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Facile Synthetic Design and Characterization of Curcumin-Metformin Adduct: Potential Insights into the Role of This Conjugate in Diseases of Aging

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Abstract: Recently, the anti-glycation and anticancer properties of curcumin longa and okra seed extract were studied alone and also in combination with the well-established drug metformin. The combined effect of curcumin with metformin and metformin with okra seed extract was found to be highly efficacious in inhibiting Advanced Glycation End-products (AGEs). In order to understand the mechanistic implications of curcumin combined with metformin and its enhanced anti-glycation activity, a Curcumin-Metformin Adduct was chemically synthesized. This adduct was fully characterized by thin-layer chromatography, Nano Drop spectrophotometry and electrospray-ionization mass spectrometry. The adduct may be helpful not only in elucidating the mechanism of anti-glycation and anti-cancer activities but also in studying the role of curcumin in binding of Aβ-oligomers and disaggregating fibrillar formation in Alzheimer's disease.

Key words: Curcumin, metformin, diabetes, anticarcinogenic.

1. Introduction

Curcumin is a major ingredient present in yellow spice turmeric as well as in the plant curcuma longa linn (Fig. 1) [1-4]. It is a major dietary polyphenol that has been characterized by various analytical and spectroscopic techniques [5-8]. The root of the turmeric plant has powerful antioxidant, potent anti-inflammatory properties and has been used as a traditional herbal medicine in India [8-10]. It is used as a natural brain protecting substance blocks aggregation and fibril formation, inhibits lipid peroxidation and scavenges nitric oxide radicals [11-15]. Suppression of NF-kB activation by curcumin and further inhibition of cyclo-oxygenase-2 has implications for the treatment of osteoarthritis [16]. In a recent study done by Lo, J. Y., and coworkers [16, 17], isolated curcumenol—a sesquiterpene from curcuma zedoaria (white root), was found to suppress Akt-mediated NF-kB activation and p38MAPK signaling pathways.

The anti-glycation and anticancer properties of curcumin longa were studied alone and also in combination with the well-established drug metformin [4, 18-20]. The combination of curcumin with metformin and metformin with okra seed extract was highly efficacious in inhibiting Advanced Glycation End-products (AGEs) [18].

The low water solubility and poor bioavailability of curcumin has resulted in limited clinical applications. Therefore, attempts have been made to enhance its biological activity by conjugating with curcumin. A careful study by Jiang, Z. et al. [19] showed the inhibitory effect of self-assembled nanohydrogel of curcumin-hyaluronic acid conjugates on amyloid

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Fig. 1 Curcuma longa linn.

B-protein aggregation and cytotoxicity. In another elegant study, the synthesis and characterization of curcumin derived pyrazoles and isoxazoles inhibiting Aβ precursor protein in Alzheimer's disease was reported by Narlawar, R. et al. [12]. Other applications of conjugates such as folate with chitosan have found enhanced biological activities in terms of cellular update of nanoparticles in HT-29 cells [21].

These studies prompted the design and synthesis of a Curcumin-Metformin Adduct as an agent for studying Alzheimer's disease and its potential applications into enhanced anti-diabetic and anti-cancer activities.

2. Material and Methods

Curcumin and metformin (1,1-dimethylbiguanide) were purchased from Sigma chemicals. Glacial acetic acid was obtained from Sigma-Aldrich. Aluminum backed plates for TLC were obtained from Machrey-Nagel, Germany. Silica Gel (63-200 µm particle size) was purchased from Sigma-Aldrich. Electrospray Ionization Mass Spectra (ESI/MS) in the (+) ion mode was recorded using electrospray quadrupole mass spectrometer. UV-visible studies were performed using a Nano Drop spectrophotometer.

3. Experimental Procedure

Curcumin (8 mg, 0.02 mM) was dissolved in methanol (2 mL) and metformin hydrochloride (25 mg, 0.15 mM) was added followed by 200 µL of triethylamine and a catalytic amount of glacial acetic acid (200 µL) (Fig. 2). The reaction mixture was vortexed for 45 seconds and left stirring at room temperature for 5 minutes. After 5 minutes, all the reaction mixture was consumed as monitored by analytical and preparatory thin-layer chromatography (aluminum backed pre-coated SIL G/UV254) TLC plates in the solvent system: $CHCl_3$:CH_3OH (24:4 v/v). The major extremely polar metformin-curcumin adduct (95%, R_f = 0.53) and the minor isomer (5%, R_f = 0.69) were visualized only in an iodine chamber. The parent curcumin starting material (R_f = 0.94) was visualized under UV light (Fig. 3).

