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Green synthesis, characterization, structure, biological activity, theoretical calculations and drug-likeness analysis of coumarins

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Abstract

Condensation of salicylaldehydes and 2-hydroxynaphthaldehyde with various β -dicarbonyl derivatives **2a-c**, in the presence of KF-Al₂O₃, leads to the synthesis of a series of coumarins **3-8** and benzo[*f*]coumarins **10** respectively by a solvent free reaction under microwave irradiation. The catalyst is recovered by a simple filtration and reused in subsequent reactions. The structure of all synthesized compounds has been established by using analytical techniques such as IR, ¹H and ¹³C NMR spectroscopic spectra and elemental analysis. Most of the coumarins exhibited significant antibacterial activity against *S. aureus* Gram-positive bacteria compared to Cefotaxime as positive control. The compounds **4a** and **7a** demonstrated moderate antimicrobial activity against compound **8a** is extremely sensitive. In addition, the compounds showed moderate to good antiradical (DPPH) activity. Theoretical calculations let us to confirm the reaction mechanism. Parameters drug-likeness and physicochemical properties including pharmacokinetic analysis of the synthesized compounds was performed using DruliTo program.

Highlights

- KF-Al₂O₃ efficient and reusable catalyst in a facile synthesis of coumarin derivatives.
- Conventional heating and microwave irradiation in free of solvent.
- Structural confirmation of the synthesized compounds was done by various spectroscopic techniques.
- Drug-Likeness analysis and Geometries of synthesized compounds were optimized by using B3LYP method with 6-31G* basis set.
- Antioxidant and antibacterial activity.

Graphical abstract

Keywords

Coumarins, Microwave irradiation, Green synthesis, Biological activity, Drug-Likeness analysis

Introduction

Curcumin, which imparts the yellow color to curry, is a natural product of the spice turmeric, *Curcuma longa* L. (Zingiberaceae). Curcumin exhibits a variety of pharmacologic activities, including anti-inflammatory, anticancer, antioxidant, wound-healing, antimicrobial effects [1], antiallergic activity [2,3] and inhibits degranulation of the RBL-2H3 tumor mast cell line in culture [4,5]. Also, it prevents biliary disorders, anorexia, coughs, diabetes, hepatic disorders, rheumatism, sinusitis, cancer and Alzheimer's disease [6,7]. The ethyl acetate extract of *Curcuma longa* L. and curcumin were found to decrease histamine release from mast cells by blocking intracellular signalling events in mast cells. The anti-allergy activities of curcumin and curcumin-related compounds in relation to their antioxidant activities. Most of these compounds were shown to inhibit histamine release from RBL-2H3 cells induced by concanavalin A or a calcium ionophore [8]. Chemically, it is 1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione; it consists of two back bones, each with a ketone and a $-\text{CH}_2$ group separating the backbones and a terminal meta-methoxy-para hydroxyl phenyl ring on each side. It has been suggested that the antioxidant activity of the curcumin molecule depends upon the presence of a phenolic group [9]. On the other hand, other studies concluded that the hydrogens of active methylene group are important for antioxidant activity [10]. Litwinienko and Ingold [11] demonstrated that active methylene group and phenolic groups are responsible as well.

Pyrimidinone derivatives are widely distributed in nature and exhibit various biological properties, such as antimalarial [12-14], antibacterial [15], antifunga [16], anti-HIV [17], antiviral [18], anticancer [19] and anti-inflammatory [20, 21] activities. Therefore, the corresponding dihydropyrimidinones (DHPMs) have exhibited important therapeutic and pharmacological properties, namely as the integral backbone of several calcium channel blockers [22], antihypertensive agents [23] and $\alpha 1\text{a}$ -antagonists [24]. A broad range of biological effects including

antiviral, antitumor, antibacterial, and anti-inflammatory activities have been described for these compounds [24-26]. Functionalized DHPMs have shown significant antibacterial [27] antiviral [28] and antitumor [29] activities. The synthesis of DHPMs has been the focus of great interest for organic and medicinal chemists [24, 27].

The development of dihydropyrimidinones/thiones scaffold as biologically active compounds contributed toward Biginelli cyclocondensation application in drug industry. The biological significance of curcumin and dihydropyrimidinones/thiones inspired us to synthesize these compounds and evaluated them as antioxidant and antibacterial agents. Earlier 3,4-dihydropyrimidin-2(*H*)-one/thione analogs of curcumin were reported and synthesized by $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ [30], chitosamine hydrochloride [31] and H_2SO_4 [32] as catalysts.

