Research Article

Clean Procedure and DFT Study for the Synthesis of 2-Amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones Derivatives: A Novel Class of Potential Antimicrobial and Antioxidant Agents

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2-Amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5** is synthesized by the two-component reaction of 4-hydroxycoumarin with ethyl-2-cyano-3-(aryl) acrylate **3**. The structures of the obtained compounds are confirmed by analytical IR, ¹H, and ¹³C-NMR spectra to elucidate the different positions of protons and carbons and as well as theoretic studies (DFT/B3LYP). All the newly synthesized compounds are screened for their antibacterial. Furthermore, these compounds showed antioxidant activities of different extents with respect to individual compounds as well as to the antioxidant methods. The compounds **5a-e** were found to be the most active antioxidant in the series then Trolox, which makes the investigated complexes a promising new class of antibacterial activity compounds.

1. Introduction

Coumarin and its derivatives represent one of the most active classes of compound possessing a wide spectrum of biological activity [1–3]. Novobiocin and chlorobiocin are established antimicrobials containing a coumarin skeleton [4]. Many of these compounds have proved to be active as antibacterial [5–7], antifungal [8], anti-inflammatory [9], anticoagulant [10], anti-HIV [11] and antitumor [12]; In addition, these compounds are used as additives to food and cosmetics [13]. Coumarin derivatives are commonly used as optical whiteners, luminescence dyes [14], active media for lasers [15] and solar collectors [16].

Several natural or synthetic coumarins with various hydroxyl and other substituents are found to inhibit lipid peroxidation and to scavenge hydroxyl radicals and superoxide anions [17]. 4H-chromenes with amino and cyano groups are also the synthons of some special natural products.

In continuation of the search for such potent molecules and as a part of our ongoing research, we plan to synthesize the hybrid structure having the lipophilic functionality on the pyran ring. The current work highlights the synthesis as well as the theoretical studies of the important coumarin scaffold. The title compound is synthesized by the reaction of 4-hydroxycoumarin and (E) ethyl-2-cyano-3-(aryl) acrylate **3** in the presence of piperidine as a base catalyst and at reflux of DMC. Condensation of **1** with arylaldehydes in the presence of piperidine at room temperature gives the corresponding (E) ethyl-2-cyano-3-(aryl) acrylate **3**, Scheme 1

TLC is used to monitor the progress of the reaction; the structures of products are characterized by ¹HNMR along with ¹³CNMR; and FT-IR spectral techniques, and they are in agreement with the structure of (E) ethyl-2-cyano-3-(aryl) acrylate **3**. The IR spectrum of compound **3a** shows a band in the region of 1664.62 cm⁻¹ which is the characteristic for C=O. It shows strong bands at 2372 cm⁻¹,

Compounds 3	Ar
3a	C ₆ H ₅
3b	p-OCH ₃ C ₆ H ₄
3c	2, 5-CH ₃ C ₆ H ₃
3d	p-NO ₂ C ₆ H ₄
3e	p-N(CH ₃) ₂ C ₆ H ₄

SCHEME 1: Synthesis of ethyl-2-cyano-3-(aryl) acrylate **3**.

1952 cm⁻¹, 3146 cm⁻¹ due to (C≡N); (C=C); (C-H)_{arom} functions, respectively. The ¹H NMR spectrum of the synthesized compound **3a** exhibits a multiplet signal in the region δ 7.37–7.93 due to the aromatic protons. A singlet in the region δ 8.0 ppm is also observed due to the CHAr proton. The quartet in the region δ 4.30 ppm and the triplet in the region δ 1.35 ppm in compounds **3a** are observed due to methyl and methylene protons of the ethyl groups. Treatment of intermediate **3** with 4-hydroxycoumarin in the presence of piperidine at reflux of dimethylcarbonate gives **5** in high yield (Scheme 2).

The FT-IR spectra of compounds **5e** shows absorption bands at 1582–1586 cm⁻¹ corresponded with C=O and C–O linkage and 3705 cm⁻¹ is observed due to the –NH group. The ¹H NMR spectra of **5e** displays a signal at δ 6.76 ppm that is ascribable to the NH₂ protons. A characteristic singlet proton signal at δ 2.49 ppm is assigned to the CHAr proton. In addition, the aromatic protons (both coumarinic and aryl) are observed between δ 6.91 and δ 7.92 ppm (see Section 4), and the expected triplet for methylic protons is observed at δ 1.26 ppm. The singlet at δ 3.03 ppm is assigned to the three protons of the methoxy group.