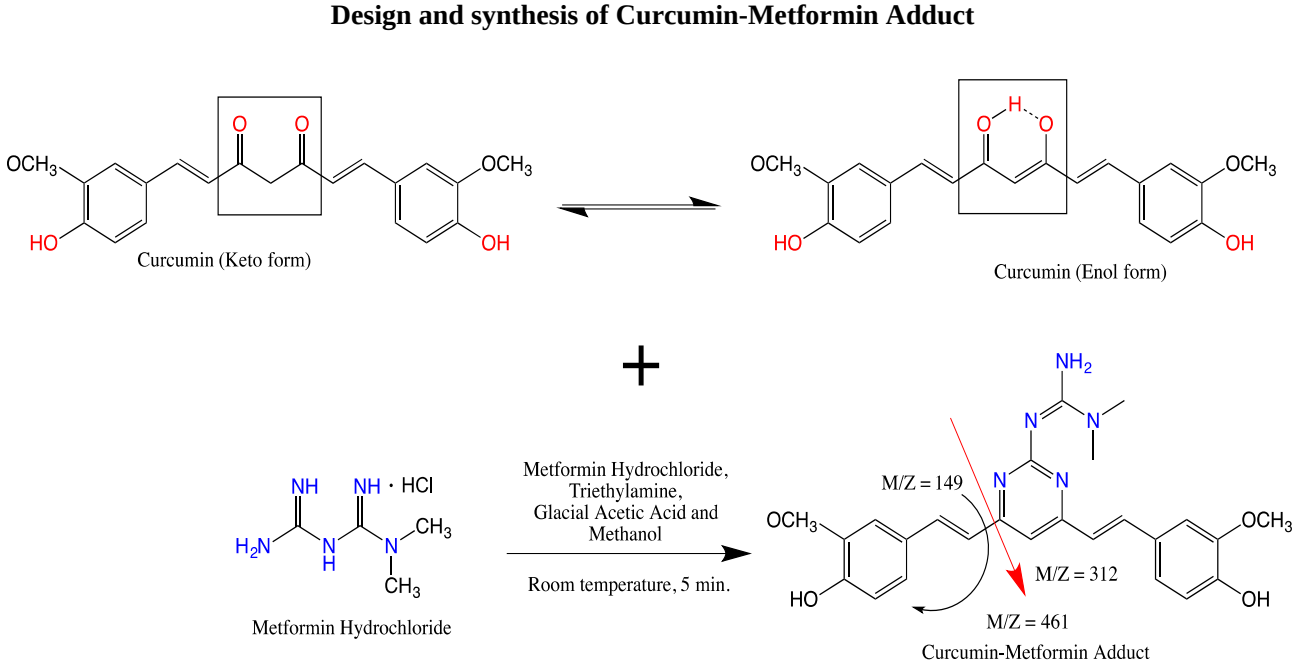

Fig. 2 Design and synthesis of Curcumin-Metformin Adduct.

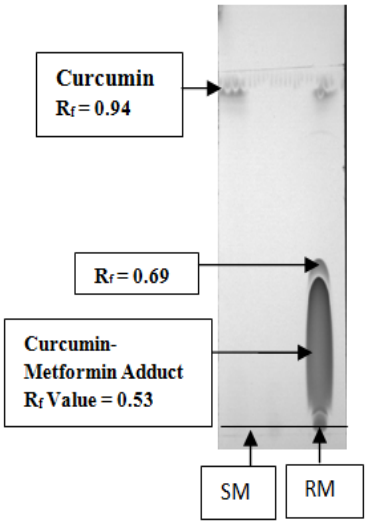

Fig. 3 TLC: $CHCl_3$:CH_3OH (24:4 v/v), curcumin: Starting Material (SM), Reaction Mixture (RM).

