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(RESEARCH ARTICLE)

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Design, synthesis and evaluation of some novel derivatives of cinnamic acid as anti-inflammatory agents

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Abstract

The role of Cinnamic acid derivatives as antioxidants, antimicrobial agents, antidiabetic drugs, anticancer drugs etc. is well reported. A thorough literature search revealed that the substitution on carboxylic acid group of Cinnamic acids by different amines imparts the potent conjugates. The quest for the design of effective anti-inflammatory drugs is still the primal focus of several research works world-wide owing to the side effects associated with the existing drugs. The side effects of the existing drugs are associated with the presence of the free carboxyl group in the molecules. In the present work, Cinnamic acid was reacted with various cyclic amines in presence of coupling agent to obtain the corresponding amide derivatives which were evaluated for anti-inflammatory activity using in vitro methods.

Keywords: Design; Synthesis; Novel; Cinnamic acid; Amines; Anti-inflammatory

1. Introduction

Inflammation is normal and necessary protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia [1]. It is caused by a variety of stimuli, including physical damage, UV irradiation, microbial attack, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many of these diseases are debilitating and are becoming increasingly common in our ageing society. Rheumatoid arthritis and degenerative arthritis are the major inflammatory diseases affecting people worldwide [2]. Rheumatoid arthritis is an inflammatory term that usually involves multiple joints. It affects 0.3–1.0% of the worldwide population and is more predominant among women in developed nations. The continual inflammation leads to joint damage; however, the disease can be inhibited with drugs uses. Degenerative joint disease, which is considered by trouncing of joint cartilage that leads to pain loss and damage the function primarily in the hips and, affects 9.6% of adult males and 18% of women aged more than 60 years. Gains in life expectancy and aging populations are required to make the fourth leading cause of handicap by the year 2020 [3].

Cinnamic acid, a natural aromatic carboxylic acid (Figure 1), is a key chemical found in plants such as *Cinnamomum cassia* (Chinese cinnamon) and *Panax ginseng*, fruits, whole grains, vegetables and honey. The presence of an acrylic acid group substituted on the phenyl ring gives cinnamic either a *cis* or a *trans* configuration with the latter being the most common of the two. Studies have reported that cinnamic acid exhibit antioxidant, antimicrobial, anticancer, neuroprotective, anti-inflammatory and antidiabetic properties. Cinnamic acid terminates radical chain reactions by donating electrons that react with radicals forming stable products. Cinnamic acid can be prepared by enzymatic deamination of phenylalanine [4].

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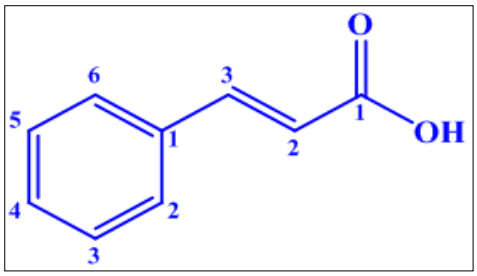


Figure 1 Structure of Cinnamic acid

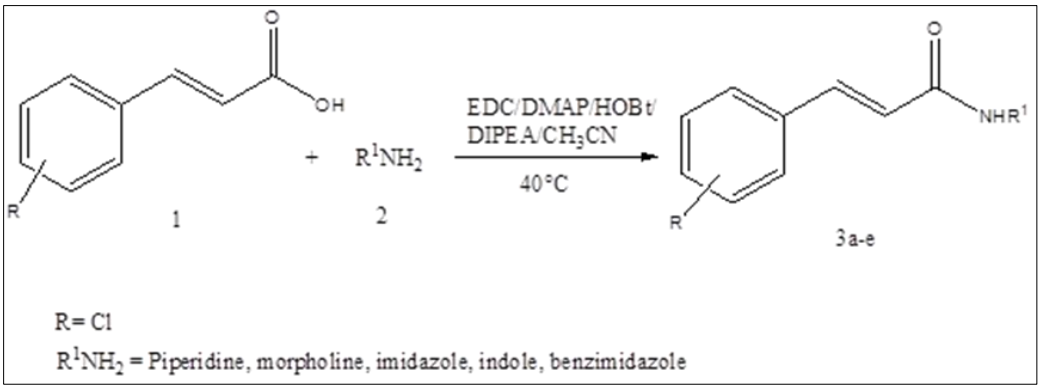
2. Material and methods

2.1. Material used

The chemicals and reagents required for the present investigation were obtained from various sources and used untreated.

