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SYNTHESIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF 5-((2-OXO-2*H*-CHROMEN-7-YLOXY)METHYL)-1,3,4-THIADIAZOL-2(3*H*)-ONE DERIVED FROM UMBELLIFERONE

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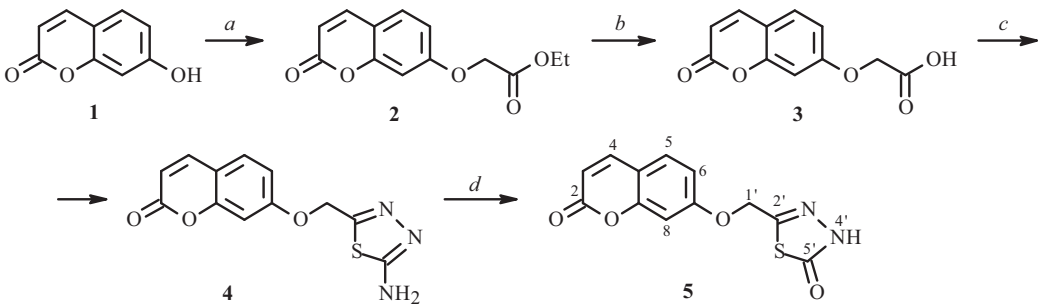
*5-((2-Oxo-2*H*-chromen-7-yloxy)methyl)-1,3,4-thiadiazol-2(3*H*)-one was synthesized and characterized by FT-IR and NMR spectra in addition to elemental analysis. The prepared compound shows considerable antibacterial and antifungal activity. The free radical scavenging activity of the synthesized compound was screened for* in vitro *antioxidant activity.*

Keywords: antimicrobial, antioxidant, 1,3,4-thiadiazole, thiosemicarbazide, umbelliferone.

The rapid evolution of bacterial and fungal resistance has led to increasing levels of resistance to classical antibiotics [1–3]. Coumarins are very important oxygen-containing heterocycles with diverse biological activity as anticoagulants and antithrombotics that are of both natural and synthetic origin [4, 5]. Some coumarin derivatives possess antimicrobial [6, 7], antitumor [8], antiviral [9], and other activities [10]. A systematic investigation of this class of compounds revealed that coumarin derivatives containing pharmacophore agents play an important role in medicinal chemistry [11]. In continuation of previous work [12–18], we focused on the synthesis of new heterocyclic compounds and here report the synthesis of 5-((2-oxo-2*H*-chromen-7-yloxy)methyl)-1,3,4-thiadiazol-2(3*H*)-one (**5**).

The preparation ethyl 2-(2-oxo-2*H*-chromen-7-yloxy)acetate (**2**) was used as a starting material for the synthesis of compounds **4** and **5** presented in this study. The chemical structures of the synthesized compounds **2–5** were confirmed. The *in vitro* antioxidant and antimicrobial activities were investigated for the novel synthesized compound **5**.

Ethyl 2-(2-oxo-2*H*-chromen-7-yloxy)acetate (**2**) was synthesized by reaction of ethyl bromoacetate with umbelliferone (7-hydroxycoumarin) (**1**) in the presence of potassium carbonate. Compound **3** was obtained by hydrolysis of compound **2** using sodium hydroxide [19]. Compound **4** was synthesized by cyclization of thiosemicarbazide (TSC) with the carboxyl group of compound **3** in the presence of phosphorus oxychloride ($POCl_3$). The IR spectrum shows evidence of the formation of compound **4**, with the absence of a hydroxyl group at 2975–3170 cm^{-1}, and the appearance of new bands at 3302 and 3343 cm^{-1} for the amino group; the lactone carbonyl stretching frequency was observed at 1747 cm^{-1}.



