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EXPLORING THE BIOLOGICAL FRONTIERS: SYNTHESIS AND EVALUATION OF CURCUMIN-AMINO ACID CONJUGATES

Lovy Sharma*[1] and Gaurav Chaudhary[2]

[1]Department of Pharmaceutical Chemistry, Parmarth College of Pharmacy, Nizampur, Hapur.
[2]Department of Pharmaceutical Chemistry, I.T.S College of Pharmacy, Muradnagar, Ghaziabad.

***Corresponding Author: Lovy Sharma**

Department of Pharmaceutical Chemistry, Parmarth College of Pharmacy, Nizampur, Hapur.

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ABSTRACT

Curcumin is a phytochemical derived from the rhizomes of curcuma longa, having a wide varieties of therapeutics effects such as anti-diabetics, anti-inflammatory, anti-oxidant, in asthma, anti-cancer, anti-viral, anti-amyloid, as neuroprotective, in cardiovascular diseases, in cardio toxicity, in arthritis, anti-septic, anti-bacterial, in peptic ulcer. Apart form these theraeutics effects there are some drawbacks associated with curcumin such as unstability, poor water solubility, less bioavailability, poor absorption, which restrict the therapeutic response of curcumin. In this work, we designed the curcumin-amino acids conjugates via modification on phenolic group of curcumin without disturbing the methoxy group, with the aim to achieve the goal of implementing certain desirable characteristics in curcumin, such as reduced side effects, increased bioavailability, and therapeutic potential in order to determine antibacterial and anti-inflammatory activity of synthesised conjugates. All the synthesized derivatives of curcumin (L1 to L6) exhibited moderate to significant biological activity in terms of anti-inflammatory and anti-bacterial activity. It may be concluded that principles of structural manipulation have been appropriately applied in the present research work. This structural manipulation may lead to the synthesis of amino acid derivates of curcumin may serve the purpose of obtaining desirable therapeutic activity, minimization of side effects and desirable changes in the properties like solubility, bioavailability and stability to the chemical structure.

KEYWORDS: curcumin, solubilities, curcumin-amino acid conjugates.

INTRODUCTION

Curcumin is a phytochemical derived from the rhizomes of curcuma longa, or turmeric (haldi), belonging to the family Zingiberaceae.[1], having a wide varites of therapeutics effects such as anti-diabetics, anti-inflammatory, anti-oxidant, in asthma, anti-cancer, anti-viral, anti-amyloid, as neuroprotective, in cardiovascular diseases, in cardio toxicity, in arthritis, anti-septic, anti-bacterial, in peptic ulcer.[2-8]

Curcumin, also known as diferuloylmethane with formula $C_{21}H_{20}O_6$ and a molecular weight, 368.38. It is a symmetric, diaryl heptanoid molecule containing 2 aromatic rings which contain o-methoxy phenolic group; these two rings are linked by seven carbon chain having α, β-unsaturated β-diketone compounds.[9]

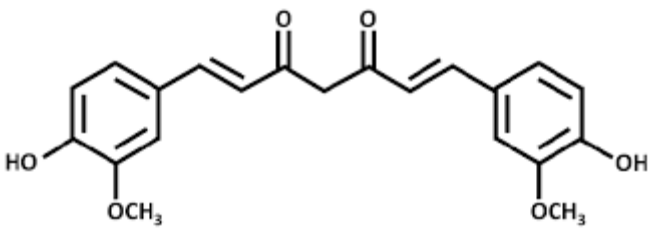


Fig: 1. Structure of Curcumin.