2.2. Preparation of Amides of cinnamic acid (General procedure) [5]

1mmol of cinnamic acid and 1 mmol of amine were dissolved in acetonitrile. To this solution was added 1equivalent of EDC, 1 equivalent of DMAP, 5 equivalent of DIPEA and 0.1 equivalent of HOBt. The reaction mixture was refluxed at 40°C for 48 hours. The completion of the reaction was monitored using TLC using hexane-ethylacetate-acetic acid (8-1-0.1) as the solvent system.



Scheme 1 General method for the preparation of amides of cinnamic acid

2.3. Chemical Characterization

All the synthesized compounds were characterized for melting point, solubility, yield and the structure was confirmed using spectral studies like NMR, Mass and IR.

2.4. Melting point determination

The melting points were determined using open capillary method using a electrically heated melting point determination apparatus and are reported uncorrected.

2.5. Thin Layer Chromatography

The purity and homogeneity of the compounds was determined by thin layer chromatography, using silica gel G as the stationary phase on glass plates. Iodine vapors were used for development of the chromatogram. The solvent system used for performing the TLC of compounds was hexane: ethylacetate: acetic acid in the ratio 9:1:0.1.

2.6. Solubility

The solubility of all the synthesized compounds was studied in solvents of varying polarity. A small amount of the sample was shaken in 1 mL of solvent in a test tube and was visually inspected for the absence of the solid particles in the test tube.

2.7. Evaluation of *in vitro* anti-inflammatory activity

A number of methods are available for *in vitro* evaluation of anti-inflammatory activity; two methods were considered for the present work.

2.7.1. Inhibition of albumin denaturation

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution.

The technique of inhibition of albumin denaturation reported previously [6, 7] was used with slight modifications. The volume of each component of the reaction mixture was reduced to half its volume.

The synthesized molecules were individually dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentration. A solution of 1% BSA in deionized water was prepared for the test.

The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solutions. Ibuprofen solution (1 µg/mL) was used in the positive control and distilled water was used in the negative control vessels instead of test solution.

The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition } = (1 - \mathrm{D/C}) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

2.7.2. Anti-proteinase action

Preparation of Tris-HCl buffer

An accurately weighed quantity of 121.44 g of Tris was dissolved in 800 mL of distilled water. The pH of the solution was adjusted to 7.0 by addition of appropriate volume of concentrated HCl and the final volume of the solution was made up to 1 L with distilled water.

The technique of anti-proteinase action reported by Oyedepo *et al* [8] and Sakat *et al* [9] was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 µg/mL). The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition of proteinase inhibitory activity was calculated by the following formula:

$$\text{Percentage inhibition } = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

2.8. Statistical Analysis

All the experiments were performed in triplicate and the results are expressed as mean ± standard deviation. The difference between the experimental groups was compared by one-way ANOVA followed by Dunnets multiple comparison test using Graph Pad Instat software.

3. Results

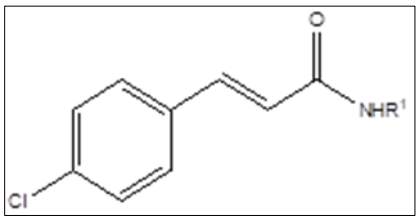


Figure 2 General structure for Amide derivatives of Cinnamic acid

Table 1 Physicochemical features of cinnamamide derivatives (3a-e)

Compound	Color	Yield (%)	Melting Point (°C)
3a	Yellow	71	167-170
3b	Brown	68	159-161
3c	Pale Yellow	72	148-151
3d	Yellow	74	173-175
3e	Brown	67	162-164

Table 2 Solubility profile of compounds 3a-e

Compound	Water	Methanol	Chloroform	DMSO
3a	Insoluble	Soluble	Soluble	Soluble
3b	Insoluble	Soluble	Soluble	Soluble
3c	Insoluble	Soluble	Soluble	Soluble
3d	Insoluble	Soluble	Soluble	Soluble
3e	Insoluble	Soluble	Soluble	Soluble