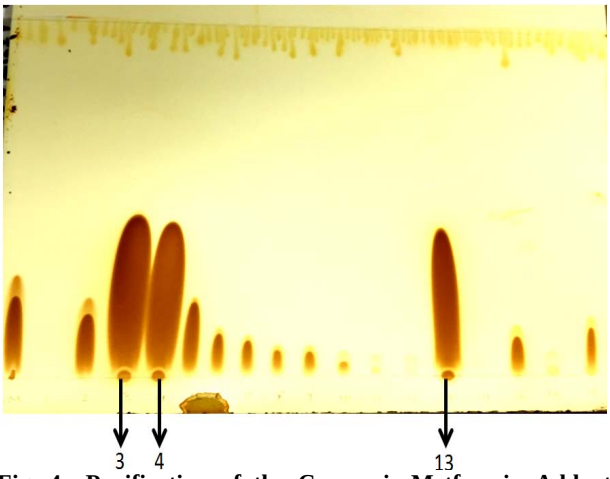

Fig. 4 Purification of the Curcumin-Metformin Adduct reaction mixture via flash column silica gel column chromatography, fractions 3 to 13 are purified fractions as monitored by thin-layer chromatography.

The yellowish mixture (10 mg) was evaporated to dryness under N_2 atmosphere at room temperature and the residue chromatographed on preparatory TLC Silica Gel plates (Fig. 3). For purification by Flash Column Silica Gel Chromatography, the reaction mixture was applied to a glass column filled with 2.1 g of Silica gel particle size 63-200 µm. Elution was performed using 100 mL of a mixture of hexane/ethyl acetate (10:90 v/v) and subsequent elution with increasing amounts of ethyl acetate with hexane (30:70 v/v) provided pure fractions 3, 4 and 13 (4.5 mg) (Fig. 4).

4. Results and Discussion

Recently, the binding and modification of proteins were studied: Lysozyme (Lys) and Human Serum Albumin (HSA) by methylglyoxal under physiological conditions [18-20]. Inhibition of the formation of Advanced Glycation End-products (AGEs) from MGO-modified ribonuclease by structurally-defined flavonoids present in Okra-Seed Extract (OSE) and curcumin bioactives was also by Dayal, B., and coworkers [19]. The analysis of Lys-MGO and HSA-MGO was achieved by AGE-associated absorbance changes via Nano Drop spectrophotometry analysis and their inhibitory effect was assessed using fluorescence spectroscopy and SDS-PAGE analysis [18] (Fig. 8). Furthermore, comparative efficacy studies using a well-established AGE inhibitor, metformin with OSE and combination of okra seed with Yellow Curcumin (YC) were studied and analyzed via specific fluorescence and SDS-PAGE analysis. The results exhibited 70%-80% anti-glycosylation activity of metformin and OSE extract while YC inhibitory activity ranged from 45%-50%. But the combinations of metformin with OSE or YC further enhanced antiglycation activity in a dose dependent manner (Fig. 8) [18-20].

The commonly used drug metformin (1, 1-dimethylbiguanide) for type-2 diabetes reduces cancer risk and tumor growth [22-24]. The mechanisms by which this happens are not completely understood. One of the proposed mechanisms suggest activation of AMP-activated protein Kinase (AMPK), inhibition of mammalian Target of Rapamycin (m-TOR) activity, Akt-dephosphorylation, disruption of UPR transcription and cell cycle arrest [22-24]. These effects may be secondary to inhibition of complex I of the mitochondrial electron transport chain [22-25]. Therefore, these studies are aimed at developing more potent anti-diabetic, anti-cancer drugs and their mechanisms. Such observations have been studied by Dayal, B. et al. [18-20], suggesting that metformin or phenformin