Indeed the ¹³C{¹H} NMR spectrum of **3e** in DMSO-d₆, shows the presence of 23 carbon atoms, such as: C₁, C_f, C₈, C_h, and C_i and C_g are, respectively, 167.6, 164.6, 61.4, 35.2, and 39.5. The signals between (92.0 and 163.4 ppm) correspond to the ethylenic and aromatic carbons.

The formation of the 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5** occur via

two steps: 4-hydroxycoumarin attacks the ethylenic double bond of the compound **3**. The nonisolated intermediate (**i2**) rearranges to give (**i3**), who is cyclized to give 2-amino-3-ethoxycarbonyl-4-(aryl) 4H-pyrano-[3,2-c]-chromene-5-ones **5** as shown in Scheme 3.

To further confirm the structure of the product **5** a systematic theoretical treatment of these compounds is performed by using the DFT/B3LYP approach implemented in the Gaussian 09 series of programs [18]. The B3LYP hybrid functional has been used in describing potential energy surfaces (PES).

The geometries of compounds **5** are fully optimized using analytic gradients. The harmonic vibrational frequencies of the stationary points of the PES have been calculated at the same level of theory in order to identify the local minima as well as to estimate the corresponding zero-point vibrational energy (ZPE) [19, 20].

For each atom no pseudopotential is used. A Def2-SVP EMSL basis set exchange is employed for each atom [21]. Optimized geometry for **5c** is depicted in Figure 1. Values of selected geometrical parameters are listed in Table 1.

The value of the dihedral angle (3-3'-5'-6' = 96.8°) shows that the aryl group is orthogonal to the plan of the tricyclic structure **5**. The bond length C_{4'-5'} in compound **5c** is more elongated than its counterparts in **5b–d**, this is caused by the steric hindrance of the two methyl groups in positions 2 and 5. Another important information concerning the nature of the groups present in the structure **5** can be obtained by calculating the vibrational frequencies of these

Compounds 5	Ar
5a	C ₆ H ₅
5b	pOCH ₃ C ₆ H ₄
5c	2,5-CH ₃ C ₆ H ₃
5d	pNO ₂ C ₆ H ₄
5e	pN(CH ₃) ₂ C ₆ H ₄

SCHEME 2: Synthesis of 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5**.

compounds. Values and intensities of the harmonic wave numbers calculated with the DFT/B3LYP method, and the corresponding experimental values of the compound **5c** are presented in Table 2.

The values of the theoretical IR frequencies are in agreement with the experimental values with a shift to larger frequency values less than 10%. This shift is due to the approximations used in the DFT/B3LYP method.

The NBO analysis allows the determination of the atomic natural charges reports the natural charges of the acid protons in compound **5c** calculated at DFT/B3LYP level of theory. We find that the protons Ha and Ha' carried by the nitrogen atom are the most acidic Table 3.

2. Antibacterial and Antioxidant Studies

2.1. Free Radical Scavenging Activity Assay. The free radical scavenging activity of the new 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5** is tested by utilizing DPPH scavenging.

DPPH is a free radical and accepts one electron or one hydrogen radical to become a stable diamagnetic molecule [22]. The reduction capability of the DPPH radical is determined by the decrease in absorbance induced by 2-amino-3-ethoxycarbonyl-4-(aryl)-4Hpyrano-[3,2-c]-chromene-5-ones **5**. Briefly, 1.5 mL ethanolic solution of the synthesized compounds (0.2 mM) is added to 1.5 mL (0.2 mM) solution of DPPH radical in ethanol (final concentration of DPPH and synthesized compounds is 0.1 mM). The mixture is shaken vigorously and left standing for 30 min. After this, the absorbance at 534 nm is determined,

and the percentage of scavenging activity is calculated using the following formula:

$$\text{Scavenging activity} = \left\{ \frac{[(A_b + A_s) - A_m]}{A_b} \right\} \times 100\%, \quad (1)$$

where A_b : absorbance of 0.1 mM ethanolic solution of DPPH at 534 nm, A_s : absorbance of 0.1 mM ethanolic solution of test compound at 534 nm, and A_m : absorbance of ethanolic mixture of the drug and DPPH at 534 nm.

Trolox was used as reference compound. All tests and analyses are done on triplicate and average on three samples. The results are illustrated in Scheme 4.

