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Original article

Synthesis, antibacterial and antiviral properties of curcumin bioconjugates bearing dipeptide, fatty acids and folic acid

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ABSTRACT

Curcumin bioconjugates, viz. di-*O*-tryptophanylphenylalanine curcumin (**2**), di-*O*-decanoyl curcumin (**3**), di-*O*-pamitoyl curcumin (**4**), di-*O*-bis-(γ,γ)folyl curcumin (**6**), C^4-ethyl-*O*-γ-folyl curcumin (**8**) and 4-*O*-ethyl-*O*-γ-folyl curcumin (**10**) have been synthesized and tested for their antibacterial and antiviral activities. The conjugates **2**, **3**, **4**, **6** and **8** have shown very promising antibacterial activity with MIC ranging between 0.09 and 0.67 μM against Gram-positive cocci and Gram-negative bacilli. Further, the conjugates **2**, **3**, **6**, **8** and **10** have been screened for their antiviral activities against HSV, VSV, FIPV, PIV-3, RSV and FHV and the molecules **2** and **3** have shown good results with EC_{50} 0.011 μM and 0.029 μM against VSV and FIPV/FHV, respectively. However, the molecules did not show expected results against HIV-1 III_B and ROD strains in MTT assay.

1. Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, commonly known as diferuloyl methane, is a natural yellow pigment derived from rhizomes of the plant *Curcuma longa* (Zingiberaceae) – a plant grown in tropical southeast Asian and Indian subcontinent, and has been proved as potent antioxidant, anti-inflammatory, antiviral and anticancer agent through modulation of multiple cellular machinery [1–6]. Turmeric, a spice used to provide specific flavor and yellow color to curry, has been used for many centuries as an Indian folklore medicine in Ayurveda – an ancient traditional system of medicine for treatment of wide range of illnesses. Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism, blood purification and rheumatoid arthritis [7–9]. Recent studies have shown curcumin as a potential molecule in the treatment of different forms of cancer, e.g., cervical cancer caused by HPV [10–12].

It has been observed that both curcumin and the oil fraction, suppress the growth of several bacteria like *Streptococcus*, *Staphylococcus*, *Lactobacillus*, etc [12,13] and human pathogenic fungi. Turmeric oil is also active against *Aspergillus flavus*, *Aspergillus parasiticus*, *Fusarium moniliforme* and *Penicilium digitatum* [14,15].

Results have shown that curcumin treatment effectively reduced Coxsackie Virus B3 replication through desregulation of Ubiquitin–Proteosome System (UPS) and COP9 Signalosome (CSN) performing a critical role in their life cycle, i.e., it strongly reduced viral RNA expression and further protein synthesis [16,17]. Curcumin has been identified as inhibitor of HIV-1 LTR directed gene expression and viral replication. A previous study has shown that curcumin, a pharmacologically safe compound, is able to block HIV replication by inhibiting HIV-integrase and protease [18,19].

The double bonds in curcumin provide definite conformational flexibility to the molecule, which accounts for its various properties. Further, blocking of phenolic groups decreases its antioxidant activity since these groups play critical role in enzymatic activity at receptor sites [20,21]. Studies have revealed that curcumin has very low bioavailability due to its poor absorption and rapid metabolism in the liver and intestinal wall [22,23]. Curcumin is highly hydrophobic and cannot be administered systemically. On intravenous administration, it disappears rapidly from the blood and quickly appears as metabolites in the bile [10,24,25]. Therefore, one of the most appreciable approaches is to make biodegradable conjugates of curcumin molecule with suitable ligands to enhance its cellular uptake. For preparing bioconjugate of curcumin, amino acids and fatty acids – natural

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components of bacterial cell wall, and folic acid – a cofactor in the synthesis of thymidine and other nucleotides, were selected [26–29]. These bioconjugates are supposed to enhance cellular uptake, lipophilicity of the molecule and sustained release of drug molecule to improve the half-life and reduce the rate of metabolism of curcumin molecule inside the cell.

We have previously reported a series of nucleosidic molecules [30–33] of significant therapeutic applications. In the present pursuance, we are focusing on naturally occurring molecules – curcumin and its bioconjugates, e.g., di-*O*-tryptophanylphenylalanine curcumin (**2**), di-*O*-decanoyl curcumin (**3**), di-*O*-pamitoyl curcumin (**4**), di-*O*-bis-(γ,γ)folyl curcumin (**6**), C^4-ethyl-*O*-γ-folyl curcumin (**8**) and 4-*O*-ethyl-*O*-γ-folyl curcumin (**10**). These bioconjugates have been screened for their antibacterial and antiviral activities.

