SYNTHESIS AND VALUATION OF MANNICH BASES OF CERTAIN NOVEL NITRO HYDROXY 1, 2 – PYRAZOLINES

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ABSTRACT:

A study was conducted to synthesise and evaluate the anti-inflammatory and anti-microbial activities of mannich bases from 1, 2 –hydroxy pyrazolines. For this, vanillin was reacted with substituted acetophenones and different vanillinyl chalcones were obtained which when coupled with phenyl hydrazine, produced hydroxy 1, 2 –pyrazolines. The hydroxyl pyrazolines were reacted with sulpha drugs and other compounds of biological interest containing free amino group in the presence of formaldehyde when mannich bases of 1, 2- hydroxyl pyrazolines were obtained. The compounds were characterized by elemental analysis, Ultra Violet (UV), Infra Red (IR) and Nuclear Magnetic Resonance (NMR) studies. All the newly synthesized compounds have been screened for Anti-inflammatory and Anti-microbial activity. Out of six compounds, para nitro hydroxy pyrazolines of sulphadiazine mannich base (A), nitrohydroxy pyrazoline of sulphacetamide (B) substituted nitro hydroxyl pyrazoline of amino anti-pyrine mannich (F) exhibited significant or oedema inhabiting oedem activity. Out of six compounds, para nitro hydroxyl pyrazoline of sulphadiazine mannich base (A) also exhibited anti-microbial activity.

KEY WORDS:-Vanillinyl chalcones, Anti-bacterial activity, Anti-inflammatory activity, Antifungal activity.

INTRODUCTION

A number of anti-inflammatory agents have been discovered and many of them have disappeared form the market due to their side effects and lack of specificity. As the types and nature of inflammation with regard to individuals vary, all the anti-inflammatory agents discovered are not effective in all types of inflammation. However, it is known that, till now a potent inhibitor of inflammation does not really exist and an intensive study seems to be definitely necessary.

Pyrazolines constitute an interesting class of organic compounds with diverse chemical and pharmacological applications. It is also interesting to note that mannich bases are also known for large profiles of biological activity such as anti-bacterial, anti-malarial, anti – fungal and anti-inflammatory. During the last decade, considerable interest has arisen in the field of anti-inflammatory agents. Many of the inflammatory conditions have aggravated due to secondary infections. It was thought worthwhile to combine a drug moiety, possessing chemotherapeutic actions with the hydroxyl pyrazolines, through mannich reaction. The hydroxyl pyrazolines were reacted with the moieties of biological interest, which have
free amino group like sulpha diazone, sulphacetamide, 4-amino antipyrine, 2-amino thiazole, 2-aminopyridine and 4-amino benzoic acid to obtain manich bases of pyrazolines3, 4, 6.

MATERIALS AND METHODS

The present work is to synthesize and analyse anti-inflammatory activity of certain novel 1, 2 pyrazolines. Vanillin was condensed with nitro acetophenones to yield the corresponding nitro vanillinnl chalcones with phenyl hydrazine to yield nitro hydorox pyrazolines.

Vanillin (1.52g) in ethanol was added to nitro acetophenones in 0.8g of Sodium hydroxide solution and cooled for 10 minutes. Then the entire mixture was poured on crushed ice. The precipitated nitro vanillinnl chalcones were filtered and crystallized from ethanol.

Nitro vanillinnl chalcones were taken in 15 ml of ethanol and added to 0.02 moles of phenyl hydrazine and were refluxed for 5-6 hours. Then 1-2 ml of glacial acetic acid was added and again refluxed for 3-4 hours. On cooling, reaction mixture was poured on crushed ice. The precipitated nitro hydroxyl 1, 2 pyrazolines were filtered and crystallized from ethanol. The purity of the compounds were established by single spot in tin layer chromatography plate (silica gel) (solvent system used was benzene: chloroform: methanol in the ratio 50:35:15).