Among the compounds from the 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5** series, **5b** showed moderate antioxidant activity. The activity exhibited by the compound **5e** is the highest. In addition, the experimental data shows that compound **5a** scavenges free radicals better than Trolox. Thus, we can conclude that substituents on the aryl group do not influence significantly the antioxidant activity.

One parameter that has been introduced recently for the interpretation of the results from the DPPH method is the effective concentration or EC_{50} value (otherwise called the IC_{50} value), which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) and corresponds to the endpoint of the titration. In all cases, any residual (yellow) color from the reduced form or any nonspecific absorbance from the sample should be considered in defining the "endpoint" of the titration, that is, the 50% point. Additionally, this IC_{50} parameter has also the drawback of that the higher the antioxidant activity,

SCHEME 3: Proposed mechanism for the synthesis of 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5**.

FIGURE 1: DFT/B3LYP optimized geometries of **5c**.

the lower is the value of EC_{50} . The EC_{50} values exhibited by 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5** are summarized in Table 4.

From inspection of Table 4, it is evident that compounds **5e** are more active than Trolox.

2.2. ABTS Radical Cation Decolorization Assay. The potential of 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones **5** to scavenge free radicals was also assessed by checking their ability to quench $ABTS^{+\bullet}$ depicts the concentration-dependent decolorization of $ABTS^{+\bullet}$.

ABTS radical-scavenging activity of 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]chromene-5-ones **5** is determined according to Re et al. [23]. The $ABTS^{+\bullet}$. The cation radical is produced by the reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution is diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. In a final volume of 1 mL, the reaction mixture comprised 950 μ L of ABTS $\pm$ solution and 50 μ L of the 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-xchromene-5-ones **5** at various concentrations. The reaction mixture is homogenized and its absorbance is recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the

TABLE 1: DFT/B3LYP optimized geometrical parameters^a.

Compounds	5c	5d	5e	5a	5b
1-2	1.385	1.383	1.387	1.386	1.386
1-8a	1.349	1.351	1.350	1.350	1.350
2-b	1.200	1.201	1.200	1.200	1.201
2-3	1.462	1.461	1.461	1.461	1.461
3-4	1.358	1.357	1.357	1.357	1.357
3-4'	1.509	1.504	1.504	1.504	1.505
4-5a	1.444	1.444	1.444	1.444	1.444
4-1'	1.358	1.357	1.358	1.358	1.358
1'-2'	1.366	1.368	1.368	1.368	1.368
2'-3'	1.370	1.371	1.369	1.370	1.370
2'-d	1.351	1.348	1.353	1.351	1.351
3'-4'	1.522	1.516	1.517	1.516	1.516
3'-1''	1.460	1.460	1.460	1.460	1.460
4'-5'	1.541	1.529	1.526	1.530	1.528
1-2-3	116.7	116.5	116.4	116.5	116.4
1-2-b	117.5	118.3	117.9	118.0	118.0
2-3-4	119.4	120.0	119.9	120.0	120.0
3-4-5a	122.5	122.0	122.2	122.1	122.2
3-4-1'	122.5	122.4	122.3	122.3	122.3
3-4'-5'	113.2	111.1	111.0	111.0	110.9
4-1'-2'	119.8	119.6	119.6	119.5	119.6
1'-2'-3'	122.0	122.0	121.8	121.9	121.9
1'-2'-d	109.4	109.8	109.6	109.7	109.7
2'-3'-4'	121.2	120.9	120.7	120.7	120.7
2'-3'-1''	123.2	124.3	124.2	124.3	124.2
3'-4'-5'	114.3	122.2	112.8	112.6	112.8
3'-2'-d	128.5	128.2	128.5	128.4	128.4
3'-1''-2''	114.8	114.8	114.7	114.7	114.7
3'-1''-c	124.4	123.7	124.2	124.0	124.1
2-3-4-1	2.9	1.4	1.8	1.1	1.0
4-5a-2-3	4.6	1.3	1.5	1.4	1.5
2-3-4-1'	-176.3	-177.6	-177.0	-177.1	-177.0
2-3-4'-5'	-67.6	-71.5	-73.5	-73.2	-73.4
3-4-1'-2'	11.4	11.9	12.8	12.8	12.8
4-5a-8a-1	-1.5	0.9	-1.0	-1.0	-1.0
4-5a-5-6	-179.0	-179.5	-179.5	-179.4	-179.4
3'-3-5'-6'	96.8	96.5	95.4	95.0	94.0
4-5a-8a-8	179.0	179.5	179.5	179.4	179.4
3-4-2'-3'	1.9	1.6	1.7	1.7	1.6
3'-4'-3-4	-14.3	-16.4	-19.5	-18.7	19.4
4-1'-2'-3'	-9.1	-10.0	-10.8	-10.7	-10.9
2'-3'-1''-2''	9.0	3.7	4.5	4.3	4.5
2'-3'-1''-d	4.5	3.1	3.6	3.5	3.6
3'-4'-5'-6'	61.7	61.2	59.1	59.2	57.5
3-4'-5'-6'	-64.9	-62.2	-61.2	-64.1	-65.8

