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Research Article

IN-VITRO ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF N-CHLOROPICOLINAMIDE

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ABSTRACT

N-Chloropicolinamide (NCP) was synthesized by reaction of picolinamide (PA) with trichloroisocyanuric acid (TCICA) and further treated with methanol and characterized by IR, UV, ^{1}HNMR, ^{13}C NMR and Mass spectral analysis. In the present paper, the *in-vitro* anti-inflammatory and antioxidant activity of synthesized compound were studied. Antioxidant property was evaluated by using 2,2-diphenyl-2- picrylhydrazyl (DPPH) free radical-scavenger method and anti-inflammatory activity was evaluated by inhibition of albumin denaturation method using diclofenac sodium as a standard.

Keywords: Anti-inflammatory, Antioxidant, N-Chloropicilinamide, Synthesis.

1. INTRODUCTION

The N-halo reagents have been extensively developed for use in organic synthesis. N-halo compounds are versatile reagents and have been employed as potentially reactive intermediates that are widely used in organic synthesis [1]. Some specific feature of N-halo reagents such as the high activity of the N-X bond and the various modes of fission of this bond determine their wide applications in organic synthesis [2]. A very simple method for the preparation of several compounds that have extensive applications in organic synthesis, industry and medicine has been developed [3, 4].

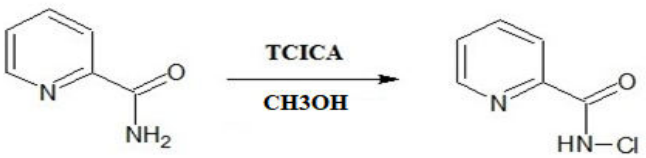
Inflammation is a disorder involving localized increase in the number of leukocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation [5]. Previously, it was reported that reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion, and peroxynitrite radicals will participate in the process of inflammation [6, 7]. These ROS produced in excess will injure cellular bio molecules such as nucleic acids, proteins, carbo-hydrates and lipids, causing cellular and tissue damage and leads to different inflammatory conditions parti-cularly, skin inflammations, broncho-inflammations and arthritis which augments the state of inflammation [8]. Antioxidants are molecules, natural or synthetic, capable of interacting with free radicals and stopping their chain reactions before essential vital molecules are damaged.

Free radicals such as superoxide, hydroxyl and nitric oxide are the oxygen centered free radicals, and they are also called reactive oxygen species (ROS) [9]. These free radicals then initiate a chain reaction which leads to the formation of various other free radicals leading to oxidative stress which in turn results in the productivity of reactive oxygen species and reactive nitrogen species causing lipid peroxidation (LPO) and cellular damage [10, 11]. The violation in the balance between oxidants and reductants in benefit for the oxidative processes is called as "oxidative stress." Antioxidants are chemical compounds that can scavenge free radicals that are formed in the body due to normal physiological process [12]. They are generated in the human body and would cause damage to lipids, proteins and DNA and thus may lead to various diseases such as carcinogenesis, drug-associated toxicity and inflammation [13]. In view of these findings, it was considered of interest to undertake the synthesis of N-Chloropicolinamide compound that might possess certain anti-oxidant and anti-inflammatory activity. The structures of the synthesized compound were deduced on the basis of ^{1}H and ^{13}C NMR, IR and mass spectra. Therefore, in the present study synthesized N-Chloropicolinamide compound were screened for their antioxidant activity by diphenylpicrylhydrazyl (DPPH) and anti-inflammatory by albumin denaturation method.

MATERIALS AND METHODS

1.1. Synthesis and characterization of n-chloro-picolinamide

N-Chloropicolinamide was synthesized by chlorination with trichloroisocyanuric acid under mild conditions at room temperature. This method is clean, fast and efficient, the yields are also good to excellent [14].



The melting point was found to be 138°C with molecular formula as $C_6H_5ON_2Cl$. NCP was found to be soluble in water, acetic acid, DMSO, DMF, sparingly soluble in ethanol, ethyl acetate, chloroform and insoluble in benzene, acetonitrile, dioxane and DCM.

1.2. Evaluation of the anti-inflammatory activity of the synthesized N-Chloropicolinamide compound

In vitro anti-inflammatory activity was performed by using inhibition of protein denaturation method according to the previously reported literature with slight modification. The reaction contained 2ml of different concentrations (100, 200, 300, 400 and 500 µg/ml) of the test compounds or diclofenac sodium standard and 2.8ml of phosphate buffer saline pH-6.4 were mixed with 0.2ml egg alumin obtained from fresh hen's egg incubated 37°C for 15min, heated at 70°C for 5min and cooled to reach room temperature. After cooling, their absorbance was measured at 660nm by using vehicle as blank. The percentage inhibition of protein denaturation was calculated using the following formula

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

1.3. In vitro- antioxidant activity of synthesized N-Chloropicolinamide compound

In vitro antioxidant activity was performed by using a free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) model system according to the methods previously reported with slight modification. The reaction mixture consisted of 0.5ml of different concentration of the test compounds. The mixture was shaken vigorously and allowed to stand at room temperature in the dark 30 min. the absorbance was then measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

Radical scavenging activity (%) = 100 - {(Ac-As)/Ac} X 100

Where $A_{C=}$ control in the absorbance and $A_{S=}$ sample in the absorbance of reaction mixture (in the presence of sample).

