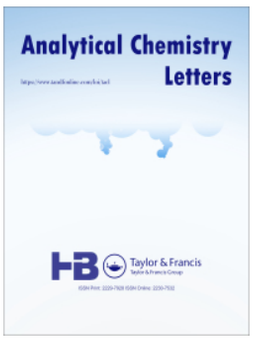

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Article

Investigation of the Anti-inflammatory potential of Monocarbonyl Analogues of Curcumin†

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†- Dedicated to Professor R.A. Mane on his 70th birthday

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Abstract: In the present investigation, we report the synthesis, anti-inflammatory activity and molecular docking of monocarbonyl analogues of curcumin. The anti-inflammatory activity of the synthesized compounds was gauzed using the protein denaturation assay using Diclofenac sodium as reference standard. Among the tested compounds, **3d**, **3e**, **3f**, **3j**, **3k**, **3l** and **3m** displayed excellent anti-inflammatory activity by exhibiting good range of percentage inhibition as compared to the standard DFS. *In silico* binding affinity study against Cyclooxygenase (COX-2) enzyme could provide valuable insight into their plausible mechanism of action. Also, *in silico* ADME prediction of synthesized monocarbonyl curcumin analogues showed excellent pharmacokinetic parameters by not violating Lipinski's rule of five.

Keywords: Monocarbonyl Curcumin analogues, Anti-inflammatory evaluation, Cyclooxygenase, Molecular docking.

Introduction

Inflammation is a complex biochemical process triggered by chemical or biological irritants, such as toxins, phenol, capsaicin, burns and bacteria [1]. Infection or inflammation may cause the development of prostaglandin E2 (PGE2). Excessive production of PGE2 is linked to a variety of diseases, including cancer, rheumatoid arthritis, atherosclerosis, and pain. A chronic or acute inflammatory state has been related to a number of diseases, including acute lung damage, sepsis, arthritis, diabetic nephropathy, cerebrovascular, Alzheimer's disease, atherosclerosis, and even cancer, according to numerous recent studies [2]. (Figure 1).

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Several non-steroidal anti-inflammatory drugs (NSAIDs) approved by FDA are currently marketed as anti-pyretic, anti-inflammatory, and analgesic agents [3]. These are used to treat muscle pain, dysmenorrhea, arthritic diseases, pyrexia, gout, and migraines, as well as opioid-sparing agents in some acute trauma situations [4]. Prostaglandins, which are inflammatory mediators, cause inflammation, pain, and pyrexia in the body. NSAIDs will effectively inhibit the synthesis of these prostaglandins. Anti-inflammatory drugs can be broadly classified as selective and non-selective COX-2 inhibitors (Figure 2). However, clinical applications of the existing NSAIDs has been limited in conventional treatment methods due to drug resistance and harmful side effects like gastrointestinal problems, headaches, allergic reactions, dizziness, kidney damage, stroke, liver damage etc [5]. Hence, there is urgent need of developing some efficient alternatives to NSAIDs with minimal side effects.

Turmeric has been used as a food spice and pigment for decades [6]. Turmeric, in addition to its distinct flavour and colour, has been traditionally used in Chinese and Indian medicine, especially as an anti-inflammatory agent. Cur-

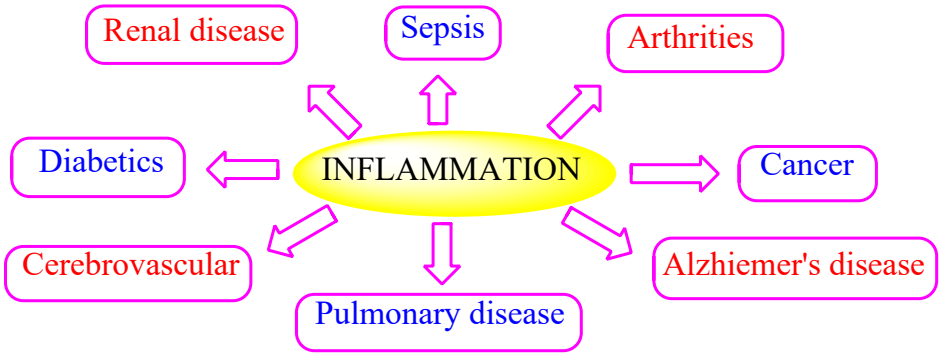
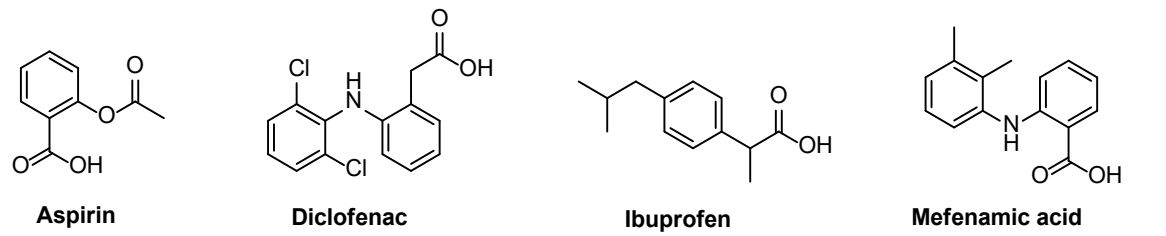
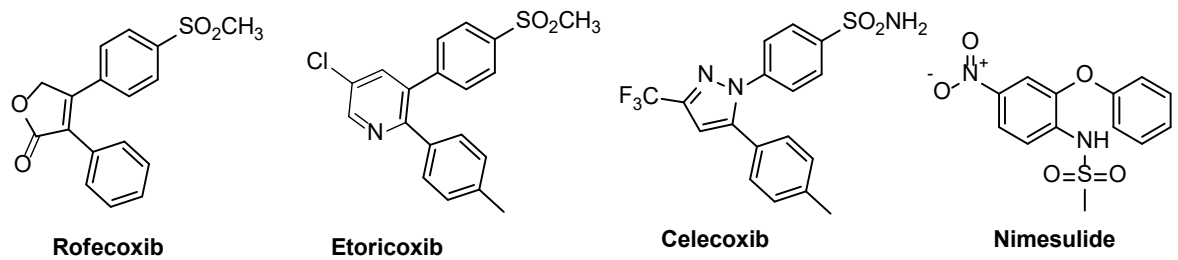