a. ethyl bromoacetate; *b*. NaOH; *c*. TSC/$POCl_3$; *d*. HNO_2

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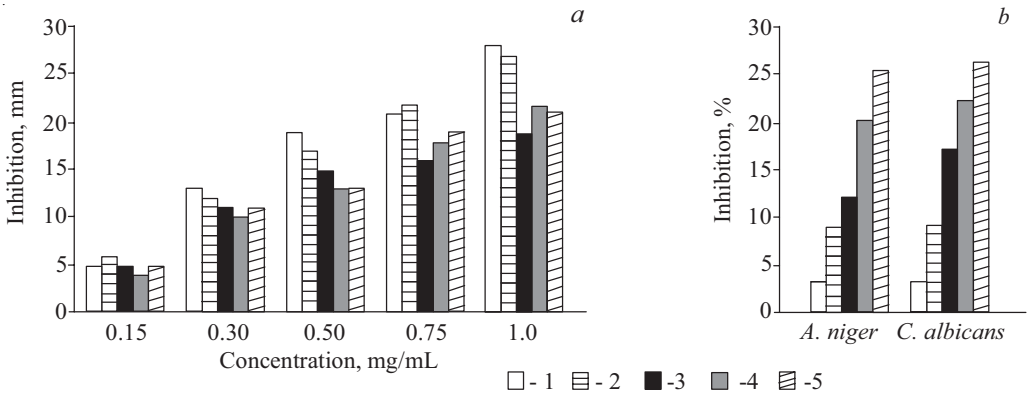

Fig. 1. The effect of test organisms (*a*) and fungi (*b*). *a*: *Staphylococcus aureus* (1), *Escherichia coli* (2), *Proteus vulgaris* (3), *Pseudomonas aeruginosa* (4), *Klebsiella pneumoniae* (5). *b*: 0.15 (1), 0.30 (2), 0.5 (3), 0.75 (4), 1.0 (5) (mg/mL)

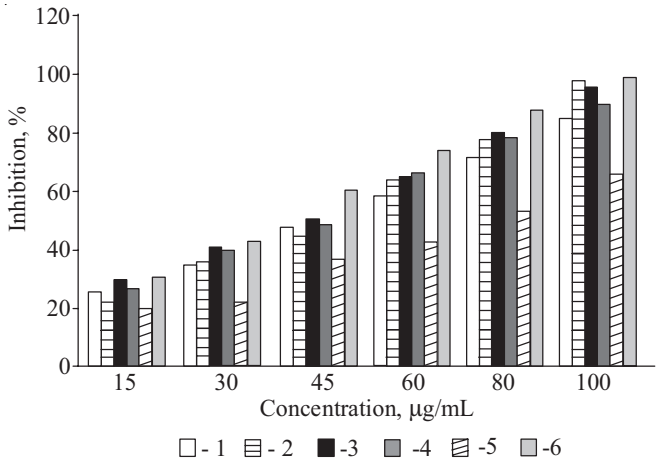

Fig. 2. The effect of compound **5** toward DPPH, nitric oxide, and hydrogen: A.A./DPPH (1), S.C./DPPH (2), A.A./nitric oxide (3), S.C./nitric oxide (4), A.A./hydrogen peroxide (5), S.C./hydrogen peroxide (6). A.A. – ascorbic acid, S.C. – synthesized compound **5**.

The ^{1}H NMR spectrum shows signals at δ 4.94 (1H, s, NH_2), 5.760, and 5.891 (1H, s, -C=C-H). In the ^{13}C NMR, 163.5 and 176.4 ppm were new and attributable to the carbon of the heterocyclic ring. The target compound **5** was synthesized by the addition of nitrous acid (HNO_2) to compound **4**, and the product was subsequently washed with water.

Although two types of tautomers, ketone or enol, might be expected from the reaction of compound **4** with nitrous acid under acidic conditions, only the ketone-type compound **5** was observed. The existence of the ketone form predominantly in the solid state is demonstrated by the presence of two absorption bands at 1713 cm^{-1} and 3297 cm^{-1}, belonging to the $\nu_{C=O}$ and ν_{NH} groups, respectively, and by the absence of ν_{OH}.

Antimicrobial Activities. *In vitro* antimicrobial screening effects of compound **5** were tested against various bacterial (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and fungal species (*Aspergillus niger* and *Candida albicans*). As shown in Fig. 1, increased inhibition of microbial growth is due to uncoordinated heteroatoms and the azomethine (>C=N) group. The mode of action of compound **5** may involve formation of a hydrogen bond with the active centers of the cell constituents [20, 21] through the azomethine group (>C=N-), resulting in interference with normal cell processes.

The results of antibacterial activity for 5-((2-oxo-2*H*-chromen-7-yloxy)methyl)-1,3,4-thiadiazol-2(3*H*)-one (**5**) indicated that it exhibited antibacterial activity against the studied bacteria at both low and high concentrations. The increased activity of compound **5** can be explained by electron delocalization over the entire molecule (Fig. 1a).

