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DESIGN, SYNTHESIS, CHARACTERIZATION AND IN VITRO ANTI DIABETIC ACTIVITY STUDIES ON SEMISYNTHETIC DERIVATIVES OF CURCUMIN

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ABSTRACT

The present work has been under taken to synthesis curcumin derivatives. The synthesized compounds were characterised by UV and IR spectroscopy and screened for *in vitro* alpha-amylase inhibitory activity. The semisynthetic derivatives of curcumin was prepared using two schemes to synthesize pyrazole and pyrimidine derivatives. Pyrazole derivative were prepared by condensation of curcumin with hydrazine hydrate, pyrimidine derivative were prepared by condensation of curcumin with urea. The structure of the compounds synthesized during the present work were established on the basis of the chemical data, IR, UV data. The purity of the compounds were established by single spot-on TLC plates. The newly synthesized compounds were evaluated for their α- amylase inhibitory activity.

KEYWORDS: Curcumin, alpha amylase, Pyrazole, Pyrimidine, Semisynthetic.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterised by a high blood glucose concentration sustained hyperglycaemia caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis.

Type 1 diabetes occurs due β-cell destruction, usually leading to absolute insulin deficiency. Type 2 diabetes may range from predominantly insulin resistance with relative insulin

deficiency to insulin secretory defect with insulin resistance. There are many digestive enzymes in humans, among them the most important one is pancreatic alpha-amylase that act as a catalyst in the reaction which involves the hydrolyses of the alpha -1,4 glycosidic linkages of the starch amylopectin, amylase, glycogen, and numerous maltodextrins and is responsible for starch digestion.[1]

Excess conversion of starch to sugars, will increase the sugar level in blood, thus the role of insulin will come into action by ordering cells to metabolise the excess sugar moieties and excess glucose is stored as glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose raises which might results in hyperglycemia. To control hyperglycemia several studies on inhibition of amylase enzyme activity is being studied.[2]

Curcumin is a beta-diketone derivative, belonging to the group of curcuminoids, which are natural phenols responsible for turmeric's yellow color. Curcumin and their derivatives constitute an important class of heterocyclic compounds.[3] They gained prominence in medicinal chemistry due to their diverse biological activities such as antiviral,[4] anticancer,[5] antioxidant,[6] antibacterial,[7,8] anti-inflammatory,[9] antifungal[10] and anti-arthritic activity.[11] Chemical literature reveals that several organic compounds containing curcumin fused, pyrimidine and pyrazoles moieties, have been reported to possess α- amylase inhibitory activity.[12-14] Hence, it was thought of interest to synthesize some newer curcumin derivatives by merging pyrimidine and pyrazoles moieties with the hope to synthesize better antidiabetic agents.[15,16]

MATERIALS AND METHODS

Chemistry

Pyrazole is an organic compound with the formula $C_3H_3N_2H$. It is a heterocycle characterized by a 5-membered ring of three carbon atoms and two adjacent nitrogen atoms. Pyrazole is a weak base, with pKb=11.5. Pyrazoles are a class of compounds that have the ring C_3N_2 with adjacent nitrogen atoms.[17-20]

Pyrimidine is an aromatic heterocyclic organic compound similar to pyridine. One of the three diazines (Six-membered heterocyclic with two nitrogen atoms in the ring) it has the

nitrogen atoms at positions 1 and 3 in the ring. The other diazines are pyrazine (Nitrogen atoms at the 1 and 4 positions) and pyridazine (Nitrogen atoms at the 1 and 2 positions).[21]

Isolation of curcumin from turmeric rhizomes

Fresh rhizomes were collected and were cleaned, washed with deionised water, boiled and dried in the sun for one week and dried, at 50˚C in a hot air oven for six hours. Dried rhizomes were powdered by electronic mill. About 10 g of turmeric rhizome powder was extracted with 200 ml of 95% ethanol in a soxhlet assembly until all the colouring matter is extracted. The obtained crude extract was concentrated by distillation process and remaining ethanol was evaporated to obtain semisolid brown coloured mass. The concentrate hence obtained was dissolved in 50 ml of benzene. Equal volume of sodium hydroxide (10% w/v) was added to benzene solution. Using a separating funnel curcumin was partitioned between the two layers. Sodium hydroxide layer was taken and curcumin was precipitated by neutralising it with dilute hydrochloric acid solution (10% w/v). The precipitate obtained was filtered using vacuum pump and dried. The isolated curcumin was spectrally (IR spectroscopy) and chromatographically (Thin layer chromatography) evaluated and further used for synthesis of semisynthetic derivatives of curcumin.[22,23]

