used in the long term symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [17, 18] whereas mefenamic acid [2-((2, 3-Dimethylphenyl) amino) benzoic acid] is an analgesic drug and has been used mostly in the short term treatment of pain in soft-tissue injuries, dysmenorrhea and rheumatoid and osteoarthritis [19, 20]. Their anti-inflammatory and analgesic action is due to the inhibition of

cyclooxygenase (COX) enzyme, which is responsible for the synthesis of prostaglandins. These reports inspired us to undertake the synthesis of Mannich bases of nicotinamide with NSAIDs like diclofenac and mefenamic acid in which both nicotinamide and diclofenac and/or mefenamic acid are present in a single molecule.

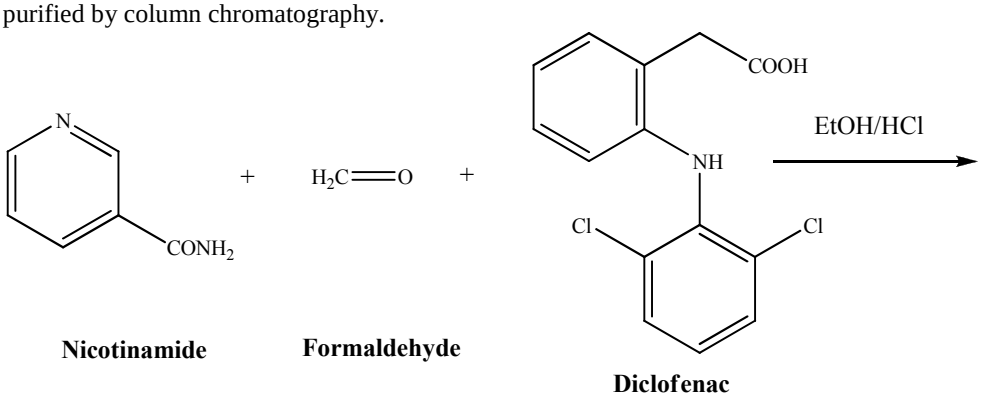
MATERIALS AND METHODS

Chemistry

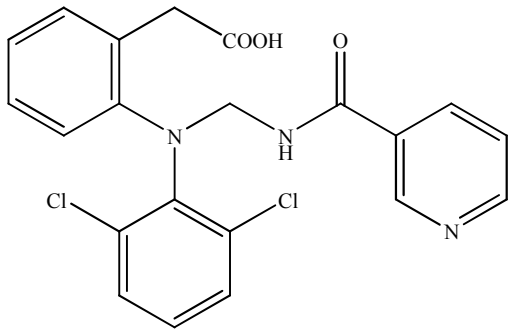
The purity of all the synthesized compounds were checked by thin layer chromatography on silica gel G as stationary phase and different solvent systems as mobile phase using iodine vapors as detecting agent. Melting points were determined by the Tempo melting point determination apparatus in open capillary tubes and are uncorrected. Elemental analyses were carried out on Perkin Elmer 2400 CHN Elemental Analyser. Infrared spectra were recorded on Shimadzu 8000 FTIR Spectrophotometer in KBr phase. Proton NMR spectra were done on Bruker Avance II 400 NMR Spectrometer using tetramethyl silane as internal standard.

General procedure for the preparation of Mannich base of nicotinamide with diclofenac and mefenamic acid

Nicotinamide (0.01 mole), formalin solution (2 ml) and diclofenac or mefenamic acid (0.01 mole) were taken in a round bottom flask. Absolute alcohol (50-60 ml) and concentrated hydrochloric acid (4-5 ml) were added to it. The contents were refluxed on water bath for 10-12 hours. Formalin solution was added to it in divided portions in order to complete the reaction. After refluxing the reaction mixture was cooled. Diethyl ether was slowly added to precipitate the compound. The solvent was evaporated under reduced pressure. The product was collected, dried and purified by column chromatography.



