Synthesis of amino acid conjugates of tetrahydrocurcumin and evaluation of their antibacterial and anti-mutagenic properties

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Tetrahydrocurcumin (THC), the hydrogenated and stable form of curcumin, exhibits physiological and pharmacological activities similar to curcumin. A protocol has been developed for the synthesis of novel conjugates of THC with alanine (2a), isoleucine (2b), proline (2c), valine (2d), phenylalanine (2e), glycine (2f) and leucine (2g) in high yields (43–82%). All the derivatives of THC exhibited more potent anti-microbial activity than THC against Bacillus cereus, Staphylococcus aureus, Escherichia coli and Yersinia enterocolitica. The MIC values of the derivatives were 24–37% of those for THC in case of both Gram-positive and Gram-negative bacteria. Derivatives 2g and 2d exhibited maximum anti-mutagenicity against Salmonella typhimurium TA 98 and TA 1538, respectively at a low concentration of 313 μg/plate, with comparable activity for THC evident only at 3750 μg/plate. These results clearly demonstrated that the conjugation of THC at the phenolic position with amino acids led to significant improvement of its in vitro biological attributes.

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1. Introduction

Curcumin is the major colour constituent of turmeric (Curcuma longa, Zingiberaceae) used in Indian cuisine mainly for its colouring and flavouring attributes. Curcumin is highly valued for its medicinal and nutraceutical attributes (Ravindran, Babu, & Sivaraman, 2007). Although curcumin has promising therapeutic potential (Corson & Crews, 2007), various studies have highlighted its instability under physiological conditions (Anand, Kunnunakkara, Newman, & Aggarwal, 2007). It is soluble in organic solvents like acetone and ethyl acetate but insoluble in water at acidic or neutral pH. At pH 7.2, curcumin is sparingly soluble in aqueous media (~20 μg per ml) but degrades rapidly with the half-life of 30 min. The stability further decreases with increase in pH (Wang et al., 1997). Tetrahydrocurcumin (THC, 1) is the major and stable metabolite of curcumin. Its glucuronidation constitutes the reported pathway of metabolism of curcumin (Pan, Huang, & Lin, 1999). Glucuronides of curcumin and THC can serve as bio-available forms of curcumin in vivo. It exhibits physiological and pharmacological activities similar to curcumin and, in some systems, does show higher antioxidant activity than curcumin. THC is reported to be more stable than curcumin in buffer solutions of physiological pH of 7.2 and also at basic pH, as well as in plasma.

THC is derived from curcumin by selective reduction of olefinic bonds alpha to the carbonyl group in a diferuloyl backbone. THC is an effective natural antioxidant (Osawa, Sugiyama, Inayoshi, & Kawakishi 1995; Sugiyama, Kawakishi, & Osawa, 1996) and anti-inflammatory compound (Rao, Basu, & Siddiqui 1982), employed in many formulations in the field of cosmetics and nutrition. THC offers protection to the skin against damage due to ultraviolet radiation and other factors which are essential for sun protection preparations and cosmetics for aged skin. Tetrahydrocurcuminoids have superoxide scavenging ability and inhibitors of fat oxidation and offers protection against rancidity of several fat components. THC produces the protective effect to cells against oxidative stress by scavenging free radicals and reactive oxygen species (Nakamura, Ohto, Murakami, Osawa, & Ohigashi, 1998). THC is a potent antioxidant under the conditions where the radical initiators are produced in the polar water medium (Khopde et al., 2000). Administration of THC to streptozotocin (STZ)-nicotinamide-induced diabetic male Wistar rats significantly improves specific insulin binding to the receptors, with the receptor numbers and affinity binding reaching near-normal levels resulting in a significant increase in plasma insulin with the effect of THC being more prominent than that of curcumin (Murugan, Pari, & Rao, 2008). THC-inhibits lipoygenase (LOX) enzyme implicated in inflammatory conditions by preventing the activation of LOX-1 (Sneharani, Sridevi, Srinivas, & Rao, 2011). It is also effective in inhibiting...
cyclooxygenase-2 and phospholipase A2 (Hong et al., 2004). THC inhibits cancer (HT1080) cell migration and invasion by down regulation of extracellular matrix (ECM) degradation enzymes and the inhibition of cell adhesion to ECM proteins (Yodkeeree, Garbisa, & Limtrakul, 2008).

Recent attempts at preparing sugar and amino acid conjugates of curcumin led to the preparation of a number of water-soluble curcumin derivatives, which exhibited potent antioxidant, antimicrobial and antimutagenic properties comparable to and in several cases superior than curcumin (Kumar, Narain, Tripathi, & Misra, 2001; Parvathy, Negi, & Srinivas, 2009, 2010; Parvathy & Srinivas, 2008). The authors demonstrated that curcumin–β-diglucoside prevented oligomer formation and inhibited fibril formation of α-synuclein, whose aggregation is centrally implicated in Parkinson’s disease (Bharathi, Parvathy, Srinivas, Indi, & Rao, 2012). Curcumin is unstable and is metabolised to THC and other reduced forms in vivo. Hence, the bioavailability of curcumin is limited. Tetrahydrocurcumin (THC) is a stable metabolite of curcumin in physiological systems and more lipophilic than curcumin. Also, in several studies THC is shown to exhibit a wide array of bioactive properties more potent than curcumin. Hence, it was envisaged that it would be of great interest to synthesise new amino acid derivatives of THC and explore their bioactive attributes. In the present study, we attempted the syntheses of selected novel amino acid conjugates of THC and evaluated their in vitro antimicrobial and antimutagenicity potential.