Curcumin is widely used in the inflammation as curcumin inhibits LOX, COX, and INOs which cause inflammation. Lipoxygenase (LOX), cyclo-oxygenease 1 and 2 (COX-1 and 2), inducible nitric oxide synthase

(INOs) are those enzymes which are responsible for inflammation.[10] Curcumin produced phototoxicity when exposed to blue light. Anti-bacterial and antibiofilm activity of curcumin is due to its auto-oxidation. When curcumin is combined with other anti-bacterial drug, it shows synergistic effects and increased the sensitivity towards gram-negative bacteria.[11]

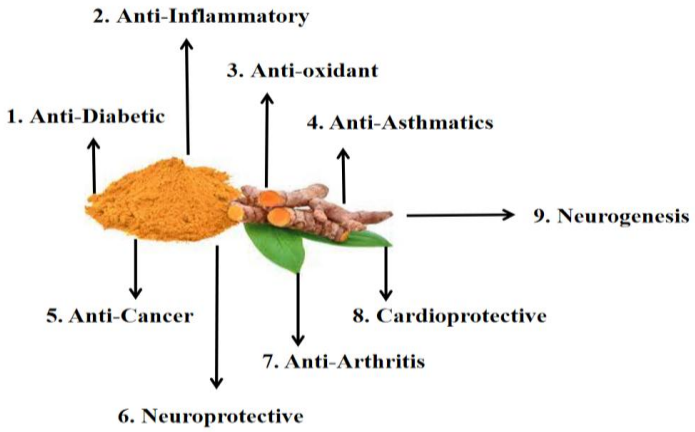


Fig: 2. Therapeutic Activities of Curcumin.

Apart form these theraeutics effects there are some drawbacks associated with curcumin such as unstability, poor water solubility, less bioavailability, poor absorption, which restrict the therapeutic response of curcumin. The main reason behind this is due to its poor absorption, quick systemic elimination, rapid metabolism and chemical instability.[12]

Several animal studies suggest that about 90% of oral curcumin is excreted in faeces.[13] Proton coupled oligopeptide transporter PepT1 is predominantly located in the proximal tubule of the kidney and the small intestine.[14] Its wide range of particular substrates includes the majority of di- and tripeptides, b-lactam antibiotics, and ACE inhibitors. Targeting PepT1 has been used in numerous instances to increase the oral bioavailability of pharmacologically active substances. Typically, this is done by altering the compounds such that they mimic the natural di- or tripeptide substrates.[15]

There are many studies which shows that the poor absorption and low bioavailabilty was improved through the peptide carrier approach by PepT1 transporter. When amino acids combined with curcumin it helps to overcome certain undesirable characteristics like poor water solubility, less bioavailability and minimization of side effects.[16]

Many studies shows that when hydrogen is substitute the methoxy group of curcumin, the resulted compounds shows less potent anti-inflammatory activity, which proof that methoxy group play a important role to reduced inflammation.[17]

In this work, we designed the curcumin-amino acids conjugates via modification on phenolic group of curcumin without disturbing the methoxy group, with the aim to achieve the goal of implementing certain desirable characteristics in curcumin, such as reduced side effects, increased bioavailability, and therapeutic potential in order to determine antibacterial and anti-inflammatory activity of synthesised conjugates. 6 novel curcumin-amino acids conjugates (LS1-6) were synthesied.

Synthetic scheme

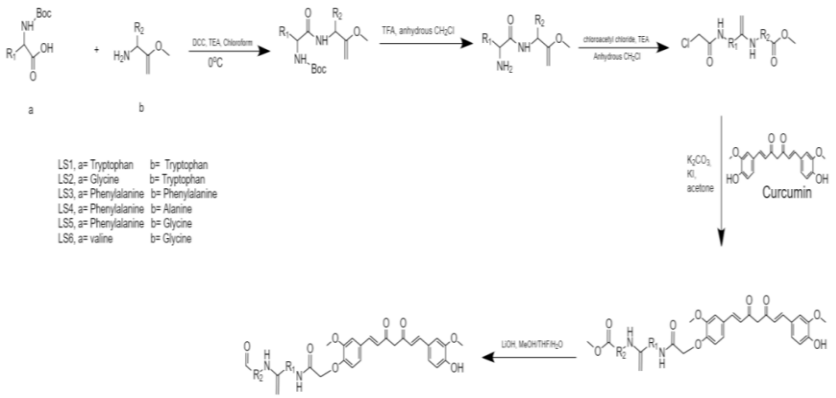


Fig: 3. Synthesis of Curcumin-Amino acid conjugates.