In continuation of our interest on the synthesis of heterocycles, using polyoxometallates as green catalysts [33-36], herein we describe a simple and efficient strategy for the synthesis of a series of 4-aryl-5-(4-Hydroxy-3-methoxyphenyl ethylene carbonyl)-6-(4-hydroxy-3-methoxyphenylethylene)-3,4-dihydropyrimidin-2(1*H*)-ones/thiones **4a-e** under mild conditions using curcumin as a key synthon. In the present study, commercial heteropolyacid Keggin type $\text{H}_3\text{PMo}_{12}\text{O}_{40}$ was used as catalyst to accomplish the synthesis by conventional method heating and under microwave irradiation, and the percentage yields of the compounds were compared with the reported one. Antioxidant and antibacterial studies were also performed.

2 Experimental

2.1. Materials and Methods

The chemicals reagents and solvents (Fluka products) used were of analytical grade and were used without further purification. Melting points were determined on a Stuart scientific SPM3 apparatus fitted with a microscope and are uncorrected. The infrared spectra were recorded in the region $4000\text{--}400\text{ cm}^{-1}$ on a BRUKER TENSOR 27 IR spectrophotometer without KBr. Electronic

spectra were measured on a JENWAY 6800 ultraviolet-visible spectrophotometer; measurements were made from 200 to 800 nm. The elemental microanalysis (C, H, N) was carried out by the Truspec 630-200-200 Elementary Analysis-Equipment, Service of Microanalysis, Department of Chemistry-University of Aveiro, Portugal. The needle voltage was set at 3000 V, with the ion source at 80 °C and desolvation temperature at 150 °C. Cone voltage was 35 V. Screening of the compounds for antimicrobial activity was done at the Pharmaceutics laboratory. The compounds were synthesized using adaptation of previous reports by J. Lal et al [30,31]. For synthesis under irradiations, the multimode microwave reactor (a modified microwave oven Candy mga20 m) used has a single magnetron (2450 MHz) with a maximum delivered power of 800 W. It was directly graduated in W (from 100 to 800 W). Experiments were carried out in a Pyrex reactor fitted with a condenser. During experiments, time, temperature and power were monitored. Temperature was monitored with the aid of an external infra-red IR thermometer (Flashpoint FZ400). The progress of the reactions was monitored throughout by TLC plates (silica gel G) using mobile phase, dichloromethane: methanol (5:1), and the spots were identified by iodine vapors or UV light.

2.2. General method for the synthesis of 3,4-dihydropyrimidin-2(H)-one/thione analogs of curcumin

A 50 ml round-bottom flask was charged with equimolar mixture of curcumin (0.002 mol; 0.736 g), substituted aldehydes (0.002 mol), and urea/thiourea (0.003 mol) dissolved in less ethanol (2 ml) and $\text{H}_3\text{PMo}_{12}\text{O}_{40}$ (5 mol %), and was refluxed for 6-9 h by conventional method / irradiated under microwaves for 150-210 s. The reaction was monitored throughout by TLC plates (silica gel G) using mobile phase, dichloromethane: methanol (5:1). After completion of reaction, the excess solvent (2/3rd) was removed, and the reaction mixture was poured into crushed ice. The precipitate was filtered washed with hot water, dried, and purified with diethyl ether. Finally the pure products of curcumin-3,4-dihydropyrimidin-2(1*H*)-one/thione derivatives (**4a–n**) were also identified by melting point measurement, UV-visible spectroscopic methods, FT-IR and elemental analysis, the

known compounds have been identified by comparison of IR spectral data and mp with those reported [30-32]. The general pattern of the reaction is depicted in Scheme 1.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (**4a**): This compound was obtained as dark red powder, mp 203–205 °C; UV/Vis: λ_{abs} (DMSO)/nm 272, 365; IR (cm^{-1}): ν 3506 (OH), 3364 (-NH), 2941 (-CH), 1596 (C=O), 1509 (C=C), 1269 (-C-O); Anal. Calcd. for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_6$: C, 69.87; H, 5.26; N, 5.62. Found: C, 69.80; H, 5.30; N, 5.56.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(4-N,N-dimethylphenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**4b**): This compound was obtained as Dark green crystals, mp 194–196 °C; UV/Vis: λ_{abs} (DMSO)/nm 270, 347, 426; IR (cm^{-1}): ν 3494 (OH), 3405 (-N-H), 2934 (-CH), 1587 (C=O), 1507 (C=C), 1261 (-C-O); Anal. Calcd. for $\text{C}_{31}\text{H}_{31}\text{N}_3\text{O}_6$: C, 68.75; H, 5.77; N, 7.76. Found: C, 68.81; H, 5.80; N, 7.67.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(4-chlorophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**4c**): This compound was obtained as Dark red powder, mp 198-200 °C; UV/Vis: λ_{abs} (DMSO)/nm 270, 369, 428; IR (cm^{-1}): ν 3471 (OH), 3368 (-NH), 2940 (-CH), 1583 (C=O), 1507 (C=C), 1265 (-C-O), Anal. Calcd. for $\text{C}_{29}\text{H}_{25}\text{ClN}_2\text{O}_6$: C, 65.35; H, 4.73; N, 5.26. Found: C, 65.31; H, 4.80; N, 5.30.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(2-hydroxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**4d**): This compound was obtained as Brick red crystals, mp 149-150 °C; UV/Vis: λ_{abs} (DMSO)/nm 271, 363, 427; IR (cm^{-1}): ν 3462 (OH), 3359 (-NH), 2961 (-CH), 1587 (C=O), 1507 (C=C), 1261 (-C-O), Anal. Calcd. for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_7$: C, 67.70; H, 5.09; N, 5.44. Found: C, 67.65; H, 5.23; N, 5.36.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(4-hydroxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**4e**): This compound was obtained as Dark green