^a Distances are in Å and angles in degrees. See Figure 1 for labelling of the atoms.

reaction mixture of standard group was obtained by mixing 950 μ L of ABTS⁺• solution and 50 μ L of Trolox. As for the antiradical activity, ABTS scavenging ability is expressed

TABLE 2: Theoretical and experimental vibrational frequencies (cm^{-1}) and theoretical infrared intensities (km mol^{-1}) of 5c.

	Experimental		Theoretical	
	Frequencies	Intensities	Frequencies	Intensities
CO ester	1631	s	1812	219
CO lactone	1682	vs	1868	453
NH sym	3215	m	3569	155
NH asym	3350	m	3719	129

TABLE 3: NBO charges of the acidic protons of compound 5c calculated at the DFT/B3LYP level of theory.

NBO charge of the acidic protons				
Ha	Ha'	Hb	Hb'	Hc
0.40	0.44	0.21	0.21	0.29

TABLE 4: The EC₅₀ values exhibited by 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5.

Compounds 5a-e	CI ₅₀ ($10^{-3} \text{ mol} \cdot \text{L}^{-1}$)
5a	0
5b	0
5c	0.001
5d	0
5e	0.034
Trolox (DCM/DMSO)	0.012/0

as EC₅₀ ($\mu\text{g/mL}$). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging effect \%} = \left\{ \frac{[(A_0 - A_1)]}{A_0} \right\} \times 100\%, \quad (2)$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the Sample at 30 min. All samples are analyzed in triplicate.

As shown for DPPH scavenging, these data indicate the higher capacity of 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5 to quench ABTS+Y as compared to the synthetic antioxidant Trolox, Scheme 5.

The variation in the percentage of inhibition (PI) is almost constant starting from a value of the concentration equal to 1.34 mM. In addition, the synthesized products of the type 5 have antioxidant activities better than Trolox. Indeed, the antioxidant capacity seems to be attenuated when the concentration increases in the medium. This can be explained by the existence of the peroxide sites which are susceptible for oxidizing when the concentration increases. We have just shown that the synthesized 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5 has a good antioxidant activity under weak concentration, but it proves to be necessary to determine the reaction time necessary to highlight the antioxidant effect to be able to use these derivatives in pharmacy.

SCHEME 4: Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of compounds 5.

SCHEME 5: Scavenging ability on ABTS radical of compounds 5.

The EC_{50} values exhibited by 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5 are summarized in Table 5. The 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5 are shown to be efficient antioxidants, showing higher free radical scavenging activity than Trolox.

These compounds have a remarkable oxidizing capacity which explains their susceptibility to fix free radicals DPPH and $ABTS^{\bullet+}$.

2.3. Antibacterial Activity. The cup-plate method [23, 24] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of **3a–e** against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Preparation of base-layer medium, agar medium, and peptone water is done as per the standard procedure. Each test compound (10 mg/mL) is prepared by dissolving 50 mg in 5 mL of dimethylformamide, and is used for testing same cup-plate method using PDA medium is employed to study the preliminary antifungal activity of **3a–e** against *Candida albicans* and *A. niger*. The PDA medium was purchased from Himedia Laboratories Ltd, Mumbai,

TABLE 5: The EC_{50} values exhibited by 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5.

Compounds 3a–e	CI_{50} ($10^{-6} \text{ mol} \cdot \text{L}^{-1}$)
3a	0.0029
3b	0.00275
3c	0.0029
3d	0.00365
3e	0.002
Trolox	0.00585

India. Preparation of nutrient broth, sub-culture, base-layer medium and PDA medium was done as per the standard procedure. For each test compound, a $50 \mu\text{g}/\text{cup}$ was used for testing.