MATERIAL METHODS

Preparation of curcumin-amino acid conjugates.[17] (LS1-6)

1. Synthesis of dipeptides

Boc-amino acids and amino acid methyl esters were synthesized using a technique described in the literature.[18] Boc-amino acids (10mmol) were mixed with 20 mL of chloroform and chilled to zero. With stirring, N,N′-dicyclohexyl carbodiimide (DCC) (2.25 g) and 10mmol of amino acid methyl ester hydrochloride were added to the mixture. about 10mmol of Triethylamine was then added . 3 hours of stirring at this temperature were followed by 12 hours of stirring at room temperature. Precipitation of dicyclohexylurea (DCU) served as a measure of the reaction's completeness.

DCU was removed via filtering, and the filtrate was then treated with 1 M HCl, 0.5 M Na2CO3, and water. The organic layer was collected dried over anhydrous sodium sulphate and evaporated under vacuum. The residue was diluted in 20 mL of acetonitrile, and the solution's remaining dicyclohexylurea (DCU) was filtered. A solid mass was produced by vacuum-evaporating the filtrate.

2. Deprotection of dipeptides

At 0 degrees Celsius, 1 mmol of prepared dipeptide were dissolved in 4 mL of anhydrous dichlormethane with constant stirring. A dropwise addition of TFA about 1 mL was added in this solution. After this, the mixture was agitated for 3.5 hours with heating to room temperature. The solvent was separated under a reduced pressure; the crude product was collected for further work.

3. N-chloroacetyl-dipeptides

In 1mmol solution of dipeptide, triethylamine was added dropwise in anhydrous dichloromethane with constant stirring untill 7 pH at 0^{o}C, then, 1mmol of chloroacetyl chloride and 2ml of anhydrous dichloromethane is added, then agitated at room temperature for about 4 hr. This reaction mixture washed with water. Filtered and concentrated the organic layer after the drying over anhydrous sodium sulphate. The solid is extracted by the separation of solvent via reduced pressure.

4. Preparation of curcumin-amino acid conjugates

Add 1mmol of curcumin in 6ml of acetone, and mixed properly to get a clear solution. Then 1.2 mmol of potassium carbonate was mixed and agitate for about 15-20 mint. Then 12 ml of acetone was mixed in 1mmol of compound (prepared in 3^{rd} step) and stirred for about 10-11 hr. After that mixture was filtered. Residue was mixed with ethyl acetate and washed with water and brine. The organic layer was dried on anhydrous sodium sulphate and the crude product was obtained through dried under vaccum. Which was then purified by column chromatography using petroleum ether and ethyl acetate as mobile phase.

5. Deprotection of conjugates

1 mmol of curcumin amino acid conjugates were allowed to dissolved in 10ml mixture of water, tetrahydrofuran and methanol in the ratio of 1:3:1 and then lithum hydroxide was added and stirred at room temperature for about 3-4 hr. Tetrahydrofuran and methanol was removed my little evaporation. Then the resulted mass was acidify with 1M HCl solution until pH 3 was attained. Then ppt was collected and washed with water and dried to get final product i.e curcumin-amino acid conjugate

Characterization of conjugates

1. LS1 (*Tryp-Tryp)*

1 H NMR analysis

δ: 9.71 (s, 1H), 8.12 (m, 2H), 7.58 (d, J = 15.81 Hz, 2H), 7.39 (s, 1H), 7.33 (s, 1H), 7.24 (d, J = 8.19 Hz, 1H), 7.17 (d, J = 8.25 Hz, 1H), 6.99 (m, 1H), 6.85 (m, 2H), 3.94–3.89 (m, 2H), 3.86(s, 3H), 3.85 (s, 3H), 2.06 (m, 1H), 0.95–0.81 (m, 6H) δ 8.16 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 15.8 Hz, 2H), 7.40 (s, 1H), 7.33 (s, 1H), 6.86 (m, 1H), 6.77 (d, J = 15.6 Hz, 2H), 6.09 (s, 1H)