Compound 3a

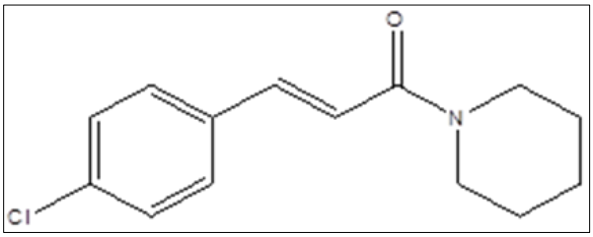


Figure 3 Structure of Compound 3 (a)

IUPAC: (E)-3-(4-chlorophenyl)-1-(piperidin-1-yl)prop-2-en-1-one

Molecular Formula: $C_{14}H_{16}ClNO$

FT-IR (cm^{-1}): 3165 (C–H str.); 1700 (C=O); 1589, 1482, 1482 (C=C ring str.), 1013 (C-N); 582 (C–Cl)

1H-NMR ($CDCl_3$) (δ ppm): 1.591-1.604 (C-H, Piperidine), 3.691 (CH methylene), 7.218-7.235 (CH Aromatic)

Mass (m/e): 283.9 (M^+)

Compound 3b

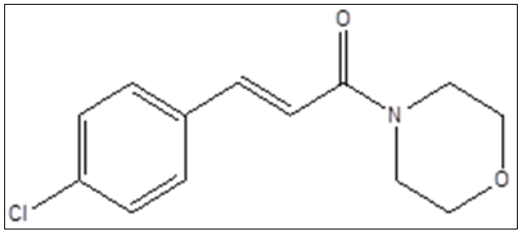


Figure 4 Structure of compound 3(b)

IUPAC: (E)-3-(4-chlorophenyl)-1-morpholinoprop-2-en-1-one

Molecular Formula: $C_{13}H_{14}ClNO_2$

FT-IR (cm^{-1}): 3119 (C–H str.); 1709 (C=O); 1478 (C=C ring str.), 1212 (C-O); 1018 (C-N); 609 (C–Cl)

1H-NMR ($CDCl_3$) (δ ppm): 3.091-3.104 (C-H morphopline), 6.635-7.670 (CH Aromatic)

Mass (m/e): 252.7 (M^+)

Compound 3c

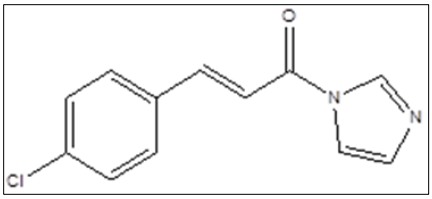


Figure 5 Structure of compound 3(c)

IUPAC: (E)-3-(4-chlorophenyl)-1-(1H-imidazol-1-yl)prop-2-en-1-one

Molecular Formula: $C_{12}H_9ClN_2O$

FT-IR (cm^{-1}): 3119 (C–H str.); 1707 (C=O); 1484 (C=C ring str.); 1015 (C-N); 608 (C–Cl)

1H-NMR ($CDCl_3$) (δ ppm): 7.297 -7.636 (CH Aromatic)

Mass (m/e): 232.9 (M^+)

Compound 3d

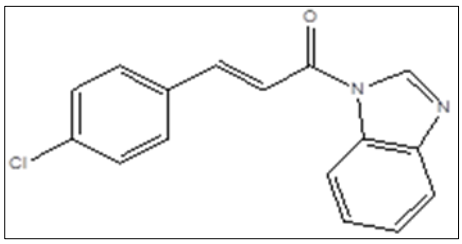


Figure 6 Structure of compound 3(d)

(E)-1-(1H-benzo[d]imidazol-1-yl)-3-(4-chlorophenyl)prop-2-en-1-one

Molecular Formula: $C_{16}H_{11}ClN_2O$

FT-IR (cm^{-1}): 3113 (C–H str.); 1709 (C=O); 1509 (C=C ring str.); 1027 (C-N); 600 (C–Cl)

1H-NMR ($CDCl_3$) (δ ppm): 7.241-7.646 (CH Aromatic)

Mass (m/e): 282.6 (M^+)

Compound 3e

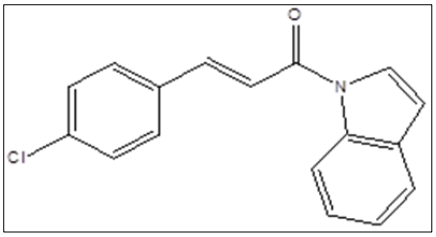


Figure 7 Structure of compound 3(e)