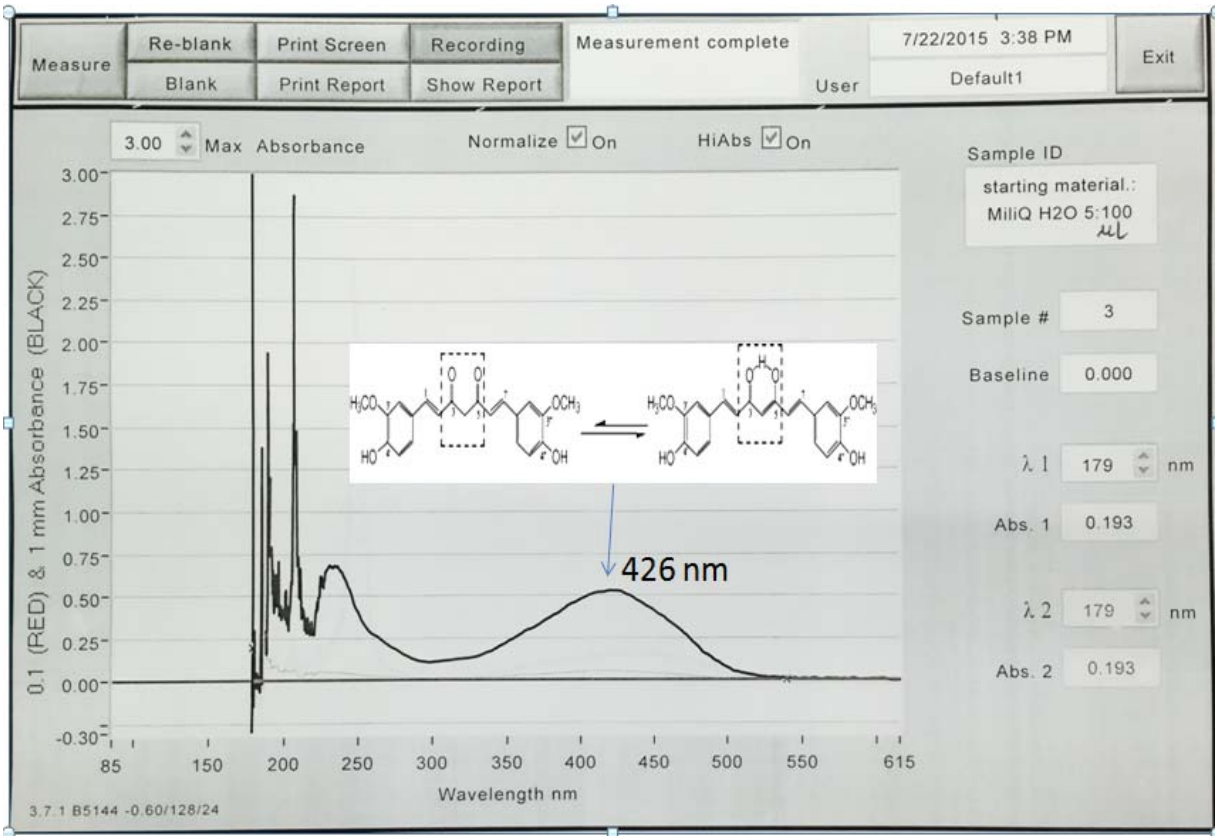

Fig. 5 UV-VIS of curcumin via Nano Drop spectrophotometery.