Figure 1. Various diseases associated with chronic inflammation



Chemical structures of some non-selective COX-2 inhibitors



Chemical structures of selective COX-2 inhibitors

Figure 2. Structures of some marketed selective & non selective COX-2 inhibitors

cumin, the active constituent of turmeric, exhibits a broad range of pharmacological activities including anti-carcinogen, immunomodulation, anti-oxidant, anti-angiogenesis, and chemoprevention [7,8]. Curcumin's anti-inflammatory properties have been thoroughly researched. However, due to its fragile nature, its possible beneficial effects on disease prevention and treatment are minimal [9]. Curcumin is quickly metabolised in liver by aldo-keto reductase enzyme due to the central-diketone moiety [10]. To address the shortcomings of curcumin, researchers used synthetic manipulation to produce more biologically active monocarbonyl analogues of the curcumin [11]. Monocarbonyl analogues of curcumin (MACs) are simply synthesized by Claisen-Schmidt type condensation of various substituted aromatic aldehydes and monoketone linker molecules under base/acid catalyst. Literature revealed that, MACs have not only better pharmacokinetics and pharmacodynamics but also superior broad spectrum of biological activities as compared to curcumin itself. MACs exhibited various biological activities like anticancer [12]. anti-oxidant [13], antitubercular [14]. anti-inflammatory [15], antimicrobial [16], antidiabetic [17] and anti-Alzheimer [18] etc. The anti-inflammatory potential of monocarbonyl analogues of curcumin is extensively studied and reported in the recent literature. Recently, Chainoglou and co-workers reviewed [19] the anti-inflammatory potential of curcumin analogues along with their mode of action. Curcumin and its analogues have been found to display potential anti-inflammatory activity by preventing the release of cytokines viz. TNF-α and IL-6 [20]. Curcumin analogues have been shown to inhibit the activation of free radical-activated transcription factors viz. nuclear factor-kappa B and AP-1, which inhibit the expression of many cytokines including IL-1, IL-6, IL-12 and TNF-α,[21].

Recent study showed that there are many monocarbonyl curcumin analogues in the literature exhibiting potential anti-inflammatory activity [22,23]. Hence, monocarbonyl curcumin analogues can act as a potential lead molecules with anti-inflammatory properties. Therefore, in search of development of new active anti-inflammatory agents and in continuation of our earlier work [24-27]. we report herein, synthesis of monocarbonyl curcumin analogues from commercially available starting materials and evaluation of their *in vitro* anti-inflammatory activity with molecular docking and ADME properties evaluation.

Materials and methods

General

All chemicals and reagents used were of analytical grade. The progress of the reactions was monitored by thin-layer chromatography (TLC) on aluminium plates coated with silica gel 60 F254, thickness: 0.25 mm (Merck). The detection of the components was made by exposure to iodine vapours or UV light. Open capillary method was used to determine the melting points and are uncorrected. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-400 MHz spectrometer. ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-100 MHz instrument.

General method for the preparation of the monocarbonyl analogues of curcumin

A mixture of substituted benzaldehyde/furfural1a-1d (2 mmol) and various monoketone linker's viz. acetone, cyclohexanone, cyclopentanone, substituted piperidin-4-ones (1 mmol) in ethanolic NaOH was taken in a 50 mL round bottom flask equipped with a mechanical stirrer. The reaction mixture was stirred at room temperature for 4-5 h. The progress of the reaction was monitored by TLC. The mobile phase of ethyl acetate: hexane was used for TLC. After the completion of reaction, the reaction mixture was poured in to 50 mL ice-water and the obtained solid was filtered, dried and recrystallized from ethyl acetate to obtain a yellow solid products.

Spectral data

1,5-Diphenylpenta-1,4-dien-3-one (3a):

Yield 87 %; mp 111-113 °C; [Lit- 113-115 °C] [28].

2,6-Dibenzylidenecyclohexanone (3b):

Yield 85 %; mp 107-109°C; [Lit- 106°C][29]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.81 (s, Ar-CH = C-, 2H), 7.47 (d, J = 8.0 Hz, Ar-H, 4H),

7.43-7.39 (m, Ar-H, 4H), 7.36-7.32 (m, Ar-H, 2H), 2.95 (t, *J* = 12 Hz, -C$\underline{H}_2$-CH_2-C$\underline{H}_2$, 4H), 1.83-1.76 (m, -CH_2-C$\underline{H}_2$-CH_2, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 190.6 (-$\underline{C}$=O), 137.1, 136.3, 136.1, 130.5, 130.4, 128.7, 128.5, 128.1, 28.6 (-$\underline{C}H_2$-CH_2-$\underline{C}H_2$), 23.2 (-CH_2-$\underline{C}H_2$-CH_2).