IUPAC: (E)-3-(4-chlorophenyl)-1-(1H-indol-1-yl)prop-2-en-1-one

Molecular Formula: $C_{17}H_{12}ClNO$

FT-IR (cm^{-1}): 3115 (C–H str.); 1708 (C=O); 1525, 1455 (C=C ring str.); 1016 (C-N); 599 (C–Cl)

1H-NMR ($CDCl_3$) (δ ppm): 7.212-7.656 (CH Aromatic)

Mass (m/e): 283.9 (M^++2)

Table 3 Inhibition of albumin denaturation by test compounds

Treatment	Inhibition of albumin denaturation (%)					
	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL	10 µg/mL
TDS1	9.39±2.002	14.37±2.099	23.63±2.228	36.17±2.069	44.36±3.754	ND
TDS2	16.36±2.165	24.35±2.314	34.64±3.821	53.83±3.292	67.36±2.998	ND
TDS3	7.5±2.291	16.1±2.156	27.3±1.194	33.5±2.167	39.6±3.869	ND
TDS4	13.37±1.657	24.97±2.623	34.69±3.034	53.05±3.165	56.64±3.194	ND
TDS5	11.01±1.659	20.18±2.617	32.86±3.009	44.29±3.616	53.93±4.209	ND
Ibuprofen	ND	ND	ND	ND	ND	52.28±3.261

Table 4 Percent inhibition of proteinase action by test compounds

Treatment	Inhibition of Proteinase Actinon (%)					
	10 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Ibuprofen	53.17±2.146	ND	ND	ND	ND	ND
TDS1	ND	6.04±0.933	9.47±0.666	14.55±1.033	19.28±2.011	28.24±2.988
TDS2	ND	9.63±1.033	13.64±1.168	21.82±2.011	34.22±2.333	45.03±3.211
TDS3	ND	4.12±0.899	7.21±1.033	13.43±1.066	15.08±1.066	20.18±1.523

TDS4	ND	7.95±1.011	13.50±1.066	19.87±2.666	26.77±3.033	38.96±3.033
TDS5	ND	7.29±1.333	12.46±0.933	16.66±2.066	23.11±1.333	36.22±3.011