In vitro antifungal screening effects of the investigated compound were tested against specific fungal species (*Aspergillus niger* and *Candida albicans)*. We found that compound **5** exhibits antifungal activity against *Aspergillus niger* and *Candida albicans* (Fig. 1b).

Antioxidant Activity. Most antioxidant compounds in a typical diet are derived from plant sources and belong to classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as monophenols, are only weak antioxidants [22]. The role of antioxidants is to remove free radicals. One important mechanism by which this is achieved is the donation of hydrogen to free radicals to reduce them to nonreactive species. Addition of hydrogen removes the unpaired electron that is responsible for radical reactivity. Free radicals have been a subject of significant interest among scientists for the past decade. Compound **5** has good antioxidant activity (Fig. 2) compared with the reference compound ascorbic acid.

EXPERIMENTAL

All chemicals used were of reagent grade (supplied by either Merck or Fluka) and used as supplied. The FTIR spectra were recorded as KBr discs on an FTIR 8300 Shimadzu spectrophotometer. The UV-visible spectra were measured using a Shimadzu UV-Vis 160 A spectrophotometer. Proton NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer. Elemental microanalysis was performed using the CHN elemental analyzer (model 5500) Carlo Erba instrument.

Synthesis of Ethyl 2-(2-Oxo-2*H*-chromen-7-yloxy)acetate (2). A suspension of umbelliferone (**1**) (6.17 mmol) in acetone (30 mL) was refluxed with ethyl bromoacetate (9.15 mmol) and K_2CO_3 (4.69 g, 33.91 mmol) for 12 h. After cooling, the mixture was evaporated to dryness, and the residue was partitioned between $CHCl_3$ (50 mL) and water (50 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was recrystallized from acetone [19]. Colorless needles (yield 76%); mp 112.5°C.

Synthesis of 2-(2-Oxo-2*H*-chromen-7-yloxy)acetic acid (3). A solution of compound **2** (2.7 mmol) and sodium hydroxide 5% (2.16 mL) in ethanol (15 mL) was stirred under reflux for 2 h. After removal of the solvent, the residue was dissolved in water and acidified with 6 M HCl. The white solid collected by filtration was washed with cold water, dried, and crystallized from ethanol [19]. White powder (yield 93%); mp 210.5°C.

Synthesis of 7-((5-Amino-1,3,4-thiadiazol-2-yl) methoxy)-2*H*-chromen-2-one (4). Phosphorus oxychloride (20 mL) was added to compound **3** (0.05 mol), and the mixture was stirred for 1 h at room temperature. Thiosemicarbazide (4.56 g, 0.05 mol) was added, and the mixture was heated under reflux for 5 h. Upon cooling, the mixture was poured onto ice. After 4 h, the mixture was stirred for 15 min to decompose the excess phosphorus oxychloride, heated under reflux for 30 min, cooled, and neutralized with 5% potassium hydroxide. The precipitate was filtered, washed with water, dried, and recrystallized from ethanol, yield 51%, mp 105°C. $C_{12}H_9N_3O_3S$. IR (ν, cm^{-1}): 3302 and 3343 (N-H, amine), 1747 (C=O, lactone). ^{1}H NMR ($CDCl_3$, δ, ppm): 4.94 (1H, s, NH_2), 5.760, 5.891 (each 1H, s, H-3, 4), 7.510–7.283 (m, C-H aromatic ring). ^{13}C NMR (DMSO-d_6, δ, ppm): 101.9, 111.0, 113.1, 114.1, 128.1, 149.5, 156.3, 160.8, 161.2, 163.5, 168.3, 176.4.

Synthesis of 5-((2-Oxo-2*H*-chromen-7-yloxy)methyl)-1,3,4-thiadiazol-2(3*H*)-one (5). A 10% aqueous sodium nitrite solution (10 mL) was added dropwise with continuous stirring over a period of 20 min to a cooled (ice-bath) suspension of compound **4** (0.01 mol) and hydrochloric acid (5 mL) in cold water (20 mL). The mixture was allowed to rise to room temperature, heated to boiling for 10 min, cooled, and allowed to stand overnight. The separated crude product was filtered, washed with water, dried, and recrystallised from ethanol, yield 47%, mp 133°C. $C_{12}H_8N_2O_4S$. IR (ν, cm^{-1}): 3397 (N-H, amine), 1713 (C=O, lactone). ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 7.81 (1H, d, J = 2.2, H-8), 7.51 (1H, d, J = 8.7, H-5), 7.29 (1H, dd, J = 8.5, 2.2, H-6), 6.43 (1H, s, H-4), 5.81 (1H, s, H-3), 5.31 (1H, br.s, H-4′), 5.21 (1H, d, J = 7.5, H-1′), 4.61 (1H, d, J = 2.1, H-1′). ^{13}C NMR (δ, ppm, J/Hz): 161.1 (d, J = 256, C-2), 115.1 (d, J = 3, C-3), 155.9 (s, C-4), 123.2 (s, C-5), 117.5 (s, C-6), 166.2 (s, C-7), 103.2 (C-8), 117.9 (s, C-1′), 155.3 (d, J = 21, C-2′), 171.2 (s, C-5′).