2,5-Dibenzylidenecyclopentanone (3c):
Yield 87 %; mp 189-191°C; [Lit- 189-193°C] [28]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, J = 8.0 Hz, Ar-CH = C- & Ar-H, 6H), 7.46-7.36 (m, Ar-H, 6H), 3.11 (s, -C$\underline{H}_2$-C$\underline{H}_2$-, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.2 (-$\underline{C}$=O), 137.1, 135.6, 133.6, 130.5, 129.2, 128.6, and 26.3 (-$\underline{C}H_2$-$\underline{C}H_2$-).

3,5-Dibenzylidenepiperidin-4-one (3d):
Yield 86 %; mp 178-180°C;[Lit- 178-179°C] [30]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.79 (d, *J* = 8.0 Hz, Ar-C$\underline{H}$ = C- & Ar-H, 6H), 7.65-7.55 (m, Ar-H, 6H), 3.30 (s,-C$\underline{H}_2$-N-C$\underline{H}_2$-, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 187.7 (-$\underline{C}$=O), 135.4, 135.0, 134.1, 132.0, 123.7, and 48.2 (-$\underline{C}H_2$-N-$\underline{C}H_2$-).

1-Benzyl-3,5-dibenzylidenepiperidin-4-one (3e):
Yield 85 %; mp 154-156°C; [Lit- 154-156°C] [31]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.82 (s, Ar-C$\underline{H}$ = C-, 2H), 7.40-7.32 (m, Ar-H, 12H), 7.23 (t, *J* = 4.0 Hz, Ar-H, 3H), 3.87 (s, -C$\underline{H}_2$-N-C$\underline{H}_2$-,4H), 3.71 (s, -C$\underline{H}_2$-Ph, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 187.9 (-$\underline{C}$=O), 137.4, 136.8, 135.3, 133.4, 130.5, 129.1, 128.7, 128.5, 127.5, 61.5 (-$\underline{C}H_2$-N-$\underline{C}H_2$-), and 54.5 ($\underline{C}H_2$-Ph).

2,6-Bis(4-bromobenzylidene)cyclohexanone (3f):
Yield 84 %; mp 162-164°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.70 (s, 2H), 7.53 (d, *J* = 8.0 Hz, Ar-C$\underline{H}$ = C- & Ar-H, 4H), 7.32 (d, *J* = 8.0 Hz, Ar-H, 4H), 2.88 (t, *J* = 8.0 Hz, -C$\underline{H}_2$-CH_2-C$\underline{H}_2$, 4H), 1.82-1.76 (m, -CH_2-C$\underline{H}_2$-CH_2, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 190.0 (-$\underline{C}$=O), 136.6, 136.0, 134.8, 131.9, 131.8, 123.1, 28.5 ($\underline{C}H_2$-CH_2-$\underline{C}H_2$), and 22.9 (-CH_2-$\underline{C}H_2$-CH_2).

2,5-Bis(4-bromobenzylidene)cyclopentanone (3g):
Yield 83 %; mp 168-170°C;[Lit- 163-166°C] [32]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.49 (t, *J* = 8.0 Hz, Ar-C$\underline{H}$ = C- & Ar-H, 4H), 7.44-7.36 (m, Ar-H, 6H), 3.02 (s, -C$\underline{H}_2$-C$\underline{H}_2$-, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.0 (-$\underline{C}$=O), 137.7, 134.5, 132.7, 132.0, 123.8, and 26.4 (-$\underline{C}H_2$-$\underline{C}H_2$-).

3,5-Bis(4-bromobenzylidene)-1-methylpiperidin-4-one (3h):
Yield 89 %; mp 141-143°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.72 (s, Ar-C$\underline{H}$ = C-, 2H), 7.55 (d, *J* = 8.0 Hz, Ar-H, 4H), 7.24 (s, Ar-H, 4H), 3.71 (s,-CH_2-N-CH_2-, 4H), 2.46 (s,-N-C$\underline{H}_3$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 186.6 (-$\underline{C}$=O), 135.4, 134.1, 133.6, 132.0, 123.6, 57.1 (-$\underline{C}H_2$-N-$\underline{C}H_2$-), and 46.1 (-N-$\underline{C}H_3$).

3,5-Bis(4-chlorobenzylidene)-1-methylpiperidin-4-one (3i):
Yield 87 %; mp 179-182°C; [Lit- 183-184°C][30]; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.75 (s, Ar-CH = C-, 2H), 7.40 (d, *J* = 8.0 Hz, Ar-H, 4H), 7.32 (d, *J* = 8.0 Hz, Ar-H, 4H), 3.72 (s, -C$\underline{H}_2$-N-C$\underline{H}_2$-, 4H), 2.47 (s, -N-C$\underline{H}_3$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 186.5 (-$\underline{C}$=O) , 135.2, 135.1, 133.6, 133.4, 131.6, 128.9, 57.0 (-$\underline{C}H_2$-N-$\underline{C}H_2$-), and 45.9 (-N-$\underline{C}H_3$).