The semisynthetic derivatives of curcumin (SDC) were prepared using two different schemes.

a) Synthesis of pyrazole derivative
b) Synthesis of pyrimidine derivative

Pyrazole derivative was prepared by condensation of curcumin with hydrazine hydrate and pyrimidine derivative was prepared by condensation of curcumin with urea.[24]

Synthesis

Synthesis of Pyrazole derivative (SDC-Ⅰ): 4-[(*Z*)-2-{5-[(*E*)-2-(4-hydroxy-3-methoxphenyl)ethenyl]-1*H*-pyrazol-3-yl}ethenyl]-2-methoxyphenol

740mg of curcumin was weighed accurately in a round bottom flask, to this 0.128 ml of hydrazine and 25ml of glacial acetic acid were added. To avoid bumping porcelain bits was added, and the refluxed for 6 hrs. After 6 hrs round bottom flask was has been removed and cooled. The solvent was distilled and then crude product was obtained. The crude product purified by recrystallization using ethanol. The purity of the compound was confirmed by single spot-on TLC plate.

Synthesis of Pyrimidine derivative (SDC-Ⅱ): 4,6-bis[(*Z*)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]pyrimidin-2(5*H*)-one

600mg of curcumin was weighed accurately in a round bottomed flask, to this 0.3g of urea and 25ml of glacial acetic acid were added. To avoid bumping porcelain bits was added, and the refluxed for 6 hrs. After 6 hrs round bottom flask was has been removed and cooled. The solvent was distilled and then crude product was obtained. The crude product purified by recrystallization using ethanol. The purity of the compound was confirmed by single spot-on TLC plate.

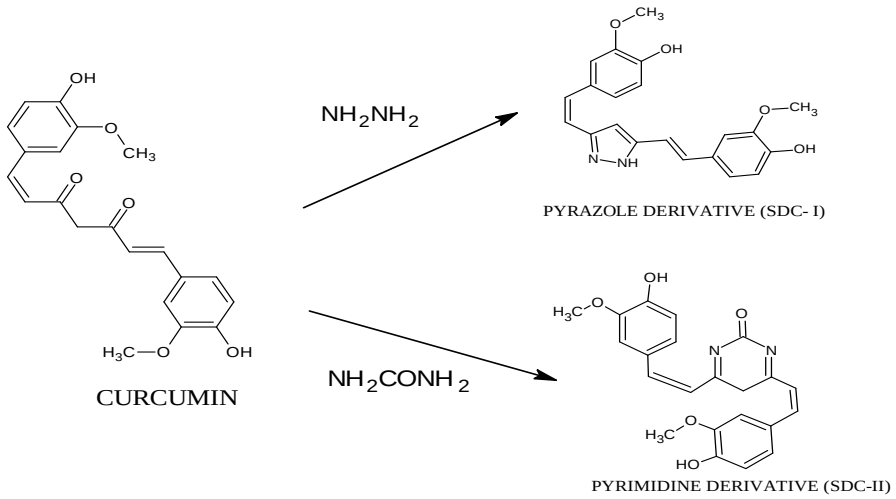

Table 1: Physicochemical properties of the isolated Curcumin and Semisynthetic derivative of curcumin.

Compound	Molecular formula	Percentage Yield (%)	Melting point	Rf value	λ_{max}
Isolated curcumin	$C_{21}H_{20}O_6$	78.5	183-185°C	0.75	421nm
Pyrazole curcumin SDC- I	$C_{26}H_{23}N_2O_4$	76	152-160°C	0.63	327nm
Pyrimidine curcumin SDC-II	$C_{21}H_{20}N_2O_4$	63	162-170°C	0.55	416nm

4-[(*Z*)-2-{5-[(*E*)-2-(4-hydroxy-3-methoxphenyl)ethenyl]-1*H*-pyrazol-3-yl}ethenyl]-2-methoxyphenol: SDC-I

IR(KBr)cm-1: 2958.82(NH stretching), 1686.51(C=O stretching), 1635.44(C=N stretching), 1102.27(C-O-C stretching), 3456.96(-OH stretching), 2951.83(Ar-H Stretchinhg), **1H NMR (500MHz, DMSO-d6, δppm):** 9.119 (s, 1H, OH), 8.80 (s, 1H, NH), 9.012 (s, 1H, NH),4.931 (s, 1H, C=CH), 3.738 (s, 3H, OCH3), 6.608-6.804 (m, 11H, Ar-H), 6.387 (s, 2H, CH), **13C NMR (500MHz, DMSO-d6, δppm):** 149.28 (C-OH), 55.05 (O-CH3), 154.75(C-OCH3),

18.65(CH), 165.96(C=C-NH), 159.15(C-OH), 136.42(C-phenyl ring), 115.77(2CH), 117.03(2CH), **MS: m/z:** 427 $[M]^+$.