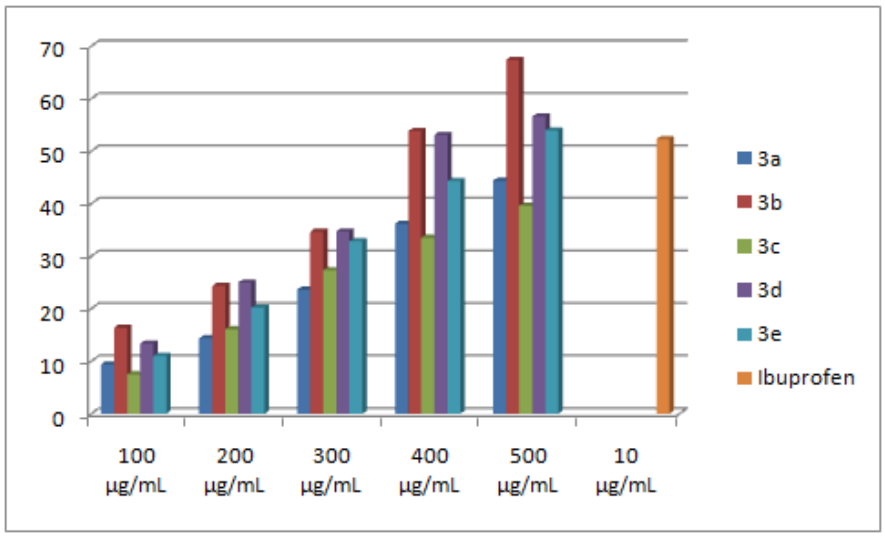


Figure 8 Comparison of percent albumin denaturation by 3a-e

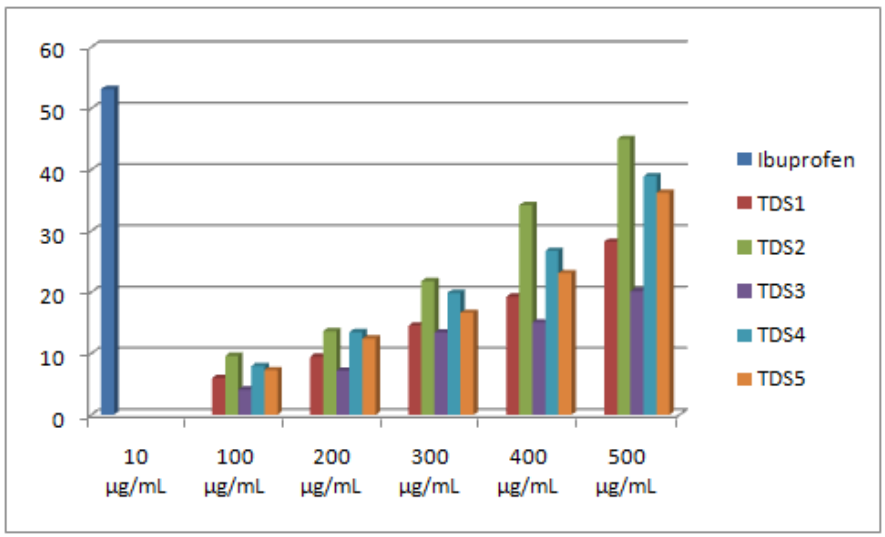


Figure 9 Comparison of percent anti-proteinase action exhibited by 3a-e

4. Discussion

The formation of an amide linkage is one of the most common features in pharmacologically active compounds. The formation of amide bond occurs by the interaction of the carboxylic group and the amino group of the reactants. EDC and DMAP are involved in the activation of the carboxyl group. Reaction of carboxylic acid with EDC in the presence of HOBt results in the formation of reactive HOBt ester. DMAP then functions as an acyl transfer agent and forms the highly reactive acyliminium ion intermediate. The reaction of the this intermediate occurs with the amine group leading to the formation of the amide linkage.

All the compounds exhibited peaks of C-H stretching (3100-3200 cm^{-1}). The vibrations of C=O stretching (1700-1710 cm^{-1}), C=C stretching (1600-1400 cm^{-1}), C-N stretching (1010-1020 cm^{-1}) and C-Cl (550-630 cm^{-1}) stretching were also found in the corresponding compounds.

The ^{1}HNMR spectra of all the compounds presented peaks at 6.6-7.9 corresponding to aromatic protons. The compounds 3a and 3b also exhibited the chemical shifts due to the protons of the saturated cyclic proton at 1.5-1.6 and 3.1-3.6

The mass spectra of the compounds exhibited peaks due to fragmentation of the molecules along with molecular ion peak or the isotopic peak.

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis. It has been said that tissue injury might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of the test compounds to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity.

It has also been reported that leukocytes protease has an important role in the development of tissue damage during inflammatory reactions and significant level of protection could be provided by protease inhibitors. Hence the inhibition of protease action by test compounds signifies its role as anti-inflammatory molecules.

All the compounds exhibited dose dependent inhibition of albumin denaturation with 3b having the highest capacity to cause the inhibition (67.36±2.998%) at the concentration of 500µg/mL. The anti-proteinase action was also dose dependent and 3b at 500µg/mL was able to inhibit (45.03±3.211 %) of proteinase activity. The results reveal that the presence of a five membered ring as a part of cyclic amide was not beneficial for anti-inflammatory action. On the other hand, it was also relevant that the cyclic amides with two hetero atoms exhibited better anti-inflammatory action in comparison to the cyclic amides with one heteroatom (3a, 3e).

5. Conclusion

In the present study, cinnamamide compounds were synthesized using the reaction of cinnamic acid and appropriate cyclic amine. The compounds were found to be of good purity and yield. The compounds exhibited anti-inflammatory activity in the *in vitro* assays. One compound **3b** was found to be significantly potent. Optimization of the amide substitution using computer aided techniques could be used to design lead molecule with potent anti-inflammatory action.

Compliance with ethical standards

Acknowledgments

We acknowledged the contributions of the Laboratory staff of Department of Pharmaceutical Chemistry, Oriental College of Pharmacy, Bhopal for creating a friendly environment for the synthesis of test compounds and for carrying out In-vitro anti-inflammatory Study.

Disclosure of conflict of interest

The authors (Ms. Geetika Vaishnav, Dr. Anup K Chakraborty, Dr. Sarita Karole, Dr. Kavita R. Loksh) declare no conflict of interest.

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