3,5-Dibenzylidene-1-methylpiperidin-4-one (3j):
Yield 86 %; mp 116-117°C;[Lit- 115-117°C] [30]; ^{1}H NMR ($CDCl^3$, 400 MHz, δ ppm): 7.98 (s, Ar-C$\underline{H}$ = C-, 2H), 7.63 (d, *J* = 8.0 Hz, Ar-H, 4H), 7.57-7.49 (m, Ar-H, 6H), 3.96 (s, -C$\underline{H}_2$-N-C$\underline{H}_2$-, 4H), 2.70 (s, -N-C$\underline{H}_3$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 187.2 (-$\underline{C}$=O), 135.9, 135.8, 134.3, 134.1, 132.3, 129.6, 57.6 (-$\underline{C}H_2$-N-$\underline{C}H_2$-), and 46.6 (-N-$\underline{C}H_3$); Mass calculated $[M + H]^+$ for $C_{20}H_{20}NO$: 290.37, found:290.44

2,6-Bis(furan-2-ylmethylene)cyclohexanone (3k):
Yield 87 %; mp 140-143°C; [Lit- 142-144°C] [33]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.55 (s, Ar-C$\underline{H}$ = C- & Ar-H, 4H), 6.66 (s, Ar-H, 2H), 6.51

(t, *J* = 8.0 Hz, Ar-H, 2H), 3.01 (t, *J* = 8.0 Hz, -C$\underline{H}_2$-CH_2-C$\underline{H}_2$, 4H), 1.91-1.85 (m, -CH_2-C$\underline{H}_2$-CH_2, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 189.2 (-$\underline{C}$=O), 152.9, 144.6, 133.1, 123.5, 116.2, 112.4, 28.1 (-$\underline{C}H_2$-CH_2-$\underline{C}H_2$), and 21.8 (-CH_2-$\underline{C}H_2$-CH_2).

2,5-Bis(furan-2-ylmethylene)cyclopentanone (3l):

Yield 86 %; mp 160-163°C; [Lit- 166-168°C] [34]; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.59 (s, Ar-H, 2H), 7.35 (s, Ar-C$\underline{H}$ = C-, 2H), 6.69 (d, *J* = 4.0 Hz, Ar-H, 2H), 6.54 (dd, *J* = 4.0, 2.0 Hz, Ar-H, 2H), 3.08 (s, -C$\underline{H}_2$-C$\underline{H}_2$-, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 195.6 (-$\underline{C}$=O), 152.9, 145.2, 136.0, 120.0, 116.1, 112.8, and 25.9 (-$\underline{C}H_2$-$\underline{C}H_2$-).

1-Benzyl-3,5-Bis(furan-2-ylmethylene)piperidin-4-one (3m):

Yield 88 %; mp 138-141°C; ^{1}H NMR (400 MHz, CDCl3, δ ppm): 7.51 (s, Ar-CH = C- & Ar-H, 4H), 7.31 (ddd, J = 15.7, 12.0, 5.3 Hz, Ar-H, 5H), 6.60 (d, J = 3.3 Hz, Ar-H, 2H), 6.49 (s, Ar-H, 2H), 3.98 (s, ,-C$\underline{H}_2$-N-C$\underline{H}_2$-, 4H), 3.80 (s, -N-C$\underline{H}_2$-Ph, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ δ ppm): 186.9 (-$\underline{C}$=O), 152.2, 145.2, 137.7, 130.2, 129.3, 128.5, 127.4, 122.3, 117.1, 112.5, 61.6 (-$\underline{C}H_2$-N-$\underline{C}H_2$-), and 54.1 (-N-$\underline{C}H_2$-Ph).

Protocol for anti-inflammatory activity

Protein denaturation assay method was used to carry out the anti-inflammtory screening using Diclofenac sodium as reference standard. The Bovine serum albumin was used for denaturation control, which was studied for prevention of denaturation measured as turbidity. The turbidity was measured using 96-Well plate reader at 450 nm, 620 nm (data not shown) and with UV visible spectrophotometer at 660 nm. 1 mL of 1% Bovine albumin and 1 mL of samples and standards were taken in the test tubes diluted to 5 mL of D/W. The solutions were heated on water bath at 70^{o}C for 10 min. The turbidity of all solutions was measured at 660 nm using a UV/VIS spectrometer (Schimadzu 1800). Deionized water was taken as the control. The protein denaturation was calculated as percentage inhibition.

% inhibition = 100 × (absorption of the control - absorption of the test)/ absorption of the control.

The results of absorbance with UV visible spectrophotometer are mentioned but showed significant resemblance with absorbance taken on Plate reader 620 nm.

Molecular docking

The 3D x-ray crystal structure of cyclooxygenase-2 (COX-2) complexed with Diclofenac was downloaded from the Protein Data Bank (www.rcsb.org/1PXX) and optimized using the protein preparation wizard while 3D structures of curcumin analogues (3a-3m) were drawn in the builder panel and refined through the ligand preparation tool of Maestro. The energy minimized structures of the curcumin analogues (3a-3m) and the refined COX-2 enzyme were subjected to molecular docking using the standard protocol implemented in the GLIDE (Grid-Based Ligand Docking with Energetics) module of the Small Drug Discovery Suite [35].

ADME prediction

All the synthesized MACs were tested for drug likeliness analysis based on Lipinski's rule of five. According to this rule, an orally active drug should not violate more than one of following four criteria: molecular weight ≤ 500, number of hydrogen bond donors ≤ 5, miLogP (octanol-water partition coefficient) ≤ 5, number of hydrogen bond acceptors ≤ 10. ADME properties were predicted by using molinspiration online property calculation toolkit. A collective property of physicochemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value known as the drug-likeness model score was computed by using MolSoft software.

