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Synthesis of Curcumin Based Mutual Pro-Drugs for Some Anti-Inflammatory Drugs

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ABSTRACT

Conjugation of the carboxylic acid group of NSAIDs while converting to prodrugs is known to be a successful approach to decrease the gastric irritation caused by NSAIDs. The crude conjugate was recrystallized from methanol to obtain curcumin-NSAID mutual prodrug. The technique of inhibition of albumin denaturation was used for evaluating the anti-inflammatory potential of the synthesized compounds. The formation of **CPD$_{1\text{-}5}$** begins with the formation of a protonated compound from the reaction between the acyl chloride and the hydroxide. Finally using the Schotten Baumann reaction mechanism, the required ester product is formed along with hydrochloric acid now that the base catalyst has absorbed the acidic proton. The albumin denaturation by the prodrugs of all the NSAIDs was higher than the corresponding NSAIDs confirming the hypothesis that the inclusion of curcumin in the prodrugs would be able to enhance the anti-inflammatory action. The objective of the present investigation was to synthesize mutual prodrugs of some NSAIDs by conjugating them with curcumin. The results indicate that the anti-inflammatory action improved significantly and further studies need to be carried out for ascertaining the decreased gastric irritation and hydrolysis.

INTRODUCTION

The diferuloylmethane (curcumin) that has been isolated from turmeric rhizomes has been reported to have several therapeutic and pharmacological benefits (Duvoix*et al*, 2005; Goel*et al*, 2008; Epstein *et al*, 2010). The most prominent action of curcumin that has been widely used is it's antioxidant and anti-inflammatory (Kohli *et al*, 2005; Jayaprakasha*et al*, 2006). The mechanism by which curcumin exerts its anti-inflammatory actions is yet to be deduced. Research has shown that peroxisome proliferator-activated receptor gamma (PPAR-γ) could be associated with anti-inflammatory effects. PPARs belong to the superfamily of nuclear receptors consisting of three genes that give rise to three different subtypes, PPAR-α, PPAR-δ, and PPAR-γ. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the management of peripheral pain but owing to the presence of the acidic carboxyl group they cause severe gastric irritation which may lead to ulcers. The presence of the carboxyl group as such is not required for the action of NSAIDs (Ullah *et al*, 2016).

Conjugation of the carboxylic acid of NSAIDs while converting to prodrugs is known to be a successful approach to decrease the gastric irritation caused by NSAIDs. Hence it was envisioned that conjugating curcumin to classical NSAIDs would be able to achieve dual advantages of decreased gastric irritation as well as enhanced anti-inflammatory action.

MATERIALS AND METHODS

Curcumin and thionyl chloride was purchased from Oxford Fine Chemicals, Mumbai. Aceclofenac, diclofenac, mefenamic, ibuprofen, and acetylsalicylic acid were purchased from Yarrow Pharmaceuticals, Mumbai. All other chemicals and reagents used were purchased from Oxford Fine Chemicals, Mumbai, and were used as obtained without further purification.

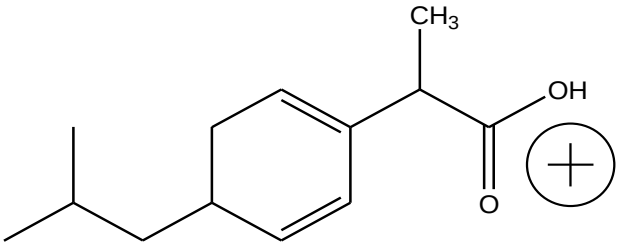
The synthesis of the mutual prodrugs was performed according to scheme 1 in two simple steps. The completion of all the reactions was monitored using silica gel G precoated aluminum-backed TLC plates. Melting points were determined by the open capillary method and are uncorrected. The solubility of each title compound was determined qualitatively in solvents of varying polarities. FTIR, ^{1}H NMR, and mass spectral studies were used to confirm the structure of the synthesized compounds.

The general method of synthesis of 2-(4-isobutylcyclohexa-1,5-dienyl)propanoyl chloride

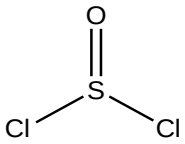
0.05 moles of NSAID were dissolved in a minimum amount of chloroform and freshly distilled thionyl chloride (0.05 mol, 6 ml) was added slowly to it. The mixture was refluxed for 15 h at 60-70ºC with continuous stirring. The viscous liquid was immediately poured on to petri dish and was vacuum dried to give yellow-colored crude acid chloride.