Nitro hydroxyl pyrazoline (0.01 mole) in 3 ml of ethanol added aromatic amino compounds (0.01 mole) and formaldehyde solution (1ml) was refluxed for 3-4 hours. On cooling, the reaction mixture was poured on crushed ice. The precipitated manich base was filtered and crystallized from ethanol. The purity of the compound was established by single spot in T.L.C. plate (silica gel). Solvent system used was benzene: chloroform: methanol in the ratio 40:30:30.

**COMPOUND A:** Nitro hydroxyl 1, 2-pyrazoline of sulphadiazine manich compound having molecular formula C33 H29 N7O6 S, had an yield of 62% with a melting point 255°C, Rf value of 0.61 and wavelength of 324nm.

**COMPOUND B:** Nitro hydroxyl 1, 2-pyrazoline of sulphadiazine manich compound having molecular formula C31 H27 N4O7 S, had an yield of 63% with a melting point 205°C, Rf value of 0.68 and wavelength of 331nm.

**COMPOUND C:** Nitro hydroxyl 1, 2-pyrazoline of aminothiazole manich compound having molecular formula C26 H23 N5O4 S, had an yield of 60% with a melting point 180°C, Rf value of 0.49 and wavelength of 266nm.

**COMPOUND D:** Nitro hydroxyl 1, 2-pyrazoline of aminopyridine manich compound having molecular formula C28 H25 N5O4, had an yield of 66% with a melting point 138°C, Rf value of 0.39 and wavelength of 282nm.

**COMPOUND E:** Nitro hydroxyl 1, 2-pyrazoline of aminobenzoic acid manich compound having molecular formula C30 H26 N4 O6, had an yield of 63% with a melting point 148°C, Rf value of 0.52 and wavelength of 306nm.

**COMPOUND F:** Nitro hydroxyl 1, 2-pyrazoline of amino antipyrine manich base having molecular formula C34 H32 N6O5, had an yield of 67% with a melting point 168°C, Rf value of 0.72 and wavelength of 290nm.
PHARMACOLOGICAL STUDIES

All the newly synthesized compounds have been screened for anti-inflammatory activity. For this, male albino rats weighing between 65-185g were used. They were divided into various groups.

ANTI-INFLAMMATORY SCREENING

ACUTE ANTI-INFLAMMATORY MODEL

Carrageenin induced rat hind paw edema method had been adopted in these experiments. The rats were divided into several groups. One group served as control while the other group received the drugs. The rats were dosed orally (100mg/kg of body weight). Ibuprofen was taken as standard drug for comparison. After one hour, a subplantar injection of 0.05ml of 1% solution of carrageenin was administered. The volume of the infected foot was measured with a water plethysmograph. the paw volume was again measured after 3 hours. The treated animals were compared with that of a group treated with the vehicle (control group) and the percentage inhibition of oedema calculated using the formula. \((1 - \frac{V_t}{V_c}) \times 100 = \text{percentage inhibition}\), where \(V_t\) was the oedema volume in treated rats and \(V_c\) in control rats.

Drugs were suspended in 4% gum acacia solution. Gum acacia4% mucilage was used as vehicle for obtaining control data.

RESULTS AND DISCUSSION

SPECTRAL STUDIES OF THE COMPOUND 7, 8

Structures of the compounds synthesized during the present work were carried out on the basis of chemical data. However the structures were established with their valuable data. The purity of all the compounds were established on TLC with their valuable data. The purity of all the compounds were established on TLC with single spot and also with \(R_f\) value. The spectra were taken at the Department of Chemistry, IIT, Chennai.

SPECTRAL DATA OF NITRO HYDROXY 1, 2 PYRAZOLINE OF SULPHADIAZINE MANNICH BASE (COMPOUND A)
ELEMENTAL ANALYSIS COMPOUND (a)

The compound A was found to have 61.5% carbon, 42% hydrogen and 13.0% of nitrogen against the calculated value of 61.8%, 45% and 131%, respectively.

U.V. SPECTRA OF COMPOUND A

Under ethanol medium, compound A was found to have wavelength of 314nm.