crystals, mp 150–152 °C; UV/Vis: λ_{abs} (DMSO)/nm 278, 349, 428 ; IR (cm⁻¹): ν 3488 (OH), 3358 (-N-H), 2940 (-CH), 1587 (C=O), 1507 (C=C), 1268 (-C-O), Anal. Calcd. for C₂₉H₂₆N₂O₇: C, 67.70; H, 5.09; N, 5.44. Found: C, 67.55; H, 5.19; N, 5.50.

5-(4-Hydroxy-3-methoxyphenylethylene carbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(3,4-dihydroxyphenyl)-3,4-dihydropyrimidin-2(1H)-one (4f): This compound was obtained as dark green crystals, mp 220-222 °C; UV/Vis: λ_{abs} (DMSO)/nm 270, 363, 429; IR (cm⁻¹): ν 3465(OH), 3358(-NH), 2934(-CH), 1573 (C=O), 1500 (C=C), 1268 (-C-O); Anal. Calcd. for C₂₉H₂₆N₂O₈: C, 65.65; H, 4.94; N, 5.28. Found: C, 65.70; H, 5.03; N, 5.32.

5-(4-Hydroxy-3-methoxyphenylethylene carbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one (4g): This compound was obtained as brown crystals, mp 157–159 °C; UV/Vis: λ_{abs} (DMSO)/nm 272, 368, 436; IR (cm⁻¹): ν 3664 (OH), 3365(-N-H), 2980 (-CH), 1587(C=O), 1513 (C=C), 1255 (-C-O); Anal. Calcd. for C₂₉H₂₅N₃O₈: C, 64.08; H, 4.64; N, 7.73. Found: C, 64.14; H, 4.70; N, 7.46.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-phenyl-3,4-dihydropyrimidin-2(1H)-thione (4h): This compound was obtained as Dark brown crystals, mp 214-216 °C; UV/Vis: λ_{abs} (DMSO)/nm 272, 365; IR (cm⁻¹): ν 3517 (OH), 3345 (N-H), 2934 (-CH), 1579 (C=O), 1513 (C=C), 1268 (-C-O); Anal. Calcd. for C₂₉H₂₆N₂O₅S: C, 67.69; H, 5.09; N, 5.44. Found: C, 67.45; H, 4.93; N, 5.26.