The cups of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish which was streaked with organisms. The solutions of each compound were added separately in the cups, and petri dishes were subsequently inoculated. Ampicillin and Griesofulvin ($6 \mu\text{g}/\text{cup}$ and $25 \mu\text{g}/\text{cup}$, resp.) were used as standard reference drugs and dimethylformamide (DMF) used as control which did not show any inhibition. Zone of inhibition produced by each compound was measured in mm, and the results are presented in Table 6.

All the tested compounds have shown antibacterial activity to some extent. Among the tested compounds **3b** and **3d**, show very good activity against the tested organisms.

Compounds **3a** and **3e** are moderate antibacterial activity. All the compounds synthesized possess electron-releasing groups, on both the aromatic rings. Therefore from the results, it is evident that compounds having electron-releasing groups like methyl, hydroxyl, and methoxy may be responsible for antibacterial activity.

3. Conclusion

In conclusion, an efficient green-chemistry method for the synthesis of 2-amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5 derivatives by condensation of 4-hydroxycoumarin and (E) ethyl-2-cyano-3-(aryl)acrylate **3** is successfully established. In this method, the potential active compounds containing chromene heteroaromatic rings can be synthesized easily by two one-step reactions. Compared to other methods [25], this new method has the advantage of good yields, mild reaction conditions, easy workup, inexpensive reagents, and environmentally friendly procedure. All the newly synthesized compounds are screened for their antibacterial effect against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

4. Experimental Section

4.1. General Procedures. Flash chromatography was carried out on 0.04–0.063 mm (Merck) silica gel, thin-layer chromatography was carried out on aluminium-backed silica plates by Merck, and plates were revealed using a UV 254

TABLE 6: Zone of inhibition produced by each compounds.

Compound code	<i>S. Aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
3a	6	8	10	7	9	7
3b	6	8	5	7	12	9
3c	12	10	9	10	9	12
3d	7	8	6	6	7	8
3e	8	10	7	9	8	12
Ampicillin	14	12	13	17	—	—
DMF	—	—	—	—	—	—

light. ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) spectra were recorded on a Varian VXR 300 instrument at 293° K in CDCl_3 or $\text{DMSO}-d_6$. Spectra were internally referenced to TMS. Peaks are reported in ppm downfield of TMS. Multiplicities are reported as singlet (s), doublet (d), triplet (t), and quartet (q), some combinations of these were made by DEPT editing of the spectra. The IR-spectra were recorded on a Philips Analytical PU 9800 spectrometer. The melting points of compounds were determined in open-glass capillaries in a paraffin bath and are uncorrected.

4.2. Materials. 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma. All other chemicals were of analytical grade purity. The 4-hydroxycoumarin, aromatic aldehydes, and ethylcyanoacetate were purchased from Fluka, DMF, were purified, dried, and distilled over CaH_2 prior to use.

4.2.1. Synthesis of (E) Ethyl-2-cyano-3-(aryl)acrylate 3. Arylaldehyde (0.0199 mol), ethylcyanoacetate (0.0175 mol) and piperidine (0.0175 mol) are mixed in round bottomed flask. The latter is stirrer at r.t for 2 h. The reaction mixture was filtered, and the residue washed with acetone.

(E) Ethyl-2-cyano-3-(4-phenyl)acrylate **3a**. Yield: 80%; mp = 80°C (MeOH); IR spectrum, cm^{-1} : 2372 (C≡N); 1952 (C=C); 3146 (C-H)_{arom}; 1105 (C-O)_{ester}; 1685 (C=O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,35 (t, 3H, CH_3); 4,30 (q, 2H, CH_2); 7,37–7,93 (m, 5H, H_{arom}); 8,00 (s, 1H, CH); ^{13}C NMR (CDCl_3 , 75 MHz): spectrum: 14,1 (C_c); 62,7 (C_b); 102,6 (C_2); 115,4 (C_a); 127,9–133,4 (C_{arom}); 155,3 (C_3); 163,0 (C_1).