Evaluation of Antibacterial Activities. The *in vitro* antibacterial effects of the synthesised compound **5** were evaluated against one species of Gram-positive bacteria (*Staphylococcus aureus*) and four Gram-negative bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Proteus vulgaris*) using the disc diffusion method [23, 24] on nutrient agar medium. The bacteria were subcultured in the agar medium and incubated for 24 h at 37°C. The 5 mm discs were soaked in test solutions (sterile filter paper discs, Whatman No. 1.0) with the appropriate amount of compound **5** dissolved in sterile dimethylsulfoxide (DMSO) at concentrations of 0.1–1.0 mg $disc^{-1}$, placed on an appropriate medium previously seeded with organisms in Petri dishes, and stored in an incubator for the above-mentioned period of time. The inhibition zone around

each disc was measured, and the results recorded in the form of inhibition zones (diameter, mm). To clarify the effect of DMSO on biological screening, parallel studies were conducted using DMSO as control, and no activity against the bacterial strains was shown.

Evaluation of Antifungal Assay. Antifungal activity [25] was examined using the determined growth inhibition rates of the mycelia of two strains (*Aspergillus niger* and *Candida albicans*) in potato dextrose broth medium (PDB). Under aseptic conditions, one milliliter of spore suspension (5×10^6 cfu/mL) of the tested fungi was added to 50 mL of PDB medium in a 100 mL Erlenmeyer flask. Appropriate volumes of compound **5** were added to produce concentrations ranging from 0.1 to 1.0 μg mL^{-1}. The flasks were incubated at 27 ± 1°C in the dark for 5 days, and the mycelium was collected on filter paper. The filter paper was dried to constant weight, and the level of inhibition relative to the control flasks was calculated using the equation

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100 \text{ , (1)}$$

where T is weight of mycelium from test flasks, and C is weight of mycelium from control flasks.

(2,2-Diphenyl-1-picrylhydrazyl) (DPPH) Radical Scavenging Activity. The DPPH radical scavenging activity of compound **5** was evaluated according to Soares et al. [21]. Initially, 0.1 mL of the samples at concentrations of 250, 500, 750, and 1000 μg/mL was mixed with 1 mL of 0.2 mM DPPH dissolved in methanol. The reaction mixture was incubated in the dark for 20 min at 28°C. The control contained all reagents without the sample, while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using the UV-VIS spectrophotometer. The DPPH radical scavenging activity of ascorbic acid was also assayed for comparison. The percentage of DPPH radical scavenger was calculated using the equation

$$\text{Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \text{ , (2)}$$

where A_0 is the absorbance of the control reaction, and A_1 is the absorbance in the presence of the samples or standards.

Nitric Oxide Scavenging Activity. Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide spontaneously and interacts with oxygen to produce nitrite ions, which can be estimated using the Griess-Ilosvay reaction [26]. In the present investigation, Griess-Ilosvay reagent was modified using naphthylethylene-diaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffered saline (0.5 mL), and compound **5** (250, 500, 750, and 1000 μg/mL) or a standard solution (0.5 mL) was incubated at 25°C for 150 min. After the incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min to complete the diazotization. Next, 1 mL of naphthyl ethylenediamine dihydrochloride (1%) was added, mixed, and allowed to stand for 30 min. A pink chromophore formed in diffuse light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Ascorbic acid was used as a standard. The nitric oxide percentage of scavenging activity was calculated using Eq. (1).

Hydrogen Peroxide Scavenging Activity. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Various concentrations (250, 500, 750, and 1000 μg mL^{-1}) of the synthesized compounds (or ascorbic acid) were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide [27, 28]. The hydrogen peroxide percentage scavenging activity was calculated using Eq. (2).

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