5-(4-Hydroxy-3-methoxyphenylethylene carbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(4-N,N-dimethylphenyl)-3,4-dihydropyrimidin-2(1H)-thione (4i): This compound was obtained as Dark green crystals, mp 195-197 °C; UV/Vis: λ_{abs} (DMSO)/nm 271, 348, 398; IR (cm⁻¹): ν 3611 (OH), 3345 (-N-H), 2940 (-C-H), 1587 (C=O), 1507 (C=C), 1261 (-C-O); Anal. Calcd. for C₃₁H₃₁N₃O₅S: C, 66.77; H, 5.60; N, 7.54. Found: C, 66.45; H, 5.93; N, 5.46.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(4-chlorophenyl)-3,4-dihydropyrimidin-2(1H)-thione (4j): This compound was obtained as dark brown powder, mp 198-200 °C; UV/Vis: λ_{abs} (DMSO)/nm 272, 370; IR (cm⁻¹): ν 3504 (OH), 3352 (-N-H), 2927 (-CH), 1579 (C=O), 1507 (C=C), 1261 (-C-O); Anal. Calcd. for C₂₉H₂₅ClN₂O₅S: C, 63.44; H, 4.59; N, 5.10. Found: C, 64.05; H, 4.83; N, 5.26.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(2-hydroxyphenyl)-3,4-dihydropyrimidin-2(1H)-thione (4k): This compound was obtained as Dark green crystals, mp 260-262 °C; UV/Vis: λ_{abs} (DMSO)/nm 272, 374, 428; IR (cm⁻¹): ν 3604 (OH), 3339 (-NH), 2940 (-CH), 1579 (C=O), 1513 (C=C), 1261 (-C-O); Anal. Calcd. for C₂₉H₂₆N₂O₆S: C, 65.65; H, 4.94; N, 5.28. Found: C, 65.49; H, 4.93; N, 5.26.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(4-hydroxyphenyl)-3,4-dihydropyrimidin-2(1H)-thione (4l): This compound was obtained as dark brown powder, mp 261-262 °C; UV/Vis: λ_{abs} (DMSO)/nm 277, 370; IR (cm⁻¹): ν 3506 (OH), 3372 (-NH), 1579 (C=O), 1507 (C=C), 1261 (-C-O); Anal. Calcd. for C₂₉H₂₆N₂O₆S: C, 65.65; H, 4.94; N, 5.28. Found: C, 65.45; H, 4.99; N, 5.42.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(3,4-dihydroxyphenyl)-3,4-dihydropyrimidin-2(1H)-thione (4m): This compound was obtained as Dark green crystals, mp 212–214 °C; UV/Vis: λ_{abs} (DMSO)/nm 269, 379; IR (cm⁻¹): ν 3505(OH), 3332 (-NH), 1573 (C=O), 1513 (C=C), 1268 (-C-O); Anal. Calcd. for C₂₉H₂₆N₂O₇S: C, 63.72; H, 4.79; N, 5.13. Found: C, 63.45; H, 4.93; N, 5.26.

5-(4-Hydroxy-3-methoxyphenylethylenecarbonyl)-6-(4-hydroxy,3-methoxyphenylethylene)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-thione (4n): This compound was obtained as Dark green crystals, mp 206–208 °C; UV/Vis: λ_{abs} (DMSO)/nm 272, 373; IR (cm⁻¹): ν 3664 (OH), 3339 (NH),

2974 (-CH), 1573 (C=O), 1507 (C=C), 1255(-C-O); Anal. Calcd. for C₂₉H₂₅N₃O₇S: C, 62.24; H, 4.50; N, 7.51. Found: C, 62.19; H, 4.63; N, 7.36.

2.3. Screening for Antibacterial Activity

The antimicrobial activities of compounds **4a-n** were evaluated for their antibacterial activities against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) bacterial strains by the agar diffusion method [37]. A sterile physiological water solution contained a bacterial colonies, was prepared at room temperature, with an optical density of 0.08–0.10 corresponding to a concentration of 10⁶ cells/mL. The bacterial solution was inoculated in the Muller-Hinton agar medium by swabbing using Petri dishes at room temperature. The tested compounds were dissolved in dimethylsulfoxide (DMSO) with a concentration of 10⁻¹ M. Twenty-five microliters of tested sample were poured onto filter paper discs 6 mm in diameter, which were then delicately placed on the surface of the agar plates. These were later maintained at 37 °C for 24 h. Activities were determined by measuring the diameter of the inhibition zone (mm).

2.4. Antioxidant activity

The antioxidant activity (free radical scavenging activity) of the synthesized compounds and curcumin was evaluated by the first time using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH.) scavenging assay [38,39]. DPPH. solution was prepared by dissolving DPPH in ethanol to give a concentration of 4 mg 100 mL⁻¹. Compounds **4a-n** and curcumin were dissolved in DMSO to obtain a solution of 10⁻¹ M. The concentration of test compounds was diluted with DMSO to get final concentrations 0.05, 0.025 et 0.0125 mol/L for all the compounds, but the standard were further diluted to give concentration solutions of 0.1, 0.05, 0.025 et 0.0125, 0.00625, 0.003125, 0.0015625 mol/l. 40 µL of each tested concentration of each compound was added to each well separately in duplicate, and then 2 mL of DPPH. solution was added. Negative control wells were loaded with 40

μ L of DMSO and 2 mL of DPPH solution each. After vortexing, the mixtures were incubated at room temperature for 1 h in darkness at 25 °C, and then the absorbance of these compounds at different concentrations was recorded at 517 nm. Ascorbic acid (AA) was used as standard for the antioxidant activity screening. A blank containing only ethanol with DMSO was used as the control. Each measurement was performed in triplicate. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as percentage of DPPH discoloration using the equation:

Equation 1

Where A_c is the absorbance of the control (absorbance of DPPH. ethanol solution without sample), and A_s is the absorbance of the tested compound after 60 min incubation.