(E) Ethyl-2-cyano-3-(4-methoxy-phenyl)acrylate **3b**. Yield: 84%; mp = 83°C (MeOH); IR spectrum, cm^{-1} : 2378 (C≡N); 1970 (C=C); 3100 (C-H)_{arom}; 1112 (C-O)_{arom}; 1685 (C=O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,29 (t, 3H, CH_3); 3,78 (s, 3H, OCH_3); 4,26 (q, 2H, CH_2); 6,88–7,90 (m, 4H, H_{arom}); 8,03 (s, 1H, CH); ^{13}C NMR (CDCl_3 , 75 MHz): spectrum: 14,1 (C_c); 55,6 (OCH_3); 62,3 (C_b); 99,4 (C_2); 116,1 (C_a); 114,7–163,0 (C_{arom}); 154,2 (C_3); 163,9 (C_1).

(E) Ethyl-2-cyano-3-(2,5-dimethylaminophenyl)acrylate **3c**. Yield: 78%; mp = 48°C (MeOH); IR spectrum, cm^{-1} : 2373 (C≡N); 1934 (C=C); 3036 (C-H)_{arom}; 1182 (C-O)_{ester}; 1681 (C=O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,38 (t, 3H, CH_3); 2,34 (s, 3H, CH_3); 2,37 (s, 3H, CH_3); 4,36 (q, 2H, CH_2); 7,12–7,21 (m, 3H, H_{arom}); 8,50 (s, 1H, CH); ^{13}C NMR (CDCl_3 , 75 MHz): 14,1 (C_c); 19,2 (CH_3); 20,8 (CH_3); 62,6

(C_b); 104,0 (C_2); 115,4 (C_a); 128,8–136,7 (C_{arom}); 153,3 (C_3); 162,5 (C_1).

(E) Ethyl-2-cyano-3-(4-nitro-phenyl)acrylate **3d**. Yield: 72%; mp = 158°C (MeOH); IR spectrum, cm^{-1} : 2382 (C≡N); 1950 (C=C); 3088 (C-H)_{arom}; 1100 (C-O)_{ester}; 1723 (C=O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,44 (t, 3H, CH_3); 4,36 (q, 2H, CH_2); 8,08–8,32 (m, 4H, H_{arom}); 8,24 (s, 1H, CH); ^{13}C NMR (CDCl_3 , 75 MHz): 13,1 (C_c); 62,3 (C_b); 106,5 (C_2); 113,5 (C_a); 123,3–148,8 (C_{arom}); 150,7 (C_3); 160,4 (C_1).

(E) Ethyl-2-cyano-3-(4-dimethylamino phenyl)acrylate **3e**. Yield: 47%; mp = 123°C (MeOH); IR spectrum, cm^{-1} : 2208 (C≡N); 1927 (C=C); 1165 (C-O)_{ester}; 1701 (C=O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,26 (t, 3H, CH_3); 3,05 (s, 6H, 2 CH_3); 4,22 (q, 2H, CH_2); (m, 4H, H_{arom}); 8,07 (s, 1H, CH). ^{13}C NMR (CDCl_3 , 75 MHz): 14,1 (C_c); 39,5 (CH_3); 39,8 (CH_3); 61,4 (C_b); 104,0 (C_2); 118,2 (C_a); 128,8–136,7 (C_{arom}); 154,1 (C_3); 163,4 (C_1).

4.2.2. Synthesis of 2-Amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones 5. The appropriate (E) ethyl-2-cyano-3-(4-arylphenyl)acrylate **3** (0.05 mol) and 4-hydroxycoumarin (0.005 mol) were dissolved in 10 mL of DMC in the presence of piperidine (0,00003 mol). The reaction mixture was refluxed for 7 h, then concentrated and cooled. The solid product was filtered off washed with the appropriate solvent.

2-Amino-3-ethoxycarbonyl-4-(phenyl)-4H-pyrano-[3,2-c]chromene-5-one **5a**. Yield: 65%; mp = 250°C (MeOH); IR spectrum, cm^{-1} : 3439 (N-H); 1090 (C-O)_{ester}; 1729 (C=O)_{ester}; 1205 (C-O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,56 (m, 3H, CH_3); 2,53 (s, 1H, CH); 3,03 (t, 2H, CH_2); 6,33 (s, 2H, NH_2); 7,10–7,88 (d, 9H, H_{arom}); ^{13}C NMR (CDCl_3 , 75 MHz): 19,5 (C_g); 36,1 (C_8); 61,6 (C_f); 103,4 (C_7); 115,4 (C_d); 152,5 (C_c); 103,4–130,8 (C_{arom}); 142,4 (C_6); 164,5 (C_1); 167,6 (C_e).