Results and discussion

Chemistry

Synthesis of monocarbonyl curcuminoids was carried out using the Claisen-Schmidt type condensation of substituted benzaldehyde/furfural with different monocarbonyl linkers.A mixture of substituted benzaldehyde/furfural 1a-1d and various monoketone linkers 2, like acetone, cyclohexanone, cyclopentanone, piperidin-4-one, 1-methylpiperidin-4-one and 1-benzylpiperi-

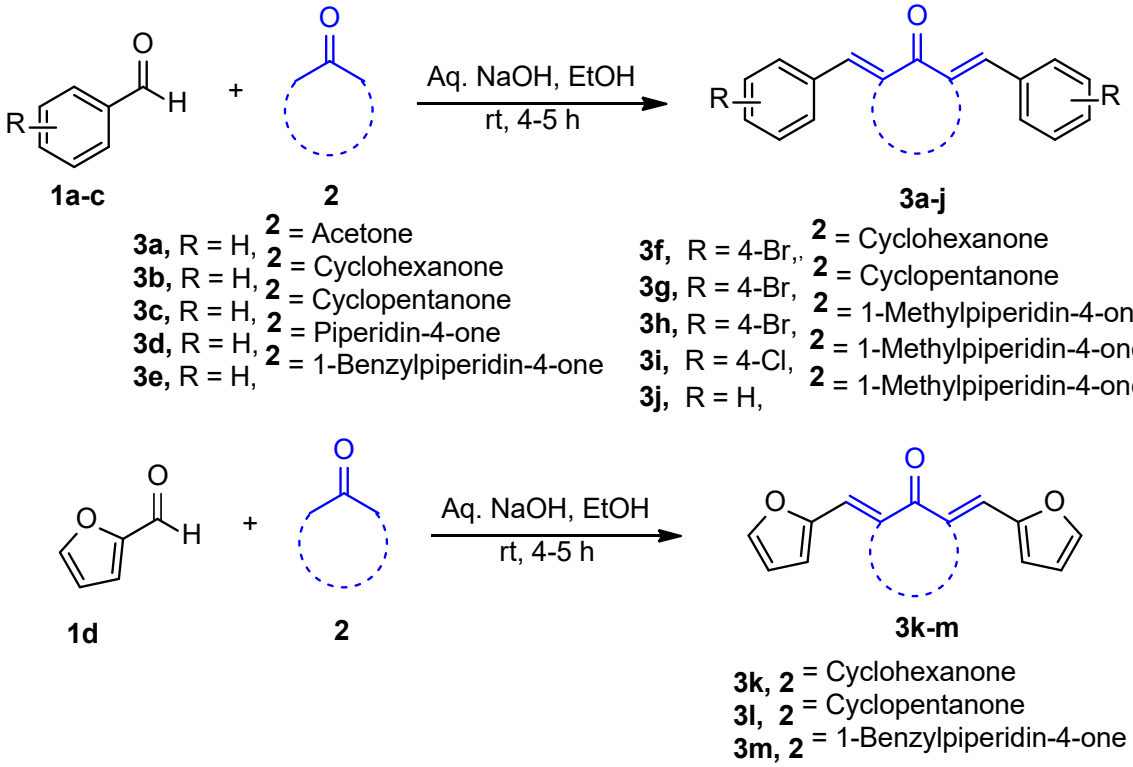
din-4-ones were stirred in presence of ethanolic NaOH as base at room temperature for 4-5h to give the corresponding monocarbonyl curcumin analogues 3a-3m (Scheme 1). The reaction was monitored by TLC. Formation of monocarbonyl curcumin analogues 3a-3m was confirmed by physical data and spectral analysis techniques such as ^{13}C NMR and ^{1}H NMR.

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity of the synthesized MACs was carried out using DFS (Diclofenac sodium) as standard. The percentage inhibition of the synthesized MACs were determined using protein denaturation assay and compared against standard DFS (Table 1). The absorbance of the standard drug DFS for anti-inflammatory activity were calculated at different concentrations and calibrated. Finally for the comparison with tested compounds, the percentage inhibition of the standard DFS were determined using 100 µg/mL solution and found to be 73.68. Among the tested compounds most of the compound displayed good % inhibition as compared to standard DFS. Particularly, MACs 3d, 3e, 3f, 3j, 3k, 3l and 3m were the most active curcumin analogues. Among the series, compound 3i displayed lowest % inhibition (09.09%) indicating least anti-inflammatory potential. The analogue 3j was found to exhibit the most active anti-inflammatory activity with % inhibition of 81.13 which was superior to the standard DFS. Also the analogue 3mwith % inhibition 76.19 was most active to standard DFS with % inhibition of 73.68.The analogue 3k displayed comparable % inhibition to that of DFS. Analogues 3d, 3e, 3f and 3l displayed significant % inhibition as compared to standard DFS.