2.5. DFT analysis

Synthesized compounds were optimized by density functional theory (DFT) employing Becke's three-parameter hybrid model, Lee–Yang–Parr (B3LYP) correlation functional method at 6-31G* level with tight SCF convergence criteria. Geometries optimization was followed by frequency calculations at the same level of theory using ORCA v4 software [40]. In order to study the reactivity of studied compounds, the optimized structures were used to calculate some DFT-based global reactivity descriptors, such as Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital energy (LUMO). The energy associated with HOMO and LUMO were used to calculate the chemical potential (μ) and hardness (η) of system using the equations (2) and (3) below:

Equation 2

Equation 3

3. Results and discussion

3.1. Chemistry

Curcumin was used as the starting material for the preparation of all compounds described in this paper (Scheme 1). A mixture of curcumin (1eq), substituted aromatic aldehydes (1eq), and urea/thiourea (1.5eq) in minimum of ethanol (2mL) using commercial heteropolyacide Keggin type H₃PMo₁₂O₄₀ as a recyclable and nontoxic acid catalyst under conventional reflux and microwave irradiation through an improved procedure to obtain 3,4-dihydropyrimidin-2(1H)-one/thione analogs of curcumin (4a-n). The reaction was monitored throughout by TLC plates (silica gel G) using mobile phase, dichloromethane: methanol (5:1).

In the initial experiments, in order to evaluate the catalytic efficiency of H₃PMo₁₂O₄₀ catalyst in the three component reaction, curcumin, benzaldehyde and urea was selected as the model reaction. It showed that only 5% mol of catalyst was sufficient to promote the reaction. Lower amounts gave a low yield even after long reaction time, and higher amounts did not improve the efficiency of this transformation. Color and physical appearance are given in table 1. Analytical and physicochemical data of synthesized Curcum-DHPMs using H₃PMo₁₂O₄₀ have been given in Table 2.

After optimization of the conditions, in order to investigate the scope of this approach, we carried out the three component cyclocondensation reaction of curcumin and urea/thiourea with a series of aromatic aldehydes under similar conditions. The reactions were completed after 6–9 h by conventional reflux and 2-3 mn under MW irradiation, affording corresponding Curcum-DHPMs in good to excellent yields. The structure of these compounds was confirmed by elemental analysis and by comparing the melting points and the spectroscopic data of FT-IR and UV-Visible with those reported in the literature.

Excellent yields are obtained with the two methods of heating; they are ranging from 80 to 98%. Earlier three different catalysts were used to accomplish the synthesis using conventional method, (SnCl₂.2H₂O; yield 92-97 %) [30], microwave assisted synthesis (chitosamine hydrochloride; yield 90-96 %) [31] and (H₂SO₄, yield 71-86%) [32]. When the same reaction carried out with

conventional method and under microwave irradiation in the presence of H₃PMo₁₂O₄₀, the yield of the compounds was ranging between 80 and 98%. The use of H₃PMo₁₂O₄₀ as catalyst in these particular reaction was comparatively more efficient than the use of H₂SO₄ and by the two heating modes, while with the use of catalyst SnCl₂.2H₂O and chitosamine hydrochloride the yields are always high.

Scheme 1

The reaction mechanism is shown in Scheme 2. In order to explain the formation of the DHPMs/thiones, we have calculated the charges of the electrophilic and nucleophilic sites of the three reagents via theoretical calculations DFT B3LYP/6-31G* using the ORCA software method [40] to identify the entities that react first and deduced the most likely intermediate (Fig. 1 reports the charges of each atom that participate in the reaction). Calculations show that the carbonyl carbon of curcumin 1 are more positive than the carbon of the aldehyde function, suggesting that the reactivity of urea 2 with curcumin 1 is the first step of the reaction, then intermediate 5 reacts with the aldehydes 1 to give 3,4-dihydropyrimidinones 4a–n (Scheme 2).