4,6-bis[(*Z*)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]pyrimidin-2(5*H*)-one: 2918.49(Ar-H stretching), 1689.31(C=O stretching), 1658.91(C=N stretching), 1092.60(C-O-C stretching), 2851.56(-OH stretching), **1H NMR (500MHz, DMSO-d6, δppm):** 3.726 (s, 3H, OCH3), 6.62-6.802 (m, 10H, Ar-H), 6.387 (s, 2H, CH), **13C NMR (500MHz, DMSO-d6, δppm):** 146.27 (C-OH), 56.04 (O-CH3), 152.72(C-OCH3), 148.39(C=O), 165.94(O=C-N), 54.03(CH2 of pyrimidinone ring), 136.40(C-phenyl ring), 115.75(2CH), 118.77(2CH), **MS: m/z:** 361[M+2]+.

Pharmacological activity

***In vitro* α- amylase inhibitory activity of curcumin derivatives**

α-amylase digests the starch in reaction mixture to yield maltose. The maltose produced would reduce the 3,5-dinitro salicylic acid in the coloring agent to 3 amino 5-nitrosalicylic acid. The reaction mixture produced a color change from orange to red. The intensity of red color will be directly proportional to the amount of maltose produced. When an enzyme inhibitor is present in reaction mixture digestion of starch, production of maltose and intensity of red color produced will be less.

The solvent used for pyrazole derivative (SDC I) was water and DMF for urea derivative (SDC II)

From 1mg/ml stock solution different concentration (5, 10, 20, 40 and 80 µg/ml) of curcumin derivatives (Pyrazole and thiourea) were prepared by adding few drops of water/DMF and volume made up with water/DMF. About 500 µl of α-amylase (0.5mg/ml) was added and was incubated for 10 minutes at room temperature. Then added 500 µl of 1.0% starch solution and incubated for another 10minutes. After that 1 ml of the colouring reagent was added to the reaction mixture and heated in a boiling water bath for 5 minutes. After cooling, 10 ml of water/DMF was added for dilution. To measure the absorbance of the interfering substances, blank was prepared for each set of concentration of test sample by replacing the enzyme solution with buffer. Control incubations representing 100% enzyme activity was prepared by replacing the test drug with water. The absorbance was then measured at 540nm. The α –amylase inhibition was expressed as percentage of inhibition and the IC_{50} values determined by linear regression plots with varying concentration of synthesized curcumin against percentage inhibition and the results were given in the table.2.[25-28]

Table 2: *In vitro* alpha amylase inhibitory activity of synthesized curcumin derivatives.

S. No	Drug	Concentration (µg/ml)					IC_{50} (µg/ml)
		5	10	20	40	80	
1	Acarbose	17.29	31.52	54.79	68.71	81.23	43
2	Pyrazole (SDC-I)	22.01	29.29	40.36	69.18	79.02	41
3	Pyrimidine (SDC-II)	30.71	41.31	57.26	77.25	81.99	38

CONCLUSION

Our attempt was focussed to prepare curcumin analogues incorporated five membered and six membered ring systems in order to study the effect of different ring systems on biological behaviour. Curcumin was used as a starting material to synthesize the target compounds. Curcumin on intermolecular cyclisation with hydrazine hydrate afforded corresponding pyrazole derivative, with urea, pyrimidinone derivative. Intermolecular cyclisation of curcumin with nitrogen nucleophiles such as hydrazine hydrate and urea, on refluxing yielded the corresponding pyrazole and pyrimidinone derivative. The reaction proceeds through initial condensation of the amino group with the carbonyl group of diketones of curcumin, followed by cyclization to produce the target compounds. The structures of the proposed compounds were found to be in consistent with the analytical studies including melting point, thin layer chromatography, Ultraviolet and Infra-red spectroscopy. Newly synthesized compounds exhibited antidiabetic activity compared to the standard, Acarbose. The curcumin derivatives showed better inhibition against alpha amylase with IC50 values of 41µg/ml and 38 µg/ml respectively. Results of alpha amylase inhibitory assay revealed that the incorporation of heterocyclic ring system to curcumin anti-diabetic activity of curcumin was increased as compared with curcumin.

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