2. Chemistry

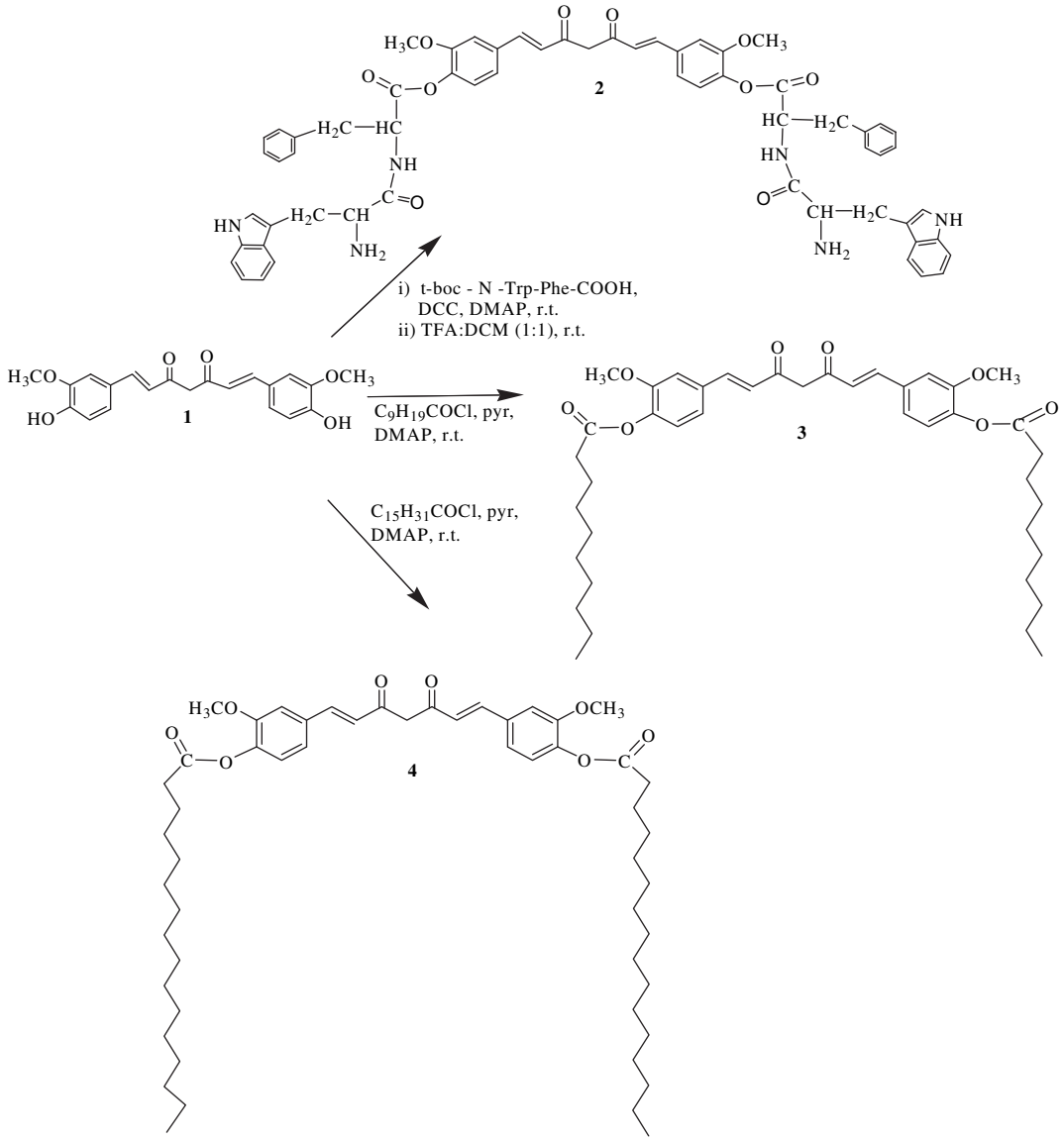
Curcumin, **1**, has two phenolic groups and one active methylene group which can be utilized as potential sites for chemical variations and covalent linkage with biomolecules. We have synthesized curcumin bioconjugates **2**, **3**, **4**, **6**, **8** and **10**, wherein the phenolic hydroxyls and active methylene group on curcumin have been utilized.

For the synthesis of compound **2** (Scheme 1), curcumin **1** was reacted with t-boc–*N*-trp–phe–COOH (indigenously synthesized) in 1:2 molar proportion using dicyclohexylcarbodiimide (DCC) coupling procedure in the presence of DMAP in anhydrous dichloromethane (DCM) to yield di-*O*-tryptophanylphenylalanine curcumin **2** in 53% yield [34].

Further, curcumin was reacted with decanoyl chloride and palmitoyl chloride in 1:2 molar proportion in the presence of DMAP in anhydrous pyridine to get the molecules **3** and **4** (Scheme 1) in 56% and 44% yield, respectively [35].

For the syntheses of conjugates **6**, **8** and **10**, folic acid was activated to *p*-nitrophenyl folate **5** (FA-PNP) (Scheme 2) using *p*-nitrophenol in the presence of DCC in pyridine and triethylamine (TEA). The completion of the reaction was assessed by precipitation of dicyclohexylurea (DCU) [36].

The activated folate **5** was reacted with curcumin **1** in 2:1 molar proportion in the presence of DCC and DMAP to get the compound **6** (Scheme 3) in 48% yield.



Scheme 1. Synthesis of curcumin bioconjugates with dipeptide, decanoyl chloride and pamitoyl chloride.