Table 1

Figure 1

Scheme 2

3.2. Spectroscopic study, Effect of the substituent on the Uv-visible spectrum

The UV-vis absorption spectra of compounds 4 and curcumin (concentration 10⁻⁵ M) were recorded immediately after products dissolution in DMSO. The absorption spectrum for the compounds 4 displays a weak band at 270-272 nm attributed to the n- π^* transition and two other broad band appears as shoulder in all cases at 331-373 nm and 398-429 nm respectively [Figure 2], attributed to the π - π^* transition related with the carbonyl groups. The bands are completely different of curcumin absorption spectrum which appears at 437 nm. These bands can be attributed to the charge transfer

between the benzo ring's and the electron withdrawing pole, constituted by the carbonyl groups that reinforce the electron attracting strength on this side of the molecules [figure 3]. In addition, the wavelength and the intensity of the absorption spectra were affected when changing the aryl substituent donor or acceptor in positions 2, 3 and 4 of the aryl ring as well as positional isomerism of the hydroxy group (compounds **4d**, **4e**, **4f** and **4k** (in dihydropyrimidinethione)). The nature and position of the substituent affects the absorbance much more than the absorption wavelength. Compounds with hydroxyl substituent show the highest intensity (**4d**, **4e** and **4k**) and -OH in position two of the benzo ring (**4d** and **4k**) presents highest wavelength (extended electron cloud). Against the compound **4f** with electron-withdrawing substituent shows the lowest intensity.

Table 2

Table 3

3.2. Antioxidant activity

The antioxidant activity of curcumin and its derivatives synthesised curcum-DHPMs 4a-n was measured in terms of their hydrogen donating or radical scavenging ability by UV–Vis spectrophotometer using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH.). DPPH radicals are stable free radicals and in the presence of molecules capable of donating H atoms, its radical character is neutralized. This is visually noticeable as the color changes from purple to yellow. When a compound is antioxidant donate protons to this radical, the initial absorbance of DPPH solution decreases. Fig. 4a-b shows the variation of absorbance versus concentration of the different compounds 4a-n and curcumin (a) and of the standard ascorbic acid (b). Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH. (or inhibition %) was calculated using equation (1).

All the compounds exhibited high scavenging activity. It is very interesting to note that RSA activity of all the synthesized compounds 4a-n is greater than curcumin (standard), the compounds 4f and 4m

showed greater antioxidant activity than ascorbic acid. The results of the antioxidant activity data are in accordance with theoretical expectations, because the number and position of the hydroxyl groups as well as the degree of conjugation of the whole molecule are important. The antioxidant potency of flavonoids of similar conjugation level is roughly proportional to the total number of -OH groups and is positively affected by the presence of o-dihydroxy moiety in the benzene ring [41].

The DPPH assay measures the ability of the sample to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity, and this was reflected in a lower IC₅₀ value. Figure 5 show the values of minimum inhibitory concentrations IC₅₀ of the tested compounds, being the most significant those of 4f and 4m as the best antioxidant agent.

Figure 2

Figure 3

3.2. Antibacterial activity

The newly synthesized curcumin 3,4-dihydropyrimidinones (4a–n) were evaluated for their in vitro antibacterial activity against three bacteria specially causing secondary infections in human being viz. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Zone of inhibition in mm was measured using disc diffusion method (Table 4). The test samples were dissolved in DMSO at a concentration of 10⁻¹ M and the antibacterial activity of curcumin-dihydropyrimidinone/thione analogues were compared with the positive control (as standard antibiotic reference drug) chosen according to the nature of the bacterial strain (Tables 4). In case of *Staphylococcus aureus* and *Escherichia coli* compounds 4a–n does not show any zone of inhibition and was completely inactive. In case of *Pseudomonas aeruginosa*; compounds 4g and 4n (with electron-withdrawing group on the benzo ring) were totally inactive whereas compounds 4a–f and 4h–m, showed much antibacterial

activity with better zone of inhibition as comparison to remaining compounds, but were less active than the standards.

The sensitivity to the different products is classified according to the diameter of the zones of inhibition as follows: Not sensitive (-) for diameter less than 8mm, Sensitive (+) for a diameter between 9 and 14 mm, highly sensitive (+ +) for a diameter between 15 and 19 mm and extremely sensitive (+ + +) for a diameter greater than 20 mm [42]. The results obtained are given in Table 4 and illustrated in Figure 6.

Figure 4

Figure 5

Table 4

Figure 6

3.3. DFT analysis

The DFT analysis was performed to gain a more insight into the electronic properties of synthesized compounds. According to the frequency calculations results of the optimized geometries, absence of imaginary frequency indicated that the structures were in stationary point. In addition, frontier molecular orbitals HOMO and LUMO play an important role to illustrate the chemical reactivity. The HOMO is considered as electron donor, because it is the outer orbital containing electrons whereas the LUMO can accept electrons [43]. Hence, the LUMO energy is directly related to electron affinity ($A = -E_{\text{LUMO}}$) and ionization potential ($I = -E_{\text{HOMO}}$) is directly related to the energy of the HOMO [44].