Nicotinamide **Formaldehyde** **Diclofenac**



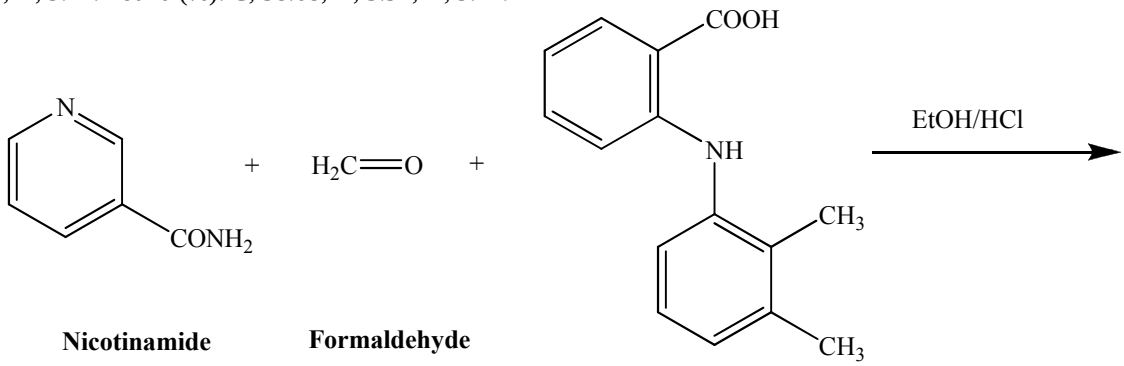
MND

Scheme 1: Synthesis of Mannich base of nicotinamide with diclofenac (MND)

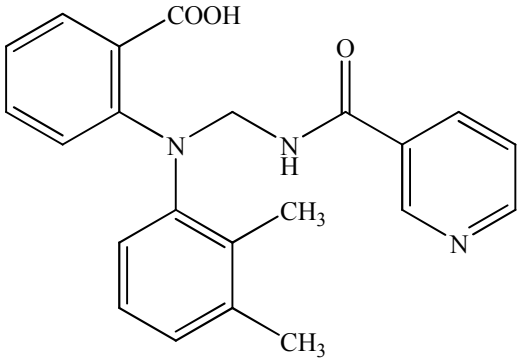
2-[2-[(2, 6-dichlorophenyl) (nicotinamidomethyl) amino] phenyl] acetic acid (MND):

Yield: 74 %; m.p.: 178-180 °C; IR (KBr, cm^{-1}): 3320, 3112 N–H *str*, 3084-3016 C–H *str* (aromatic and hetero-aromatic), 1693 C=O *str* (carboxylic acid), 1645 C=O *str* (amide I for 2^o amide), 1551 N–H *def* (amide II for 2^o amide), 804 & 716 C–H *def* (aromatic and hetero-aromatic). ^{1}HNMR ($CDCl_3$): δ (ppm) 8.23-7.04 (m, 11H, Ar**H**),

4.75 (s, 2H, =N–C**H**$_2$–NH–), 3.72 (s, 2H, Ar–C**H**$_2$–COOH). Anal.: Calculated (%) for $C_{21}H_{17}Cl_2N_3O_3$: C, 58.62; H, 3.98; N, 9.77. Found (%): C, 58.68; H, 3.91; N, 9.72.



Nicotinamide **Formaldehyde** **Mefenamic acid**



MNM

Scheme 2: Synthesis of Mannich base of nicotinamide with mefenamic acid (MNM)

2-[(2, 3-dimethylphenyl) (nicotinamidomethyl) amino] benzoic acid (MNM):
Yield: 77 %; m.p.: 118-120 °C; IR (KBr, cm^{-1}): 3322, 3115 N–H *str*, 3080-3015 C–H *str* (aromatic and hetero-aromatic), 1695 C=O *str* (carboxylic acid), 1641 C=O *str* (amide I for 2^o amide), 1556 N–H *def* (amide II for 2^o amide), 809 & 712 C–H *def* (aromatic and hetero-aromatic). ^{1}HNMR ($CDCl_3$): δ (ppm) 8.26-7.01 (m, 11H, Ar**H**), 4.73 (s, 2H, =N–C**H**$_2$–NH–), 2.32 (s, 3H, Ar–*m*-C**H**$_3$), 2.14 (s, 3H, Ar–*o*-C**H**$_3$). Anal.: Calculated (%) for $C_{22}H_{21}N_3O_3$: C, 70.38; H, 5.64; N, 11.19. Found (%): C, 70.31; H, 5.69; N, 11.13.

Anti-inflammatory Activity
Anti-inflammatory activity of the synthesized Mannich bases was carried out by hind-paw edema method [21] using carrageenan as a phlogistic agent on Wistar rats. All the protocols for animal studies were approved by Institutional Animal Ethics Committee (IAEC) of Department of Pharmaceutical Sciences, Guru Jambheshwar University, Hisar The animals were divided into different groups (control, standard and test groups) each consisting of six animals. The initial left hind paw volume of each animal was measured by plethysmograph. The animals were starved over night and only water was given *ad libitum*. A dose of 20 mg/kg body weight was given orally as suspension of tween 80 (1% w/v, in distilled water). Nicotinamide, Diclofenac and mefenamic acid were used as the standard drugs. The test and the standard groups were treated with the synthesized Mannich bases and the standard drugs respectively, whereas the control group received the vehicle (tween 80, 1% w/v, in distilled water) only. After thirty minutes, the animals were injected with 0.1 mL of carrageenan (1% w/v, in sterile saline) in the plantar region of left hind paw of the rats. The final paw volume was measured plethysmographically after 02 h and 04 h post administration of the drug. The percent inhibition of the inflammation was calculated by the following formula:

$$\% \text{ inhibition} = 1 - \frac{V_t}{V_c} \times 100$$

where V_t and V_c are the mean relative changes in the volume of paw edema in the test and control respectively. Data were statistically analyzed [22] and statistical significance was denoted as P value. The P value for all the compounds was found to be less than 0.001. The results are shown in table 1.