I.R. SPECTRA OF COMPOUND a

| Type of Vibration | GROUP Frequency in wave number (Cm⁻¹) |
|------------------|--------------------------------------|
| -C=N             | 1580                                 |
| -OCH₃            | 1370,740-800                         |
| -CH₂             | 1460                                 |
| -OH              | 2950-3500                            |
| -SO₂-NH          | 1150                                 |

The I.R. spectrum of the above compound showed characteristic absorption bands in the following region. The hydroxyl group showed a vibrational frequency between 2980 and 3500 cm⁻¹ while carbazo group at 1580 cm⁻¹ methoxy group showed in the region of 1370 cm⁻¹ and between 740 and 800 cm⁻¹. The sulpha amino group showed in 1150 cm⁻¹ and methylene group showed in 1460 cm⁻¹.

PROTON MAGNETIC RESONANCE SPECTRUM OF COMPOUND

δ 6.3 to δ 8.1 corresponding to the aromatic protons account for 15 protons. δ 8.3 – δ8.5 were due to the 3 protons of the pyrimidine, δ 8.9 correspond to the phenolic proton. δ 4.2 to δ5.2 correspond to the – CH which appears as triplet and the three – NH protons appear as humps δ 3.0- δ 4.0 peaks were due to – CH₂₂, –CH₂, -OCH₃ protons consisting of 7 protons.

From elemental analysis, IR data, NMR data, the structure of the compounds were found to be in conformity with the desired product.

PHARMACOLOGICAL SCREENING

The control group rats showed increased paw volume of 0.30. In the standard group (Ibuprofen) showed an increased paw volume of 0.12. The compound A and compound B showed almost the same increased paw volume of 0.11 and 0.12, respectively when compared with the standard drug. But compound C and D showed an increased paw volume of 0.18 and 0.17, respectively. The compounds were tested at the dosage level of 100 ml/kg.

The percentage of inhibition of activity shown by the standard group is 60%. The compounds A, B, D, E and F showed 61%, 59%, 44%, 45%, respectively and is statistically significant. The percentage of inhibition of compounds C and E are 37%,
7%, respectively but found to be statistically not significant.

**ANTIMICROBIAL SCREENING**

All the Mannich bases were screened for Anti-microbial activity at 50 µg and 100 µg concentrations against the organisms Bacillus subtilis, staphylococcus aureus, Escherichia coli and aspergillus niger. Disc and plate methods were used for the inhibition studies. Ampicillin was used as a standard drug for comparison. Anti fungal screening was carried out using readymade sabouraud dextrose agar medium. Nystatin was used as standard drug for comparison.

None of the compounds screened showed any anti-bacterial activity at100 µg concentration against the test organisms except nitro hydroxyl 1, 2 – pyrazoline of sulphadiazine mannich base (A), which showed slight activity against bacillus subtilis.

None of the compounds screened showed any anti-fungal activity against Aspergillus niger except bromohydroxy 1, 2-pyrazoline of sulphadiazine mannich base (A).

From the elemental analysis IR data, NMR data the structure of the newly synthesized mannich bases were in confirmation with the desired product.

Significant edema inhibiting activity was exhibited by compounds, Nitro hydroxyl pyrazoline of sulphadiazine mannich compound (A), Nitro hydroxyl pyrazoline of sulphacetamide mannich compound(B), Nitro hydroxyl pyrazoline of Aminopyridine mannich compound(D), Nitro hydroxyl pyrazoline of antipyrine mannich compound(F) and were as active as the (comparable to the ) standard drug used in present study viz., ibuprofen (200mg/kg).

The Compounds nitro hydroxyl pyrazoline of thiazole mannich compound (c), nitro substituted hydroxyl pyrazoline of benzoic acid mannich base, E do not exhibit any beneficial effect.

Nitro hydroxyl pyrazoline of sulphadiazine Mannich compound (A) showed slight anti-bacterial activity against Bacillus subtilis and also showed slight anti fungal activity against Aspergillus niger.

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