2. Materials and methods

2.1. Apparatus and materials

All the solvents and reagents used for the synthesis were of analytical grade. Curcumin (~95% purity) was procured from Ms. Spicex Chemicals Pvt. Ltd., Mysore, India. Palladium on barium sulphate catalyst, t-Boc amino acids, 4-dimethylaminopyridine and N,N-dicyclohexylcarbodiimide were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Dry 1,4-dioxane, trifluoroacetic acid, and methanol in chloroform as the developing solvent. The products were purified by triturating using ethyl acetate and hexane mixture. The structure of curcumin–amino acid conjugates was confirmed by 1D and 2D-NMR and mass spectral analyses.

2.2. General method for synthesis of curcumin–t-Boc amino acid conjugates

A solution of curcumin (2 g, 5.43 mmol) in dioxane (25 ml) was taken in a round-bottomed flask. t-Boc amino acid (a–g, 13.58 mmol) was added gradually to ensure its complete solubility. 4-Dimethylaminopyridine (5.43 mmol) was added to reaction mixture and stirred for 5 min. After this N,N-dicyclohexylcarbodiimide (13.58 mmol) was added and set for stirring until the completion of reaction (~7–12 h) under nitrogen atmosphere. The progress of the reaction was monitored by TLC analysis. After the completion of reaction dicyclohexylurea was filtered off. The filtrate was diluted with chloroform (25 ml) and subjected to aqueous workup. The organic layer was separated, passed through anhydrous Na2SO4 and concentrated. Further, the product was purified by triturating using ethyl acetate and petroleum ether (60–80 °C) mixture to afford t-Boc protected amino acid derivatives of curcumin in 75–95% yield.

2.3. General method for deprotection of t-Boc group in curcin–t-Boc amino acid conjugates

Each of the curcumin–amino acid t-Boc conjugates (~3 g) was dissolved in CH2Cl2 (20 ml). To this solution, trifluoroacetic acid (TFA, 4 ml) in CH2Cl2 (20 ml) was added slowly under ice cold condition. The reaction mixture was allowed to stir at 25 °C till the completion of the reaction (3–5.5 h). It was followed by neutralisation of TFA by gradual addition of solid K2CO3. The product gets separated from the mixture and settled at the bottom of the flask. It was filtered, washed with water, dried and then kept in a vacuum desiccator over KOH pellets for 12 h. The pure products were obtained in the 60–90% yield. The structure of curcumin–amino acid conjugates (1a–1g) was confirmed by 1D and 2D-NMR and mass spectral analyses.

2.4. General method for the synthesis of THC–amino acid conjugates (2a–2g)

The solution of curcumin–amino acid conjugates (1 g, 1a–1g) in methanol (15 ml) was taken in hydrogenation flask and charged with Pd/BaSO4 (10 mg, 10% w/w). The mixture was agitated at 30 psi H2 pressure till completion of the reaction (0.5–1.5 h), as inferred by disappearance of golden yellow colour. Additionally, it was confirmed by TLC analysis. After completion of the reaction, the catalyst was filtered off and the filtrate was concentrated under reduced pressure to afford pure products (2a–2g) in quantitative yields. The products were characterised by 1D and 2D-NMR and high resolution mass spectral analyses. The physical and spectroscopic data of the novel THC–alanine conjugate (2a) are presented below with numbering of the carbon atoms as depicted in Table 1. The physical and spectroscopic data of other conjugates (2b–2g) are provided in the Supplementary information.

2a: [(Z)-5-hydroxy-1.7-bis(4-O-L-alaninoyl-3-methoxyphenyl)hept-4-en-3-one]; off-white solid; m. p. 74–76 °C; 1H NMR (500 MHz, CD3OD): δ 1.70 (d, J = 7.2 Hz, 6H, H-17 & H-17‘), 2.55–2.62 (m, 2H, H-6), 2.72–2.83 (m, 4H, H-1 & H-2), 2.84–2.89 (m, 2H, H-7), 3.76 (s, 6H, H-14 & H-14‘), 4.35 (q, J = 7.3 Hz, 2H, H-16 & H-16‘), 5.56 (s, 1H, H-4), 6.72–6.81 (m, 2H, H-13 & H-13‘), 6.88–6.94 (m, 2H, H-9 & H-9‘), 6.95–7.01 (m, 2H, H-12 & H-12‘), 13C NMR (125 MHz, CD3OD): δ 16.52 (C-17 & C-17‘), 30.11 (C-1), 32.33 (C-7), 40.59 (C-6), 45.70 (C-5), 49.89 (C-16 & C-16‘), 56.47 (C-14 & C-14‘), 100.86 (C-4), 113.99 (C-9 & C-9‘), 121.54 (C-13 & C-13‘), 123.12 (C-12 & C-12‘), 138.61 (C-11), 138.70 (C-11‘), 142.14 (C-8), 142.31 (C-8‘), 151.92 (C-10 & C-10‘), 169.65 (C-15 & C-15‘), 194.15 (C-5), 206.01 (C-3). Mass: Calculated for formula C27H32N2O7: 514.2315; Found: [M+1] = 515.4629.

2.5. Antibacterial studies of tetrahydrocurcumin–amino acid conjugates

The antibacterial activity of THC–amino acid conjugates was tested essentially by the method described by Negi, Jayaprakasha, Rao, and Sakariah (1999). Bacillus cereus (F 4810, Public Health Laboratory, London, UK), Staphylococcus aureus (FRI 722, Public Health Laboratory, The Netherlands), Escherichia coli (MTCC 108, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) and Yersinia enterocolitica (MTCC 851, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) were sub-cultured in BHI broth and incubated for 24 h at 37 °C. After incubation cells were harvested by centrifugation
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