Structure Activity Relationship (SAR)

The SAR based on the anti-inflammatory activity revealed that, furfur aldehyde based monocarbonyl curcumin analogues are more potent than analogues with substituted benzaldehyde as a side chain. Also, it was found that monoketone linker played crucial role in imparting anti-inflammatory potential. The analogue 3a with acetone as a linker showed percentage inhibition 33.33 while, compound 3d (% inhibition 66.67) with piperidone as a linker enhanced percentage



Scheme 1. Synthesis of monocarbonyl curcumin analogues

Table 1. *In vitro* Anti-inflammatory activity of the MACs (100 µg concentration)

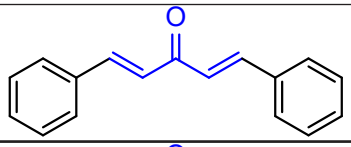
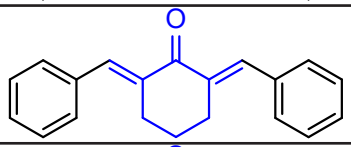
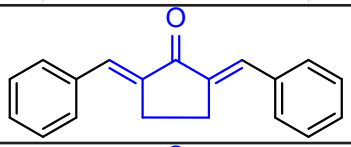
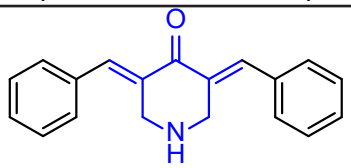
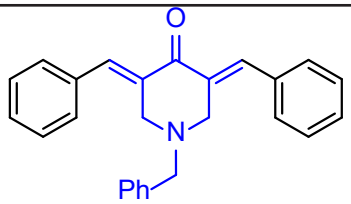
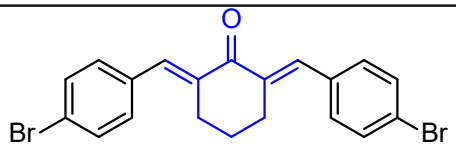
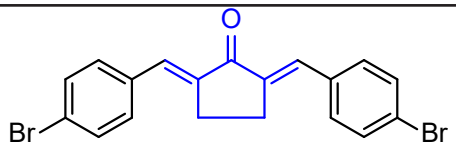
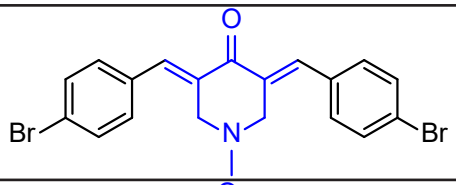
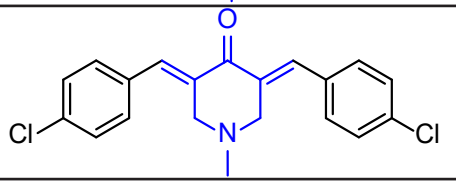
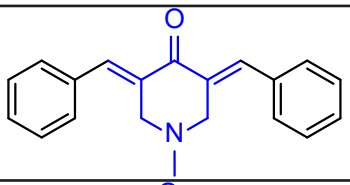
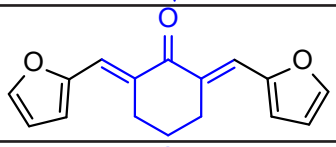
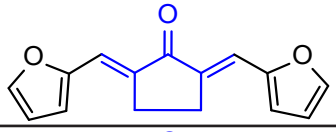
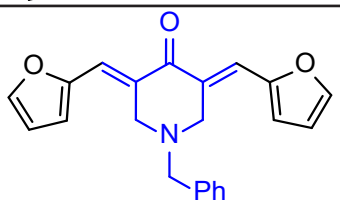
Entry	Structure of compound	Absorbance	Percent inhibition (%)
3a		0.15	33.33
3b		0.17	41.18
3c		0.18	44.44
3d		0.3	**66.67**
3e		0.27	**62.96**
3f		0.29	**65.52**
3g		0.25	60.00
3h		0.17	41.18
3i		0.11	09.09

table 1. (continued).

Entry	Structure of compound	Absorbance	Percent inhibition (%)
3j		0.53	**81.13**
3k		0.34	**70.59**
3l		0.29	**65.52**
3m		0.42	**76.19**
DFS		0.38	73.68

inhibition by two-fold. Furthermore, percentage inhibition again increases when N-methyl piperidone was used as monoketone linker (compound 3j, % inhibition 81.13). Also it was observed that substitution of halogen (-Br) on benzene enhances the % inhibition in compound 3f (% inhibition 65.52) and 3g (% inhibition 60.00) as compared to compound 3b (% inhibition 41.18) and 3c (% inhibition 44.44), this shows that halogen (-Br) substituent on benzene ring could play significant role in the binding of active sites of enzyme Cyclooxygenase 2. Surprisingly, chloro (-Cl) substitution on aromatic ring decreases the % inhibition in spite of having piperidone as a linker (compound 3i, % inhibition 09.09). Among the furfural based MACs, compound with N-benzyl piperidone as a monoketone linker, showed highest percentage inhibition and more than standard drug DFS (compound 3m, % inhibition 76.19). The graphical representation of *in vitro* anti-inflammatory activity of the synthesized curcumin analogues is shown in the following figure 3.