IR: vmax cm-1: 3200–3500 cm^{-1} (C-H), 1493 cm^{-1} (aromatic C=C), 1246 cm^{-1} (C-O), 21301(N-H)

2. LS2 (*Gly-Tryp)*

1 H NMR analysis

δ: 10.60 (s, 1H), 9.69 (s, 1H), 8.46 (d, J = 8.5 Hz,1H), 7.89 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 15.8 Hz, 2H), 7.39 (s, 1H), 7.25 (s, 1H), 7.17 (d, J = 8.2 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 6.92–6.82 (m, 2H), 6.77 (d, J = 15.8 Hz, 1H), 6.09 (s, 1H), 4.65 (s, 2H), 4.31 (m, 1H), 3.85(s, 3H), 3.86 (s, 3H), 3.80 (d, J = 5.8 Hz, 1H), 3.76 (d, J = 5.8 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 15.7 Hz, 2H), 7.40 (s, 1H), 7.33 (s, 1H)

IR: vmax cm-1: 3200–3500 cm-1, 1491 cm-1 (aromatic C=C), 1246 cm-1 (C-O), 2134 (N-H)

3. LS3 (*Phen-Phen)*

1 H NMR analysis

δ: 12.74 (s, 1H), 9.71 (s,1H), 8.29 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 15.7 Hz, 2H), 7.36 (d, J = 15.5 Hz, 2H), 7.23 (s, 5H), 7.16 (m, 2H), 6.86 (m, 2H), 6.76 (m, 2H), 6.11 (s, 1H), 4.79 (m, 1H), 4.52 (s, 2H), 4.20 (m, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.07 (m, 1H), 2.85 (m, 1H), 2.09 (m, 1H), 0.91 (m, 6H), 8.16 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 15.8 Hz, 2H), 7.40 (s, 1H), 7.33 (s, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H)

IR: vmax cm-1: 3200–3500 cm-1, 1490 cm-1 (aromatic C=C), 1246 cm-1 (C-O), 2130 (N-H)

4. LS4 (*Phen-Ala)*

1 H NMR analysis

δ: 8.53 (d, J = 7.3 Hz, 1H), 8.12(d, J = 7.2 Hz, 1H), 7.35 (d, J = 13.6 Hz, 2H), 7.31 (s, 1H), 7.28 (s, 1H), 7.21 (d, J

= 8.4 Hz, 1H), 7.06 (d, J = 7.29 Hz, 1H), 6.95 (m, 1H), 6.71 (d, J = 14.6 Hz,1H), 6.09 (s,1H), 4.60 (s, 2H), 4.41 (m, 1H), 4.21 (m, 1H), 3.86 (s, 3H), 7.53 (d, J = 14.6 Hz, 2H), 7.32 (s, 1H), 7.24 (s, 1H), 6.97 (m,1H), 6.79 (m, 2H), 6.74 (d, J = 14.6 Hz, 1H), 6.10 (s,1H), 4.63 (s, 2H), 4.42 (m, 1H), 4.27 (m, 1H), 3.79 (s, 3H)

IR: vmax cm-1: 3300–3500 cm-1, 1491 cm-1 (aromatic C=C), 1240 cm-1 (C-O), 2134(N-H)

5. LS5 ***(****Phen-Gly)*
1 H NMR analysis
d 1.69 (d, J = 6.9 Hz, 6H, H-17 & H-170), 2.56– 2.65 (m, 2H, H-6). 2.70–2.82 (m, 4H, H-1 & H-2), 2.86–2.88 (m, 2H, H-7), 3.75 (s, 6H, H-14 & H-140), 4.34 (q, J = 7.3 Hz, 2H, H-16 & H-160), 5.59 (s, 1H, H-4), 5.99–6.79 (m, 2H, H-13 & H-130), 6.89–7.01 (m, 2H, H-9 & H-90), 7.33 (s, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.86 (m, 1H), 6.77 (d, J = 15.8 Hz, 2H), 6.09 (s, 1H)