The value of HOMO, LUMO, chemical potential (μ) and chemical hardness (η) for various compounds are given in Table 5. It is worth noting that, large HOMO - LUMO gap or when hardness increases the reactivity should decrease and small HOMO - LUMO gap or when hardness decreases the reactivity should increase. According to the calculated chemical hardness (η) of synthesized

compounds (equation 3), $\eta(4m) = \eta(4f) < \eta(4h)$ which indicate that **4f** and **4m** are more reactive than **4h**.

Table 5

On the other hand, the average values of the HOMO and LUMO energies have been defined as chemical potential (μ), equation 2. The negative of the chemical potential was known as the electronegativity ($\chi = -I$). The electronegativity represents the power of molecule to attract the electrons. According to values reported in table 5, the electronegativity value of **4h** is higher than those of **4f** and **4m**.

The visualization of HOMO and LUMO of the most potent compounds (**4f** and **4m**) and the least active compound (**4h**) are displayed in Figure 7. The positive and negative phases of molecular orbitals are represented in red and green color, respectively. The HOMO of the compound **4f** is localized on the substituted phenyl ring at position 4 of the dihydropyrimidinone ring whereas the LUMO is mainly focused on styryl and acryloyl groups.

Figure 7

HOMO and LUMO of the compound **4m** are localized in the same sites as in the compound **4f**. In the case of the least active compound **4h**, the HOMO is mainly localized on sulfur atom and double bond of dihydropyrimidinone ring and along the 4-hydroxystyryl group whereas, LUMO is displayed on the styryl and acryloyl groups. It was interesting to observe that LUMO was never located on the phenyl at position 4 of the dihydropyrimidinone ring which mean that this group have a less likely chance to attract electrons.

4. Conclusion

A series of 3,4-Dihydropyrimidin-2(1H)-one/thione analogs of curcumin were synthesized in good yield using simple, efficient, and improved Bignelli reaction by a one-pot multicomponent cyclocondensation using curcumin, substituted aromatic aldehydes, and urea/thiourea in ethanol and

H3PMo12O40 Keggin type heteropolyacid as catalyst. All the compounds were evaluated for antioxidant and antibacterial activity and showed excellent antioxidant activity but moderate antibacterial activity.

Curcumin-3,4-dihydropyrimidinones/thiones derived from 2-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde showed a high intensity in UV-visible absorption spectrophotometry but the wavelengths are less affected by the nature of substituents of the benzaldehyde. The compound derived from 3,4-dihydroxy benzaldehyde and urea showed maximum antioxidant activity, while antioxidant activity decreases in compounds derived from 2-hydroxybenzaldehyde, 2-hydroxybenzaldehyde, and others.

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Scheme 1. Synthesis of 3,4-dihydropyrimidinones:thiones of curcumin under conventional reflux and MW irradiation in less of ethanol conditions

Scheme 2 Proposed mechanism for the synthesis of 3,4-dihydropyrimidin-2(H)-one/thione analogs of curcumin catalysed by $\text{H}_3\text{PMo}_{12}\text{O}_{40}$

Benzaldehyde 3

Urea 2

Curcumin 1

Figure 1. Partial charges of curcumin, benzaldehyde and urea, in ethanol using the ORCA software method

Fig.2. Absorption spectra of compounds **4** and curcumin in DMSO

Figure 3. Possible resonance structures of compounds **4a-n**

(a)

(b)

Figure 4 Absorbance at 517 nm vs concentration of **4a-n** and curcumin (a) and of the standard ascorbic acid (b).

Figure 5. IC₅₀ values of the antioxidant compounds tested

Figure 6 Antibacterial activity of compounds **4a-n** and the reference antibiotics

Figure 7: HOMO, LUMO surfaces of compounds **4f** (A,B), **4m** (C,D) and **4h** (E,F).

Equation 1

$$\text{RSA (\%)} = [(\text{Ac} - \text{As})/\text{Ac}] \times 100 \quad \text{Eq (1)}$$

Equation 2

$$\mu = \frac{E_{\text{LUMO}} + E_{\text{HOMO}}}{2} \quad \text{Eq (2)}$$

Equation 3

$$\eta = \frac{E_{\text{LUMO}} - E_{\text{HOMO}}}{2} \quad \text{Eq (3)}$$

Table 1. 3,4-dihydropyrimidinones of curcumin **4a-n** synthesized under conventional reflux and MW irradiation in less of ethanol conditions using H₃PMo₁₂O₄₀.