Table1: Anti-inflammatory activity of nicotinamide, diclofenac, mefenamic acid and synthesized Mannich bases

Compound	Change in paw volume (mL) (Mean ± SEM)		% Anti-inflammatory activity (Mean ± SD)	
	After 02 h	After 04 h	After 02 h	After 04 h
Control	0.717 ± 0.025	0.692 ± 0.015		
Nicotinamide	0.492 ± 0.015	0.517 ± 0.017	31.38 ± 0.30	25.31 ± 0.84
Diclofenac	0.333 ± 0.017	0.358 ± 0.015	53.51 ± 0.75	48.22 ± 1.05
Mefenamic Acid	0.367 ± 0.017	0.392 ± 0.015	48.85 ± 0.59	43.38 ± 0.94
MND	0.250 ± 0.018	0.275 ± 0.011	65.15 ± 1.30	60.25 ± 0.73
MNM	0.350 ± 0.018	0.375 ± 0.021	51.18 ± 0.81	45.82 ± 1.86

RESULTS AND DISCUSSION

Chemistry

Synthesis of Mannich bases of diclofenac and mefenamic acid with nicotinamide were carried out as outlined in the scheme **1** and scheme **2** respectively. Nicotinamide (active hydrogen containing compound) was reacted with secondary amine (diclofenac and mefenamic acid) in presence of formalin and dilute hydrochloric acid to furnish the Mannich bases. These were characterized on the basis of their elemental and spectral analysis. Infrared spectra of each compound showed bands for N–H stretching vibrations at about 3320 cm^{-1} and 3315 cm^{-1} and C–H stretching vibrations for aromatic and hetero-aromatic moiety were observed in the range of 3080-3015 cm^{-1}. Amide I band (C=O *str*) for secondary amide was observed near 1640 cm^{-1} whereas amide II band (N–H *def*) was observed near 1555 cm^{-1}. Stretching vibrations (C=O *str*) for carboxylic acid moiety was observed near 1695 cm^{-1}. Aromatic deformation vibrations were found near 805 and 715 cm^{-1}. In case of ^{1}HNMR spectra the chemical shift values for aromatic protons was found in the range of 8.26-7.01 δ (ppm) and appeared as multiplet. Methylene protons attached to two nitrogen atoms (=N–C**H$_2$**–NH–) was absorbed at 4.75-4.73 δ (ppm) as singlet. Methylene protons of **MND** attached to carboxylic group (Ar–C**H$_2$**–COOH) was appeared as singlet at 3.72 δ (ppm). Aromatic methyl groups of **MNM** (Ar–*o*-C**H$_3$** and Ar–*m*-C**H$_3$**) were found at 2.14 and 2.32 δ (ppm) as singlet respectively. The results of elemental analyses were found in good agreement with the calculated values.

Anti-inflammatory Activity

The synthesized Mannich bases were screened for their anti-inflammatory activity. The anti-inflammatory activity in Wistar rats by carrageenan induced hind paw-edema method. Nicotinamide, diclofenac and mefenamic acid were used as standard drugs. Both the Mannich bases showed greater anti-inflammatory activity than the corresponding parent drugs. Nicotinamide showed least anti-inflammatory activity. Mannich base of diclofenac showed greater activity than the Mannich base of mefenamic acid. The order of anti-inflammatory activity was found as MND > MNM > diclofenac > mefenamic acid > nicotinamide. It appears that the synthesized Mannich bases showed synergistic anti-inflammatory activity.

CONCLUSION

Mannich bases of Nicotinamide with diclofenac and mefenamic acid were prepared by the classical Mannich reaction and the structure was established on the basis of their elemental, IR and NMR spectral analyses. The anti-inflammatory activity was examined by carrageenan induced hind paw of edema method in Wistar rats. Synthesized Mannich bases showed better anti-inflammatory activity than the parent drugs. It is suggested that ulcerogenic potential of the synthesized Mannich bases may be studied to explore the possibility of these Mannich bases as the potential anti-inflammatory drugs devoid of common side effects of NSAIDs.

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