The general method of acylation of curcumin with NSAID acid chloride, CPD_{1-5}

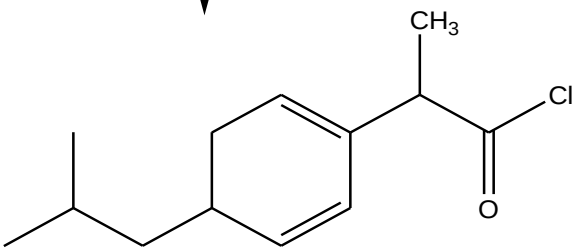
Ice cold, aqueous sodium hydroxide solution (5%) was taken in a 250 ml beaker and curcumin (0.05 mol) was added to it. The reaction mixture was mechanically stirred for 30 min at room temperature, after which the beaker was transferred to an ice bath kept on a mechanical stirrer, maintaining the temperature at 10ºC. NSAID acid chloride (0.05 mol) was added in small portions with continuous stirring for 7-8 hrs. The solid that separated was filtered using a vacuum pump and dried. The crude conjugate was recrystallized from methanol to obtain curcumin-NSAID mutual prodrugs.



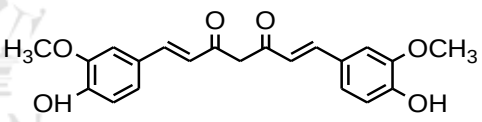
ibuprofen



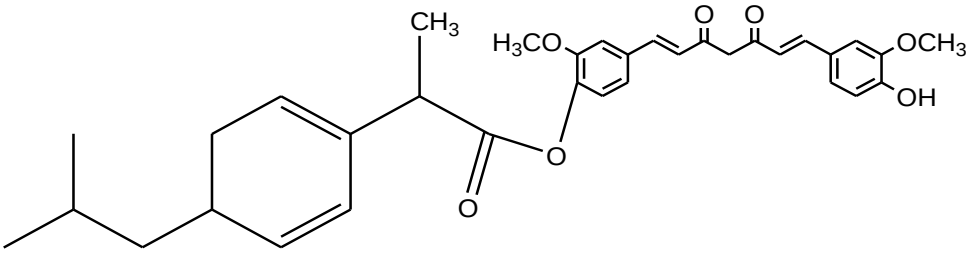
thionyl chloride



2-(4-isobutylcyclohexa-1,5-dienyl)propanoyl chloride



curcumin



4-((1*E*,6*E*)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl 2-(4-isobutylcyclohexa-1,5-dienyl)propanoate

Scheme 1 Reaction scheme for the synthesis of curcumin-NSAID conjugates *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 (Singh and Mishra, 2020) was used for evaluating the anti-inflammatory potential of the synthesized compounds.

The prodrugs were dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400, and 500µg/mL. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control.

The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS, and 1000 µL of the drug solution. Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of the extract 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 a constituent of each vessel was analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

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

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

Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one-way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result was considered statistically significant when P- value less than 0.001 ($P<0.001$) *vs* control.

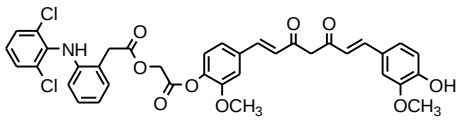
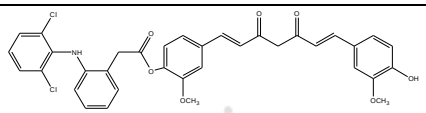
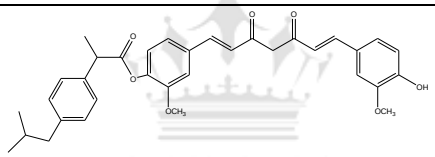
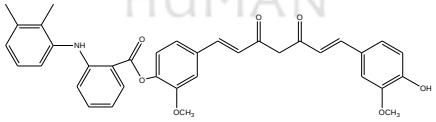
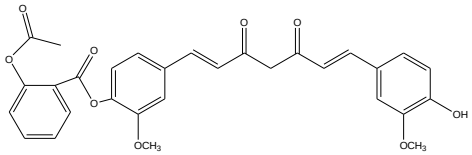
RESULTS AND DISCUSSION

The formation of **CPD$_{1\text{-}5}$** begins with the formation of a protonated compound from the reaction between the acyl chloride and the hydroxide. First, the oxygen atom creates a lone pair of electrons towards the formation of a carbon-oxygen bond followed by the absorption

of the acidic proton by the catalyst (base). Finally using the Schotten Baumann reaction mechanism, the required ester product is formed along with hydrochloric acid now that the base catalyst has absorbed the acidic proton. This HCl is neutralized by the base catalyst as well.