2. RESULT AND DISCUSSION

The title compound was screened for its in *vitro* model using anti-inflammatory and antioxidant activity. The antioxidant activity was done using the free radical DPPH- scavenging activity model system. It has been found that, the inhibition of protein denaturation activity and DPPH- radical scavenging activity of the synthesized compound was concentration dependent as shown in the fig. 1 and fig. 2. The IC_{50} values of the biological activity of the N-Chloropicolinamide compounds calculated from the data were displayed in table 1 & 2.

Table 1: Protein Denaturation activity of N-Chloropicolinamide synthesized compounds

Samples	Concentrations (µg/ml)					IC_{50} Value
	100	200	300	400	500	
N-Chloropicolinamide	22.80±1.59	41.86±2.93	55.70±3.89	65.79±4.61	84.85±5.93	271.68
Standard (Diclofenac sodium)	26.72±1.87	48.78±3.41	63.55±4.44	78.13±5.46	94.95±6.64	225.07

Values are expressed as Mean ± SD for triplicates

Table 2: DPPH free radical scavenging activity of N-Chloropicolinamide synthesized compounds

Samples	Concentrations (µg/ml)				IC_{50} Value
	20	40	60	80	
N-Chloro picolinamide	21.65±1.51	38.26±2.67	64.38±4.51	78.47±5.49	49.29
Standard (Ascorbic acid)	25.72±1.81	40.27±2.81	74.72±5.23	90.62±6.34	43.16

Values are expressed as Mean ± SD for triplicates

In vitro anti-inflammatory activity of NCP was evaluated by protein denaturation method using Diclofenac sodium as reference standard. In the protein denaturation method, synthesized compound showed inhibition in the increasing order of concentration. Synthesized compounds showed good anti-inflammatory activity when compared to the standard diclofenac sodium. The percentage of membrane stabilization for synthesized compound and Diclofenac sodium were done at 100, 200, 300, 400, 500 µg/ml. It showed the maximum inhibition 84.85±5.93at 500µg/ml. The inhibition level of protein denaturation activity of the synthesized compounds shows IC_{50} values 271% when compared with standard Diclofenac sodium as 225% as shown in the above table 1 and fig.1. Therefore, the title compound has potent anti-inflammatory activity.

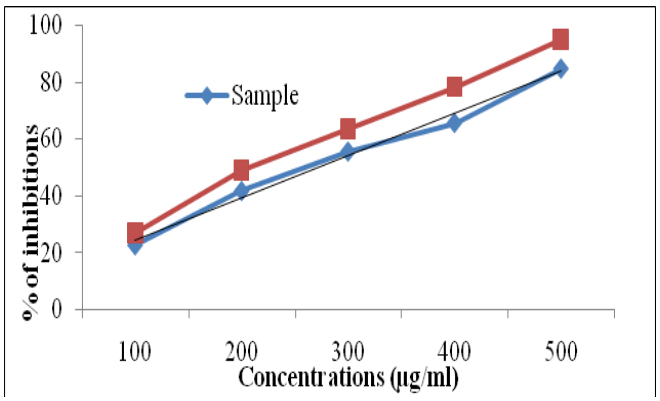


Fig. 1: Egg albumin activity of N-Chloropicolinamide compared with Std. Diclofenac sodium

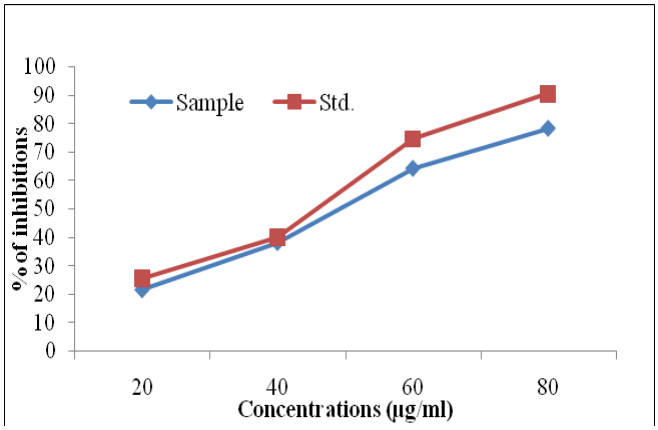


Fig. 2: DPPH activity of N-Chloropicolinamide compared with Std. (Ascorbic acid)

Various researchers have used the scavenging effect of a chemical on DPPH radical assay as a quick and reliable parameter to assess the *in vitro* antioxidant activity. The results of free radical scavenging activity of synthesized compound at different concentrations are shown in table 2 and fig. 2. DPPH scavenging activity of the compounds increasing concentration from 21.65±1.51 to 78.47±5.49% and compared with standard drug Ascorbic acid range from 25.72±1.81 to 90.62±6.34 % respectively. In DPPH assay synthesized compound with IC_{50} value of 49.29% showed significant activity when compared to standard drug ascorbic acid with IC_{50} value of 43.16%. Therefore, N-Chloropicolinamide compounds showing scavenging activities towards these ROS may expect to have therapeutic potentials towards inflammatory diseases.

3. CONCLUSION

A new compound N-Chloropicolinamide (NCP) derivatives was synthesized successfully and evaluated for their anti-inflammatory activity through inhibition protein denaturation and antioxidant activity employing free radical DPPH scavenging activity method compound containing NCP moiety which shows the highest anti-inflammatory. The activity was comparable to diclofenac sodium. While compound containing NCP moiety exhibited the highest anti-oxidant activity. The compound exhibited moderate antioxidant activity compared to ascorbic acid as standard.

Conflict of Interest

None declared

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