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Research Article

Synthesis and Biological Evaluation of Cinnamaldehyde Analogues for Anti-Arthritic Activity

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ABSTRACT

The present study is aimed to evaluate the anti-arthritic activity of novel Schiff bases of cinnamldehyde using in-vitro inhibition of protein denaturation model. All the compounds synthesized were characterized by running TLC, UV, IR & MS spectra. Diclofenac sodium was used as a standard drug for anti-arthritic activity. Results revealed that the derivative Cinnan 2 possessed significant anti-arthritic activity as compared to standard drug Diclofenac sodium. The remaining derivatives showed dose dependent activity.

Keywords: cinnamaldehyde, Schiff bases, Diclofenac sodium.

INTRODUCTION

Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability[1]. It caused by number of pro-inflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines[2]. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions[3].

The production of auto antigens in certain arthritic diseases may be due to in vivo denaturation of proteins[4]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding[5]. So, by controlling the production of auto antigen and inhibiting denaturation of protein and membrane lysis in rheumatic disease leads to anti-arthritic activity. Hence, inhibition of protein denaturation and membrane lysis were taken as a measure of the in vitro anti-arthritic activity[6].

Cinnamaldehyde occurs naturally in the bark of Cinnamon zylanicum of family laureaceae[7]. The important uses of cinnamaldehyde are fungicide, mild astringent, antimicrobial[8], anti-inflammatory[9], anti-septic[10] etc. The Schiff bases usually synthesize from a primary amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by dehydration to generation imines [11]. We describe synthesis of three new Schiff bases cinnamaldehyde using n-Hexane as solvent.

EXPERIMENTAL

1. *In silico* molecular study

In silico molecular study of the probable derivatives was determined whether all the candidates will follow the Lipinski Rule of '5' is carried out. Different soft wares like ACD/ILAB, Mol inspiration were employed to determine the physicochemical descriptors are given in Table 1.

2. Experimental procedure: Method I (conventional method)[12]

2.1. Synthesis of (1*E*, 2*E*)-*N*, 3-diphenylprop-2-en-1-imine: Cinnamaldehyde (0.01 mol) and Aniline (0.01 mol) were refluxed in 50 ml of n-Hexane. UV λ max (methanol) 394 nm, IR (KBr): 1725, 1630, 1513, 1462, 1251, 791 $cm^{-1,}$ MS m/z 207 [M^+ 100%]. The synthetic route is represented in Scheme 1.

2.2. Synthesis of 4-{(*E*)-[(2*E*)-3-phenylprop-2-en-1-ylidene] amino} phenol: Cinnamaldehyde (0.01 mol) and 4-amino phenol (0.01 mol) were refluxed in 50 ml of n-Hexane. UV λ max (methanol) 383

nm; IR (KBr): 3370, 1739, 1590, 1509, 1250, 833, 791 $cm^{-1,}$ MS m/z 223[M^+ 100%].

2.3. Synthesis of (1*E*, 2E)-*N*-(4-chlorophenyl)-3-phenylprop-2-en-1-imine: Cinnamaldehyde (0.01 mol) and 4- chloro aniline (0.01 mol) were refluxed in 50 ml of n-Hexane. UV λ max (methanol) 373 nm; IR (KBr): 1725, 1630, 1513, 1462, 1251, 791 $cm^{-1,}$ MS m/z 241[M^+ 100%].

3. Evaluation in-vitro anti-arthritic activity

For the evaluation invitro anti-arthritic activity of cinnamaldehyde analogs the method used was "inhibition of protein denaturation"[13-16] using Diclofenac sodium a standard.

Biological procedure:

The test solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of test solution (different derivatives). The test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium.

Various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) of different analogs of cinnamaldehyde (test solution) and diclofenac sodium (standard) of were taken respectively. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°c for 20 minutes and the temperature was increased to keep the samples at 57°c for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation of different concentrations was tabulated in Table 2.

The percentage inhibition of protein denaturation can be calculated as-

% of Inhibition = [100 - (O.D of test solution – O.D of product control)] X 100.

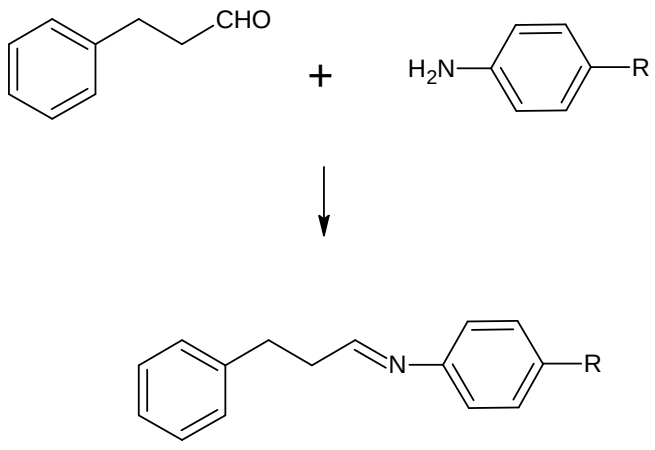
(O.D of test control) The control represents 100% protein denaturation .The results were compared with Diclofenac sodium.

RESULTS AND DISCUSSIONS

Conventional method for the synthesis of these compounds requires 2-6 hours reflux and suitable solvents with catalytic amount of n-hexane. Structures of compounds were characterized by IR, and mass spectral data. All the imines show the characteristic band around 1620 cm^{-1} for C=N stretching and C=C stretch around 1720-1740 cm^{-1}. Different concentrations of cinnamaldehyde derivatives and Diclofenac sodium were tested for anti-arthritic activity and found to have significant activity.

Table1: Physicochemical parameters

compound	Molecular formula	Formula Weight	Molar refractivity	Molar volume	Polarizability
Cinnan 1	$C_{15}H_{15}N$	209.28	69.63±0.5cm^2	221.7±7.0cm^3	27.60±0.5cm^2
Cinnan 2	$C_{15}H_{15}NO$	225.28	70.48±0.5cm^2	219.0±7.0cm^3	27.94±0.5cm^2
Cinnan 3	$C_{15}H_{14}ClN$	243.73	74.23±0.5cm^2	231.0±7.0cm^3	29.42±0.5cm^2



Scheme 1

Table 2: Percentage inhibition of protein denaturation

Concentration in µg/ml	Percentage inhibition of protein denaturation			
	Cinnan 1	Cinnan 2	Cinnan 3	Diclofenac
50	42.82	50.62	46.41	50.73
100	53.65	74.54	55.94	75.34
250	60.85	79.66	67.72	80.21
500	72.92	85.53	76.18	86.06
1000	79.68	90.42	80.57	91.93
2000	82.71	93.84	85.61	94.51

CONCLUSION

The Cinnamaldehyde derivative Cinnan 2 has showed significant activity at various concentrations and its effect was compared with the standard drug Diclofenac sodium. The maximum percentage inhibition of protein denaturation was observed as 93.84% and 85.61% at 2000 µg/ml respectively as shown in Table 1. When compared to standard Diclofenac sodium was found out to be Cinnan 2 is having the similar activity as that of Diclofenac sodium.

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