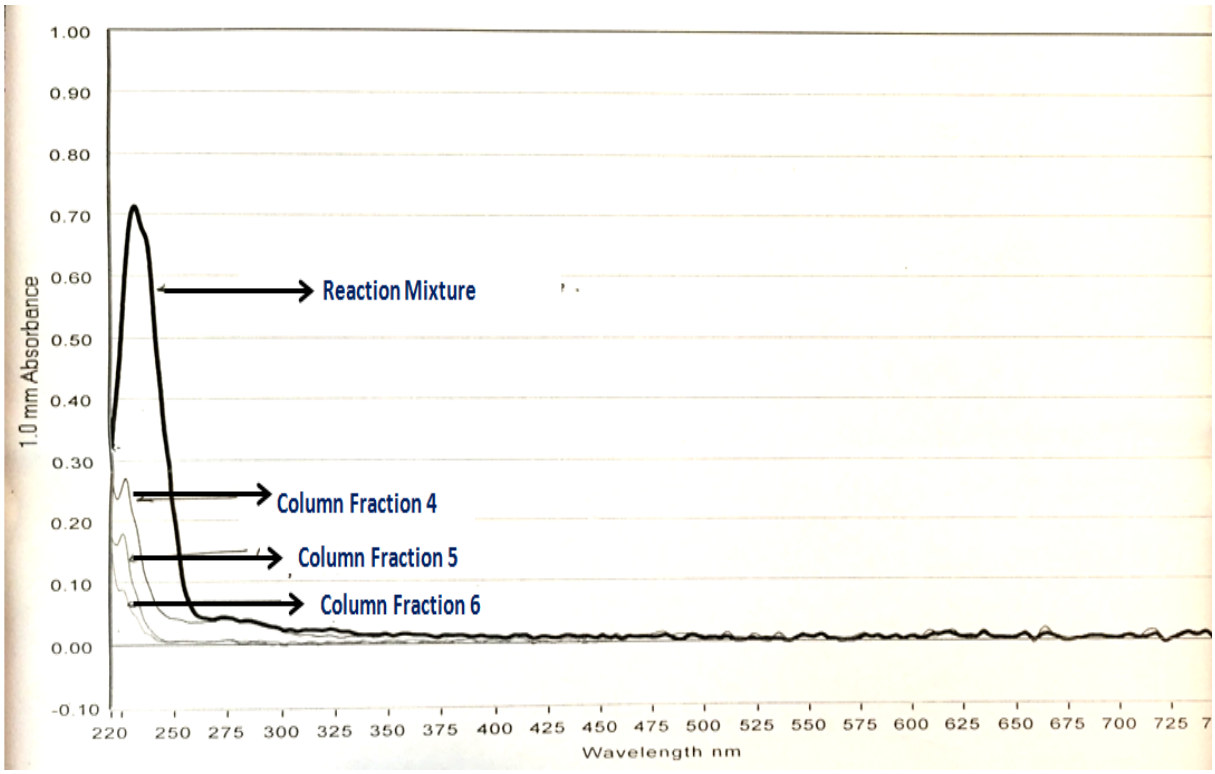

Fig. 6 UV-Vis Nano Drop spectrophotometry analysis of pure fractions of Curcumin-Metformin Adduct isolated from flash column chromatography.