Entry	Compound	R	X	Color and physical appearance
1	4a	H	O	Dark red powder
2	4b	4-N(CH ₃) ₂	O	Dark green crystals
3	4c	4-Cl	O	Dark red powder
4	4d	2-OH	O	Brick red crystals
5	4e	4-OH	O	Dark green crystals
6	4f	3,4-OH	O	Dark green crystals
7	4g	3-NO ₂	O	Brown crystals
8	4h	H	S	Dark brown crystals
9	4i	4-N(CH ₃) ₂	S	Dark green crystals
10	4j	4-Cl	S	Dark brown powder
11	4k	2-OH	S	Dark green crystals
12	4l	4-OH	S	Dark brown powder
13	4m	3,4-OH	S	Dark green crystals
14	4n	3-NO ₂	S	Dark green crystals

Table 2 Analytical and physicochemical data of synthesized Curcum-DHPMs/thiones 4a-n using $\text{H}_3\text{PMo}_{12}\text{O}_{40}$ as catalyst in 2 mL of ethanol

Compound	Time	Yield %						Mp °C	
		Found				Reported		Found	Reported
		Conv	M.W	Conv	M.W	H_2SO_4	SnCl_2	Chitosamine	
	(h)	(s)			[32]	[30]	hydrochloride	[31]	
4a	9	150	90	97	79	97	91	203-205	206-207 [31,32]
4b	9	180	80	93	-	-	-	194-196	-
4c	6	150	79	96	82	94	-	198-200	201-203 [31]
4d	9	150	83	97	-	96	93	149-150	150-152 [32]
4e	9	150	84	96	74	95	92	150-152	151-152 [32]
4f	9	150	81	96	-	-	-	220-222	-
4g	9	210	88	98	-	-	-	157-159	-
4h	9	150	85	95	73	95	91	214-216	217-219 [32]
4i	9	150	98	97	-	-	-	195-197	-
4j	6	150	80	96	76	96	-	198-200	202-204 [31]
4k	9	150	98	94	-	-	95	260-262	262-263 [32]

4l	9	150	93	95	71	93	93	261-262	261-263 [32]
4m	9	150	92	98	-	-	-	212-214	-
4n	9	210	95	97	-	-	-	206-208	-

Table 3 UV-Visible spectroscopic characteristics of compounds **4a-n** and curcumin in DMSO

Compound	λ abs (nm)	Compound	λ abs (nm)
Curcumin	437 π - π^*	4g	272 π - π^*
4a	272 π - π^*		368 π - π^*
	365 π - π^*		436 π - π^*
4b	270 π - π^*	4h	272 π - π^*
	347 π - π^*		365 π - π^*
	426 π - π^*	4i	271 π - π^*
4c	270 π - π^*		348 π - π^*
	369 π - π^*		398 π - π^*
	428 π - π^*	4j	272 π - π^*
4d	271 π - π^*		370 π - π^*
	363 π - π^*	4k	272 π - π^*

	427 π - π^*		374 π - π^*
4e	278 π - π^*		428 π - π^*
	349 π - π^*	4l	277 π - π^*
	428 π - π^*		370 π - π^*
4f	270 π - π^*	4m	269 π - π^*
	363 π - π^*		379 π - π^*
	429 π - π^*	4n	272 π - π^*
			331 π - π^*
			373 π - π^*

Table 4. Diameters of inhibition zones (in mm) for the compounds **4a-n** and the references antibiotics

Bacterial	Diameters of inhibition zones (in mm)														ATB
(Ref ATB)	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	
<i>S. aureus</i>	6	6	6	6	6	6	6	6	6	6	6	6	6	6	20
(Rif5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++
<i>E. coli</i>	6	6	6	6	6	6	6	6	6	6	6	6	6	6	25
(Cot 25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++

<i>P. Aeruginosa</i>	12	12	12	13	10	11	6	11	11	14	13	11	13	6	30
(CL 25)	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+++

ATB : antibiotic ; CL25: Colistine ; Cot25 : Cotrimoxazole ; Rif5 : Rifampicine

Table 5: Calculated DFT descriptors in (eV) for the synthesized compounds

Compound	HOMO(eV)	LUMO(eV)	μ	η
4a	-5,41	-2,16	-3,79	1,62
4b	-4,99	-2,05	-3,52	1,47
4c	-5,50	-2,25	-3,88	1,62
4d	-5,37	-2,03	-3,70	1,67
4e	-5,35	-2,15	-3,75	1,60
4f	-5,02	-2,19	-3,61	1,42
4g	-5,57	-2,33	-3,95	1,62
4h	-5,46	-2,32	-3,89	1,57
4i	-5,09	-2,20	-3,65	1,44
4j	-5,55	-2,41	-3,98	1,57
4k	-5,33	-2,19	-3,76	1,57
4l	-5,39	-2,30	-3,84	1,55
4m	-5,19	-2,34	-3,76	1,42
4n	-5,64	-2,48	-4,06	1,58