IR: vmax cm-1: 3200–3500 cm-1, 1495 cm-1 (aromatic C=C), 1246 cm-1 (C-O), 2133 (N-H)

6. LS6 *(Val-Tryp)*
1 H NMR analysis
δ: 8.10 (d, J = 7.2 Hz, 1H), 7.95 (d, J = 7.1 Hz, 1H), 7.45 (d, J = 14.8 Hz, 2H), 7.41 (s, 1H), 7.35 (s, 1H), 7.26 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 8.1 Hz, 1H), 7.01 (m, 1H), 6.89–6.84 7.80 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 14.8 Hz, 2H), 7.30 (s, 1H), 7.32 (s, 1H), 7.20–7.15 (m, 2H), 6.90 (m,1H), 6.85 (m, 2H), 6.78 (d, J = 14.8 Hz, 1H), 6.09 (s,1H), 4.63 (s, 2H), 4.41 (m, 1H), 4.32 (m, 1H), 3.82 (s, 3H)
IR: vmax cm-1: 3200–3500 cm-1, 1490 cm-1 (aromatic C=C), 1250 cm-1 (C-O), 2132 (NH)

RESULT AND DISCUSSION

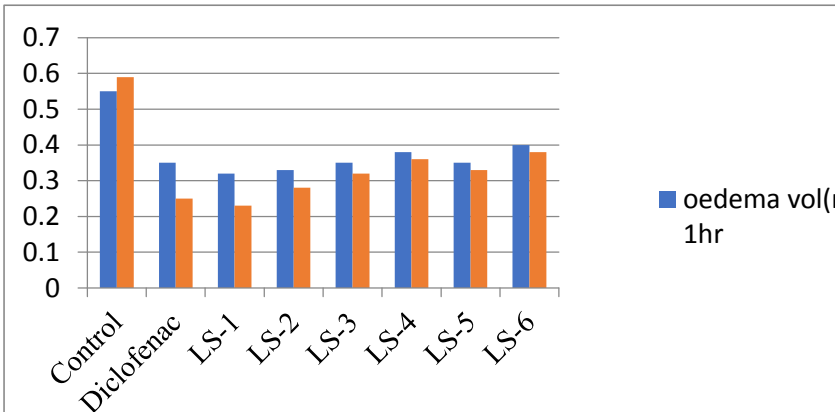
Anti-Inflammatory Activity

Carrageenan-induced edema

For the anti-inflammatory activity of synthesized compounds the rats were divided into 6 groups of 6 rats per groups. One group of animals received a placebo, while the other received the standard treatment (Diclofenac sodium).

The test substances were assigned to the remaining groups. 1% CMC suspension of standard compound and synthesised compounds were given orally to the groups (standard, control and test compound). After 30 minutes, the quantity of 0.1 mL newly generated carrageenan suspension in 0.9 percent NaCl solution injected subcutaneously in the paw was quantified. Foot volume was measured again at 2 and 4 hours, and the mean increase in paw volume in each group was determined.

The paw volume was measured by using a water plethysmometer. The quantity of oedema that had been created was calculated using the volume difference. The following calculation is used to calculate the percentage of inhibition value:
[1- Dt/Dc] *100
Dt =Paw volumes of oedema in test
Dc==Paw oedema volumes in control.



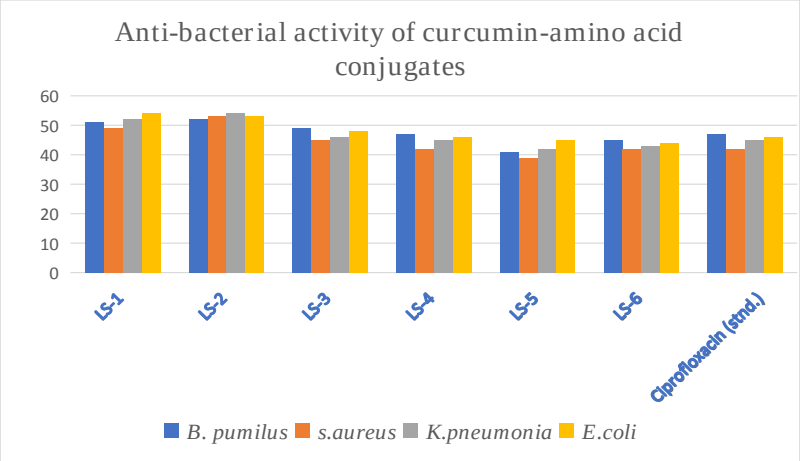
Graph 1: Anti-inflammatory screening (% inhibition) of synthesized compounds.