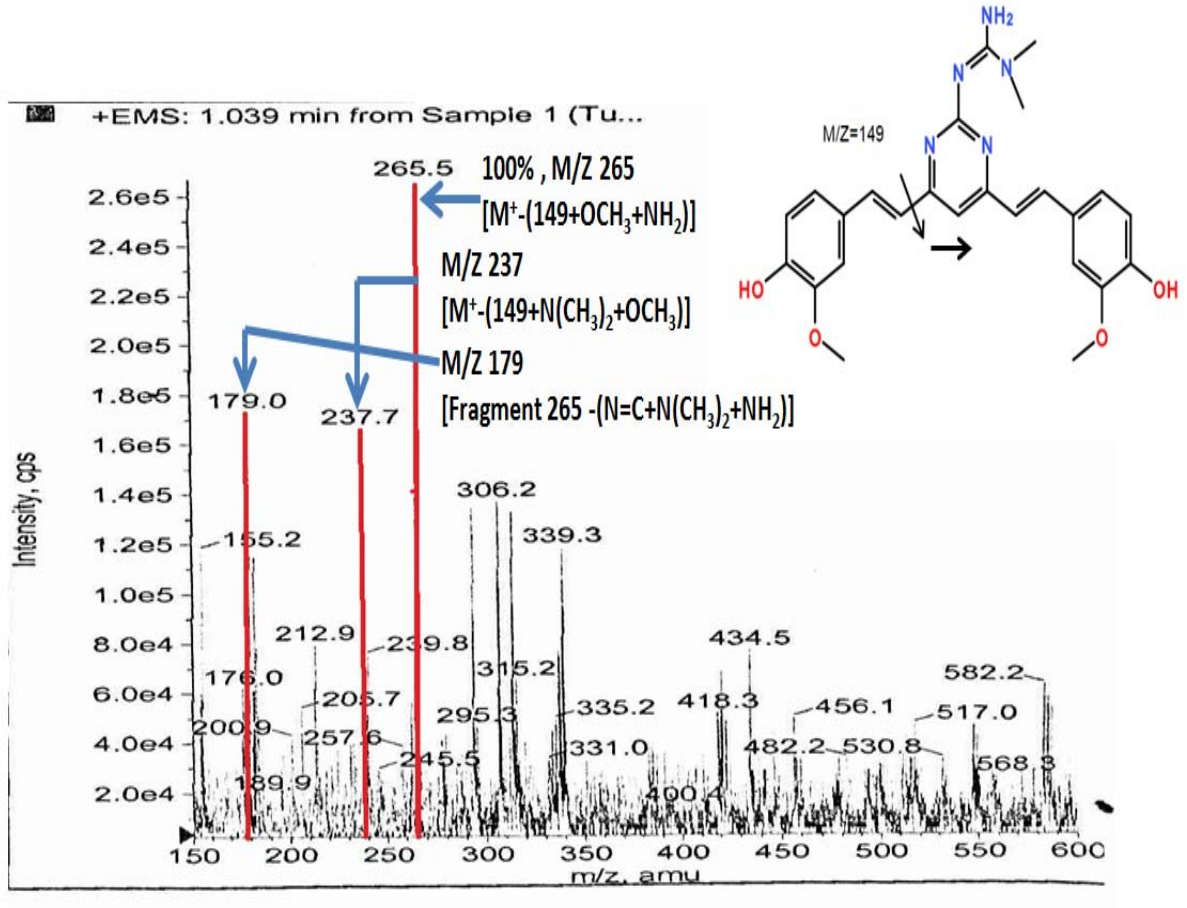

Fig. 7 Positive ion mode Electrospray-Ionization Mass Spectra (ESI/MS) of Metformin-Curcumin Adduct.

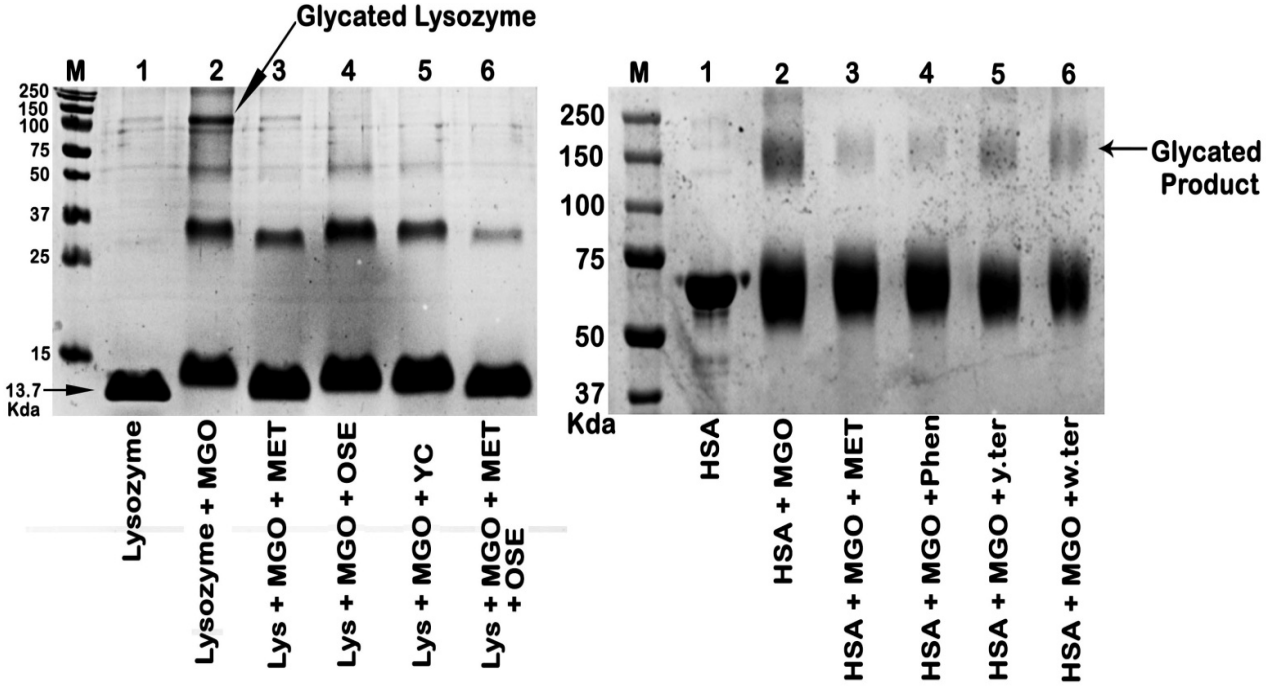

Fig. 8 Methylglyoxal-induced modification of lysozyme and HSA via SDS PAGE analysis.