The NSAIDs used, structures of the title compounds, R_f value, and solubility of the curcumin-NSAID conjugates are presented in table 1.

Table No. 1: Properties of the NSAID-curcumin conjugates

Prodrug code	NSAID Used	Structure	Rf Value	Solubility
CPD$_1$	Aceclofenac		0.54	DMSO
CPD$_2$	Diclofenac		0.56	DMSO
CPD$_3$	Ibuprofen		0.49	DMSO
CPD$_4$	Mefenamic acid		0.57	DMSO
CPD$_5$	Acetylsalicylic acid		0.46	DMSO, Chloroform

4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl 2-(2-(2,6-dichlorophenylamino)phenacetoxy)acetate, CPD$_1$

Color: Light Yellow; Yield: 75%; Melting point: 218-220°C; ^{1}H-NMR (400 MHz, DMSO): 7.2-6.5 (CH, benzene), 4.9 (O-H), 4.0 (N-H), 3.44 (CH_3); Mass: *m/z* = 705 [M + 1]; FTIR (cm^{-1}): C=O (1680-1630) 1651.13; C-H (3150-3020) 3112.47; C=C (1675-1600) 1613.97; N-H (3500-3100) 3232.34; C=C (1600-1450) 1585.45, 1477.24; C-H (2960-2850) 2933.32; C-H (3100-3010) 3112.47

4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl 2-(2-(2,6-dichlorophenylamino)phenyl)acetate, CPD₂

Color: Yellow; Yield: 69%; Melting point: 246-248°C; ^{1}H-NMR (400 MHz, DMSO): 7.9-6.3 (CH benzene), 4.9 (O-H), 4.0 (N-H), 2.40 (CH_3); Mass: *m/z* = 647 [M + 1]; FTIR (cm^{-1}): C=O (1680-1630) 1676.80; C-H (3150-3020) 3042.73; N-H (3500-3100) 3397.17, 3297.55; C=C (1600-1450) 1584.79,1477.79; O-H (3400-3200) 3297.55, 3202.53

4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl 2-(4-isobutylphenyl)propanoate, CPD₃

Color: Dark Yellow, Yield: 71%, Melting point: 220-222°C; ^{1}H-NMR (400 MHz, DMSO): 7.9-6.5 (CH benzene), 4.9 (O-H), 4.0 (N-H), 2.01 (CH_2); Mass: *m/z* = 557 [M^+]; FTIR (cm^{-1}): C=O (1680-1630) 1652.15; C-H (3150-3020) 3045.31; C=C (1675-1600) 1613.27; N-H (3500-3100) 3112.55; C=C (1600-1450) 1585, 1481; C-H (3100-3010) 3045.31

4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl 2-(2,3-dimethylphenylamino)benzoate, CPD₄

Color: Dark Yellow; Yield: 69%; Melting point: 216-218°C; ^{1}H-NMR (400 MHz, DMSO): 7.7-6.3 (CH benzene), 4.01 (NH), 2.39 (CH_3); Mass: *m/z* = 593 [M + 1]; FTIR (cm^{-1}): C=O (1680-1630) 1687.76; N-H (3500-3100) 3099.37; C=C (1600-1450) 1613.90; C-H (3000-2850) 2992.19; C-N (1360-1180) 1339.01, 1299.77, 1233.37, 1188.71; C-N (1360-1180).

4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dienyl)-2-methoxyphenyl 2-acetoxybenzoate, CPD₅

Color: Pale Yellow; Yield: 67%; Melting point: 228-230°C; ^{1}H-NMR (400 MHz, DMSO): 7.2-6.67 (CH benzene), 4.71 (CH_2), 4.01 (NH); Mass: *m/z* = 531 [M^+]; FTIR (cm^{-1}): C=O (1680-1630) 1653.56; C-H (3150-3020) 3107.54; C=C (1675-1600) 1614.34; N-H (3500-3100) 3554.99; C-H (2960-2850) 2933.06; C=O (1725-1700) 1718.69

Anti-inflammatory action (albumin denaturation assay)

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis. Tissue injury during life might be due to denaturation of the protein constituents of cells or intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein

signifies the obvious potential for anti-inflammatory activity. The result of the anti-inflammatory action of the NSAID and the corresponding prodrugs are presented in table 2.