Anti-Bacterial Activity

The preliminary antibacterial activity was determined using the agar-diffusion technique. The medium was autoclaved for about 15 min at 15 lbs pressure at 121°C and then cooled in a water bath to 50-55°C after being removed from the autoclave**.** The cooled medium was poured in to sterile petriplates and stored for solidification. The culture was injected on the medium in a laminar air flow after the medium had solidified. A sterilised cotton swab was dipped into standardised bacterial suspension or injected with 1mL of the organism suspension within 15 minutes of altering the density of the inoculums. To ensure a uniform dispersion of the inoculums, a sterile swab was employed on the top of the nutrient agar medium. To ensure that extra moisture was absorbed, the plates were left undisturbed for 3 to 5 minutes. To produce agar wells, a sterile 7mm cork borer was used, and concentrations of 25, 50, 75, 100, and 200 g/ml of diluted test compound stock solutions, as well as 100 percent DMSO, were poured into each well. Plates were incubated at 37°C in the incubator for bacterial growth and at 20-25°C in the BOD incubator. The presence of zones of inhibition

surrounding the agar wells was noticed. Zone diameters were measured in millimetres.

The following formula can be used to measure the percentage of inhibition:
% inhibition = **I** (Diameter of inhibition in mm)/**90**(diameter of petriplates in mm**)**



Graph 2: Anti-bacterial effect of synthesized compounds.

Compound LS-1 and LS-2 shows the potent anti-inflammatory effect after 1hr and 2hr both than standard. Compound LS-3 and LS-5 have almost similar effect than standard. Compound LS-4 and LS-6 does not much effective than standard.

While The anti-bacterial properties of newly synthesised compounds (LS1-LS6) were tested in vitro against microorganisms such as gram-positive strains (Bacillus pumilus, Staphylococcus aureus) and gram-negative bacterial strains (Escherichia coli, Klebsiellapneumoniae) using the Agar Diffusion Method. At 1000 g/mL concentrations, all six synthesised derivatives were tested for anti-bacterial activity.

Derivative LS-1 had the maximum activity against E.coli than the k.pneumonia, B.pumilus and S. aureus.
Derivative LS-2 had the maximum activity against k.pneumonia than the E.coli, B.pumilus and S. aureus
Derivative LS-3 had the maximum activity against B.pumilus than the E.coli, k.pneumonia and S. aureus
Derivative LS-4 had the maximum activity against B.pumilus than the E.coli, k.pneumonia and S. aureus
Derivative LS-5 had the maximum activity against E.coli than the k.pneumonia, B.pumilus and S. aureus
Derivative LS-6 had the maximum activity against B.pumilus than the E.coli, k.pneumonia and S. aureus.

CONCLUSION

The present research work is focussed on the attachment of amino acids at the active sites at Curcumin molecule and may suggest new strategies and ways for the synthesis of novel amino acid derivatives with desirable therapeutic potential. All the synthesized derivatives of curcumin (L1 to L6) exhibited moderate to significant biological activity in terms of anti-inflammatory and anti-bacterial activity. It may be concluded that principles of structural manipulation have been appropriately applied in the present research work. This structural manipulation may lead to the synthesis of amino acid derivates of curcumin may serve the purpose of obtaining desirable therapeutic activity, minimization of side effects and desirable changes in the properties like solubility, bioavailability and stability to the chemical structure.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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