Molecular docking

To gauze the plausible mechanism involved in the anti-inflammatory activity demonstrated by the monocarbonyl analogues of curcumin (3a-3m), molecular docking study against Cyclooxygenase 2 (COX-2) was performed. COX-2 is involved in the bioconversion of arachidonic acid to inflammatory prostaglandins (PGs) which mediate the inflammation and pain. A perusal of docked complexes revealed very clear preference for the monocarbonyl curcumin analogues (3a-3m) wherein all the molecules could snugly fit into the active site engaging a series of bonded and non-bonded interactions. Their docking scores (Glide scores) correlated well with the anti-inflammatory activity, with active compounds exhibiting higher binding compared to compounds with moderate activity (Table 2). The glide docking score and glide binding energy for the co-crystallized ligand (Diclofenac) was found to be -9.980 and -52.762kcal/mol respectively. A detailed analysis of per-residue interactions is elaborated for one of the most active

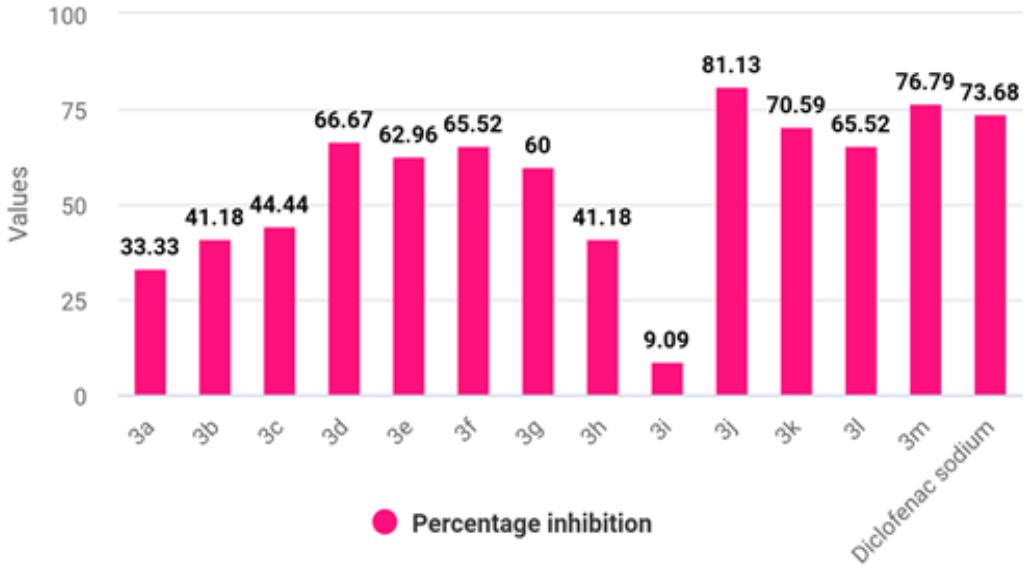


Figure 3. Percentage inhibition of synthesized MACs when compared with standard DFS

Table 2. The per-residue interaction analysis based on docking study with Cyclo-oxygenase 2 (COX-2) for monocarbonyl curcumin analogues & standard DFS

Entry	Docking Score	Glide Interaction Energy (kcal/mole)	Per-Residues interactions π-π stacking (Å)	H-bond (Å)
3a	-7.207	-34.324	Tyr385(1.849), Tyr355 (1.933)	Ser530(1.953)
3b	-7.946	-36.114	Trp387(2.314), Tyr385(2.209)	Ser530(1.984)
3c	-8.181	-38.458	Trp387(2.364), Tyr385(2.012)	Ser530(2.108)
3d	-9.141	-41.445	Tyr385(1.942)	Ser530(2.272)
3e	-8.408	-39.998	Trp387(2.849), Tyr385(2.291)	-
3f	-8.821	-40.191	Tyr385(1.908)	-
3g	-8.312	-39.736	Tyr385(2.146), Tyr355 (2.479)	-
3h	-7.685	-36.258	Arg120(1.996)	-
3i	-7.066	-28.335	Arg120(1.98)	-
3j	-9.575	-47.731	Trp387(2.365), Tyr385(1.976)	Ser530(1.922)
3k	-9.218	-43.149	Tyr385(2.534), Tyr355 (1.894)	Ser530(2.751)
3l	-8.851	-40.441	Trp387(2.142), Tyr355 (2.664)	Ser530(2.532)
3m	-9.436	-46.845	Trp387(2.898), Tyr385(2.057)	-
DFS	-9.980	-52.762	-	-

analogue 3jto identify the most significantly interacting residues and the various thermodynamic interactions governing their affinity to COX-2.

The best docked conformation of 3j into COX-2 enzyme produced a Glide docking score of -9.575 and Glide binding energy of -47.731 kcal/mol (Figure 4). The compound could aptly fit into the active site of COX-2 at co-ordinates close to the co-crystallized ligand engaging in a series of non-bonded and bonded interactions. The enhanced binding affinity of 3j is attributed to significant Van der Waals interactions with Ser530 (-1.765 kcal/mol), Tyr355 (-1.256 kcal/mol), Ser353 (-1.554 kcal/mol), Leu352 (-1.709 kcal/mol) and Val116 (-1.664 kcal/mol) residues through the central 1-methylpiperidin-4-one ring