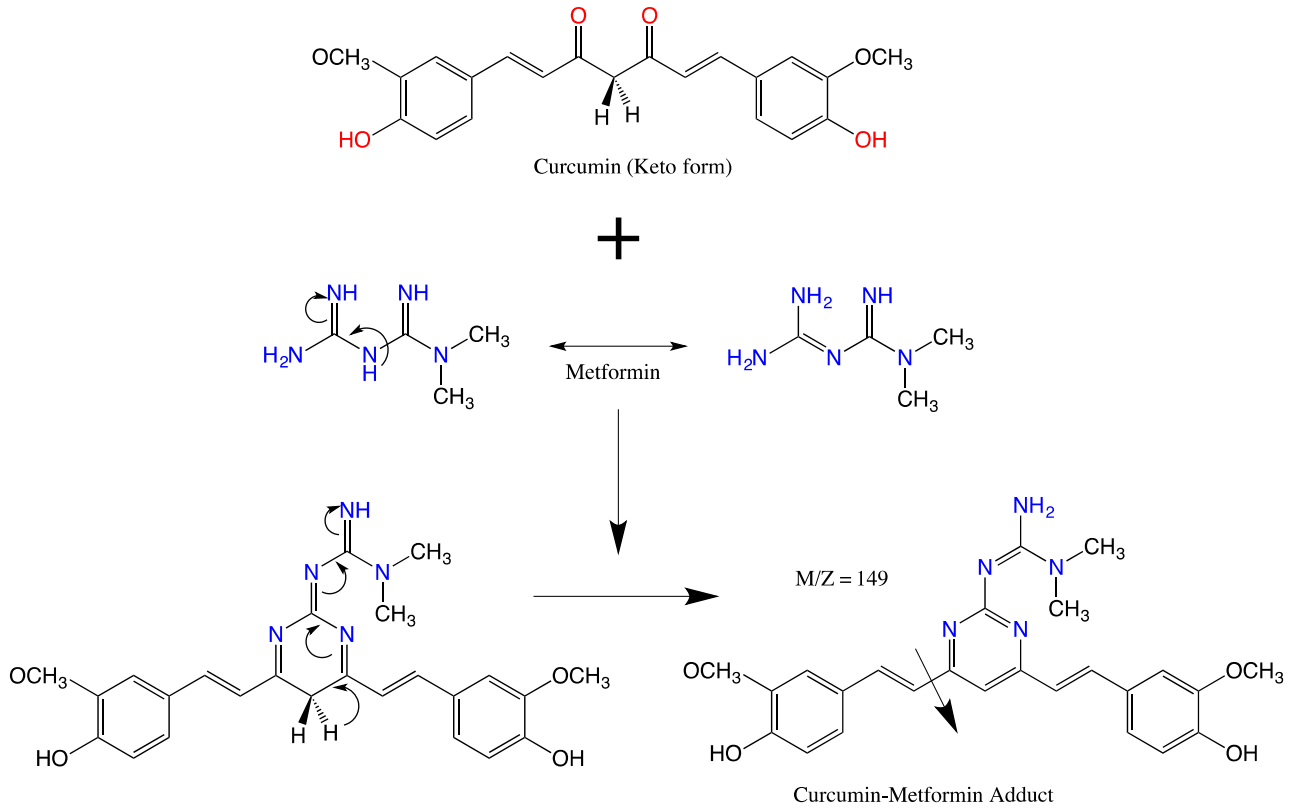

Fig. 9 Reaction mechanism: Curcumin-Metformin Adduct formation.

with OSE and curcumin shows an enhanced antiglycation activity (Fig. 8).

Therefore, combination of metformin with curcumin may have the potential not only to prevent the side-effects of lactic acidosis a potential effect of biguanides, but also may show synergistic anti-diabetic and anti-cancer effects [24, 25]. Substitution of 1,3-dicarbonyl moiety in curcumin by pyrazole has been demonstrated to inhibit gama-secretase activity [12]. The reaction of a biguanide, metformin, a well-known diabetes drug, with curcumin may also have the potential to bind Aβ-oligomers and disaggregate fibrillar formation in Alzheimer's disease as well. Structural insights into the mechanism of the formation of metformin adduct with 1,3-dicarbonyl curcumin keto-enol tautomeric form is exhibited in Fig. 9.

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