Table No. 2: Percent albumin denaturation by NSAIDs and curcumin-NSAID conjugates

Treatment	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
Aceclofenac	11.5±3.291	20.1±2.657	32.6±2.194	51.5±3.167	58.9±2.869
Diclofenac	14.36±2.165	25.35±2.243	38.64±3.128	57.83±3.692	70.36±3.899
Ibuprofen	9.29±3.163	14.24±2.196	22.06±2.695	29.37±3.068	37.46±2.162
Mefenamic acid	13.01±2.596	22.18±2.967	34.86±3.089	45.29±3.216	54.93±3.209
Acetylsalicylic acid	12.39±2.022	18.37±3.899	27.63±3.128	43.17±2.165	52.36±3.163
CPD_1	14.37±2.657	25.97±3.167	35.99±2.869	56.05±2.165	61.64±2.194
CPD_2	17.18±2.243	27.51±3.692	44.82±3.899	60.18±2.165	74.66±3.128
CPD_3	11.5±2.196	19.43±3.068	24.11±2.162	34.93±3.163	41.5±2.695
CPD_4	15.36±2.967	29.85±3.216	36.26±3.209	49.11±2.596	57.25±3.089
CPD_5	15.69±3.899	24.77±2.165	29.02±3.163	46.08±2.022	51.37±3.128

Results are expressed as mean ± SEM, n=6

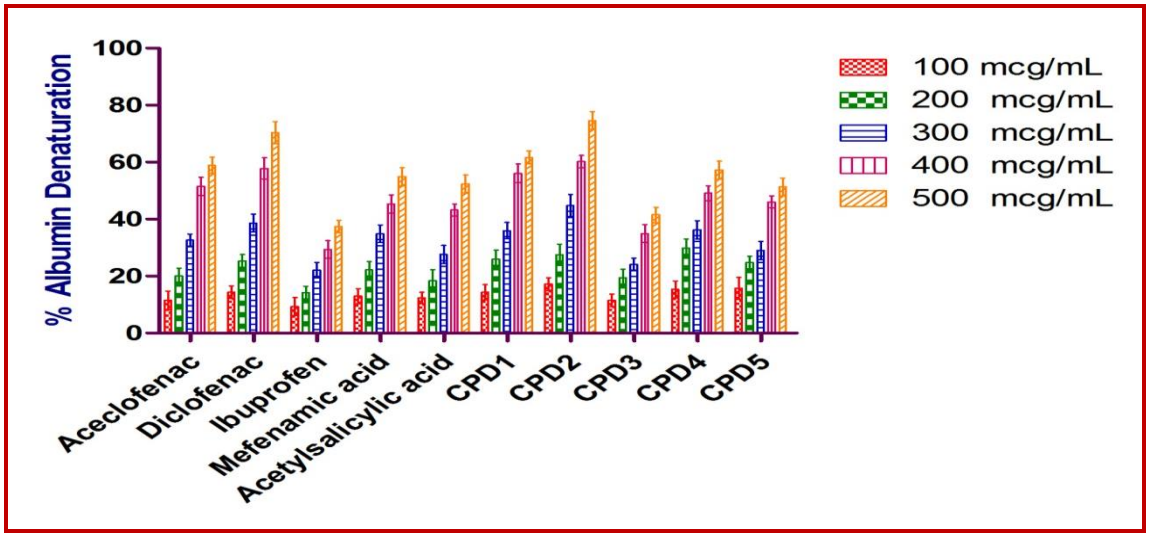


Figure No. 1: Percent albumin denaturation by NSAIDs and curcumin-NSAID conjugates

As indicated from the results, the albumin denaturation by the prodrugs of all the NSAIDs was higher than the corresponding NSAIDs confirming the hypothesis that the inclusion of curcumin in the prodrugs would be able to enhance the anti-inflammatory action.

CONCLUSION

The objective of the present investigation was to synthesize mutual prodrugs of some NSAIDs by conjugating them with curcumin to improve the anti-inflammatory action and possibly reduce the gastric irritation that is caused due to the free carboxyl group in NSAID. The results indicate that the anti-inflammatory action improved significantly and further studies need to be carried out for ascertaining the decreased gastric irritation and hydrolysis.

ACKNOWLEDGMENTS

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