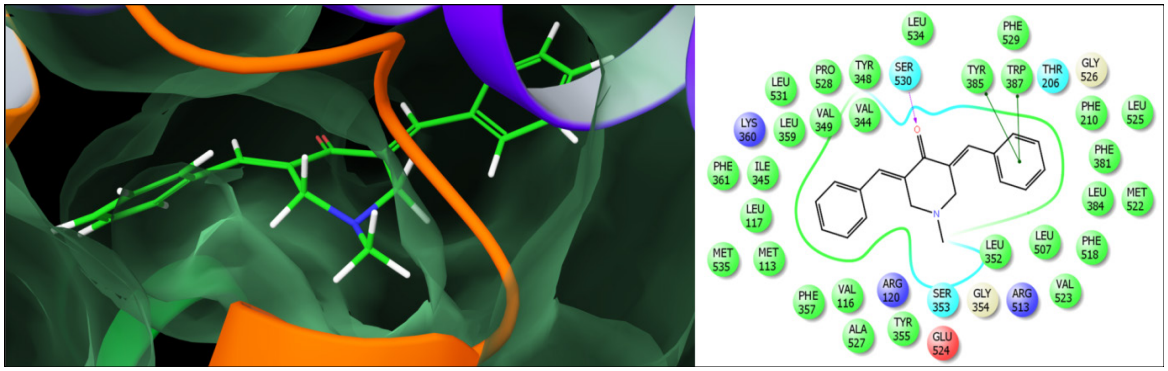


Figure 4. Mode of binding for **3j** into COX-2 (on right side: the pink lines signify the hydrogen bonding while green lines represent π-πstacking interactions)

while the 3,5-dibenzylidene side chains around it were also found to be involved in similar interactions with Leu531 (-1.393 kcal/mol), Ala527 (-3.866 kcal/mol), Gly526 (-2.255 kcal/mol), Val523 (-2.341 kcal/mol), Met522 (-1.416 kcal/mol), Phe518 (-1.065 kcal/mol), Trp387 (-1.803 kcal/mol), Tyr385 (-1.821 kcal/mol), Phe381 (-1.069 kcal/mol), Leu359 (-1.485 kcal/mol), Val349 (-2.532 kcal/mol), Ile345 (-1.726 kcal/mol) and Met113 (-1.039 kcal/mol) residues.

Furthermore another driving force for mechanical interlocking of 3j was found to be favourable electrostatic interactions with Ser530 (-1.994 kcal/mol), Glu524 (-1.437 kcal/mol) and Arg120 (-1.906 kcal/mol) residues lining the active site. The compound also engaged in a very close hydrogen bonding interaction with Ser530 (1.922Å) residue through the ketonic (=O) function on the central 1-methylpiperidin-4-one ring while one of the benzylidene side chain exhibited a prominent π-π stacking interactions with Trp387 (2.365Å) and Tyr385 (1.976Å) residues. These interactions serve as ″anchor″ to guide the orientation of ligand into the 3D space of active site and facilitate the non-bonded (Van der Waals and electrostatic) interactions. A similar network of bonded and non-bonded interactions was observed for other monocarbonyl curcumin analogues (Figure S1-S12, ESI) suggesting that these monocarbonyl analogues of curcumin could serve as a pertinent starting point for further structure-based lead optimization against COX-2.

ADME properties

Pharmacokinetic parameters of monocarbonyl curcumin analogues are summarised in table 3. It was observed that 3e, 3f, 3g, 3h and 3idid not show more than one violation of Lipinski's rule of five. All other analogues does not show any violation. Hence, all the tested compounds followed the criteria for orally active drug and therefore, these newly synthesized monocarbonyl curcuminoids may have a good potential to develop as good oral active drug candidates.

Conclusions

A small library of monocarbonyl curcumin analogues was synthesized and evaluated for in vitro anti-inflammatory activity. Compounds 3d, 3e, 3f, 3j, 3k, 3l and 3m displayed good percentage inhibition as compared to the standard DFS. Molecular docking study and ADME prediction of the synthesized MACs could provide insights into binding affinity, mode of interactions and drug likeliness.

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Table 3. Pharmacokinetic parameters of the synthesized analogues

Entry	n-atoms	TPSA (A^2)	n-ROTB	MV	MW	Mol LogP	n-ON	n-OHNH	Lipinski's violations	Drug-likeness model score
Rule	-	-	-	-	<500	≤5	<10	<5	≤1	-
3a	18	17.07	04	229.27	234.30	4.18	1	0	0	-1.30
3b	21	17.07	02	268.83	274.36	4.97	1	0	0	-0.94
3c	20	17.07	02	252.03	260.34	4.46	01	0	0	-0.88
3d	21	29.10	02	264.43	275.35	3.36	02	1	0	-1.28
3e	28	20.31	04	353.02	365.48	5.35	02	0	1	+0.62
3f	23	17.07	02	304.60	432.15	6.59	01	0	1	-1.08
3g	22	17.07	02	287.80	418.13	6.08	01	0	1	-1.03
3h	24	20.31	02	317.15	447.17	5.57	02	0	1	-0.59
3i	24	20.31	02	308.45	358.27	5.31	02	0	1	-0.23
3j	22	20.31	02	281.38	289.38	3.95	02	0	0	-0.28
3k	19	43.35	02	231.97	254.28	3.12	03	0	0	-0.53
3l	18	43.35	02	215.17	240.26	2.62	03	0	0	-0.85
3m	26	46.59	04	316.16	345.40	3.51	04	0	0	-0.38

ABS: Absorption; TPSA: Topological polar surface area; n-ROTB: Number of rotatable bonds; MV: Molecular volume; MW: Molecular weight; Mol LogP: Logarithm of partition coefficient; n-ON: Number of hydrogen bond acceptors; n-OHNH: Number of hydrogen bonds donors.

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Compliance with ethical standards

Author declares that there is no involvement of human participant and/or animals while conducting this work.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary data

Figure S1-S12, ESI are given as supplementary information.

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