2-Amino-3-ethoxycarbonyl-4-(4'-methoxyphenyl)-4H-pyrano-[3,2-c]-chromene-5-one **5b**. Yield: 70%; mp = 212°C (MeOH); IR spectrum, cm^{-1} : 3414 (N-H); 1128 (C-O)_{ether}; 1717 (C=O)_{ester}; 1186 (C-O)_{ester}; ^1H NMR spectrum (CDCl_3 , 300 MHz): 1,32 (t, 3H, CH_3); 3,02 (t, 3H, OCH_3); 3,70 (s, 1H, CH); 4,32 (q, 2H, CH_2); 6,26 (s, 2H, NH_2); 6,75–7,87 (d, 8H, H_{arom}); ^{13}C NMR (CDCl_3 , 75 MHz): 22,1 (C_g); 35,3 (C_8);

54,8 (OCH₃); 62,0 (C_f); 79,1 (C₇); 103,6–154,4 (C_{arom}); 156,8 (C₆); 162,3 (C_c); 164,6 (C₁); 167,6 (C_e).

2-Amino-3-ethoxycarbonyl-4-(2,5-dimethylphenyl)-4H-pyrano-[3,2-c]-chromene-5-one 5c. Yiled: 75%; mp = 260°C (MeOH); IR spectrum, cm⁻¹: 3436 (N–H); 1115 (C–O)_{ether}; 1728 (C=O)_{ester}; 1230 (C–O)_{ester}; ¹H NMR spectrum (CDCl₃, 300 MHz): 1,36 (m, 3H, CH₃); 1,78 (s, 3H, CH₃); 1,90 (s, 3H, CH₃); 2,74 (t, 2H, CH₂); 5,81 (s, 1H, CH); 6,52–7,59 (m, Harom et Hethyl); 7,97 (s, 2H, NH₂); ¹³CNMR (CDCl₃, 75 MHz): 24,4 (C_g); 26,4 (CH₃); 26,8 (CH₃); 40,8 (C₈); 49,0 (C_f); 84,4 (C₇); 108,4–146,2 (C_{arom}); 157,6 (C_c); 169,4 (C₁); 173,2 (C_e).

2-Amino-3-ethoxycarbonyl-4-(4'-nitrophenyl)-4H-pyrano-[3,2-c]-chromene-5-one 5d. Yiled: 63%; mp = 142°C (MeOH); IRv (KBr, cm⁻¹): 3000 (N–H); 1091 (C–O)_{ether}; 1720 (C=O)_{ester}; 1200 (C–O)_{ester}; ¹H NMR spectrum (CDCl₃, 300 MHz): 1,30 (t, 3H, CH₃); 3,68 (s, 1H, CH); 4,22 (q, 2H, CH₂); 6,32 (s, 2H, NH₂); 6,62–7,82 (d, 8H, H_{arom}); ¹³CNMR (CDCl₃, 75 MHz): 22,2 (C_g); 34,7 (C₈); 62,2 (C_f); 78,2 (C₇); 102,6–153,4 (C_{arom}); 157,8 (C₆); 163,3 (C_c); 164,2 (C₁); 167,4 (C_e).

2-Amino-3-ethoxycarbonyl-4-(4'-dimethylaminophenyl)-4H-pyrano-[3,2-c]-chromene-5-one 5e. Yiled: 46%; mp = 122°C (MeOH); IRv (KBr, cm⁻¹): 3705 (N–H); 1084 (C–O)_{ether}; 1695 (C=O)_{ester}; 1227 (C–O)_{ester}; ¹H NMR spectrum (CDCl₃, 300 MHz): 1,26 (t, 3H, CH₃); 3,00 (s, 6H, 2CH₃); 4,00 (s, 1H, CH); 4,24 (q, 2H, CH₂); 6,94 (d, 2H, NH₂); 6,20–6,78 et 7,20–7,92 (m, Harom et Hethyl); ¹³CNMR (CDCl₃, 75 MHz): 14,1 (C_g); 35,2 (C₈); 39,5 (N(CH₃)₂); 61,4 (C_f); 92,0 (C₇); 103,8 (C_b); 111,6 (C₁₀ et C₁₄); 115,3 (C_c); 118,3 (C₅); 122,7 (C₂); 127,2 (C₃); 130,6 (C₄); 133,7 (C₁₁ et C₁₃); 148,2 (C₉); 152,5 (C₁₂); 153,6 (C_d); 154,0 (C₆); 163,4 (C_a); 164,6 (C₁); 167,6 (C_e).

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