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SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL SERIES OF 1,3-DICOUMARINYL-5-ARYL-2-PYRAZOLINES

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Abstract – In the present paper novel 1,3,5-trisubstituted 2-pyrazolines (**4a-q**) were synthesized *via* condensation of different substituted 3-cinnamoyl-2-oxo-2*H*-chromenes (**2a-q**) with 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide (**3**). Chalcones were prepared *via* Claisen-Schmidt condensation by refluxing 3-acetyl-2-oxo-2*H*-chromen (**1**) with corresponding aldehydes in ethanol, in the presence of piperidine. All of these compounds were characterized by means of their IR, ^{1}H NMR and LC/MS/MS spectroscopic data and elemental microanalysis. Chalcones and pyrazolines were screened for their antioxidant and iron chelating activity.

INTRODUCTION

Coumarins comprise a very large and important class of organic compounds found in various plants[1] and have been recognized to possess variety of biological activities, anti-inflammatory,[2] antioxidant,[3] antithrombotic,[4] antiallergic, hepatoprotective,[5] antiviral[2] and anticarcinogenic,[5] respectively. Natural, semi-synthetic and synthetic coumarins are useful substances in drug research. Numerous coumarin derivatives can be utilized for the synthesis of different valuable heterocyclic ring systems. Chalcones with coumarin moiety obtained by the reaction 3-acetylcoumarins with corresponding aromatic aldehydes[6-12] proved to be especially important compounds for this purpose. Chalcones are an important class of naturally occurring flavonoid compounds, largely distributed in plants, fruits and vegetables that exhibit a wide spectrum of biological activities. Chalcones in many cases, serve in plant defense mechanisms against reactive oxygen species (ROS), thus preventing molecular damage and damage by microorganisms, insects, and herbivores.[13] Chalcones have been used in the treatment of atherosclerosis and they also prevent low density lipoproteins (LDL) oxidation. Some natural chalcones, as well as

synthetic ones, possessing hydroxyl and methoxy substituents, are proven to exhibit significant antioxidant activity.[14] Natural chalcones which have exhibited antioxidant activity include isoliquiretigenin, isoprenyl chalcone, butein, homobutein, and naringenin chalcone.[14] Chalcones are useful synthons in the synthesis of a large number of bioactive molecules, such as 1,5-benzodiazepines[15] and 1,5-benzothiaazepines,[16,17] pyrazolines[18,19] and isoxazoline.[12]

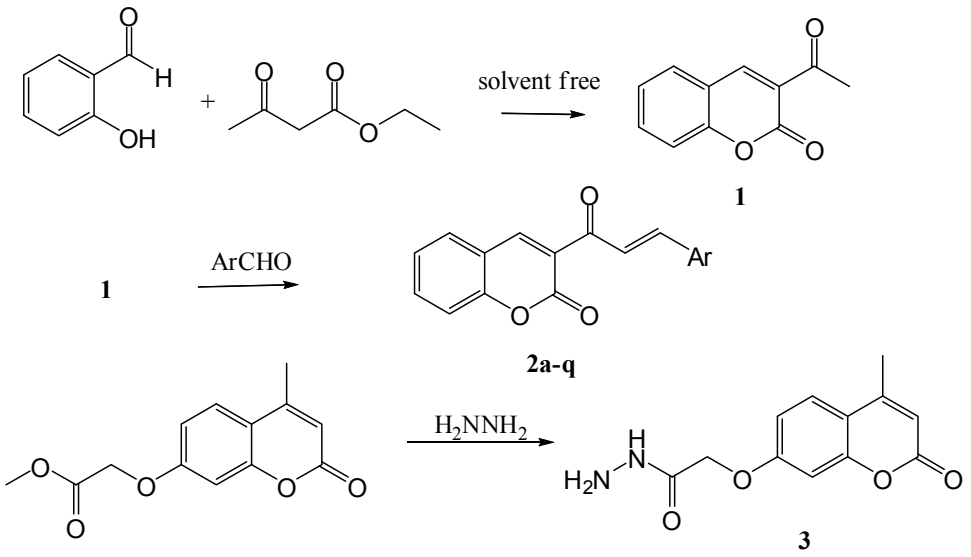
Pyrazolines are important nitrogen-containing five-membered heterocycles and some of them possess significant biological activitiy e.g., activity on the central nervous system of a mammalian host,[20] immunosuppressive,[21] and antimicrobial[22] activity. 2-Pyrazolines display a broad spectrum of potential pharmacological activities and are present in a number of pharmacologically active molecules such as phenazone/amidopyrene/methampyrone (analgesic and antipyretic), azolid/ tandearil (anti-inflammatory), indoxacarb (insecticidal), anturane (uricosuric), etc. and some 1,3,5-substituted pyrazolines have also proven to be an excellent antioxidants, compared to ascorbic acid.[23]

Therefore, both coumarins and pyrazolines possess significant bioactivities, which single them out as useful substances in drug research. On this basis, Levai *et al.*[19] have recently described the synthesis of 1-substituted-5-aryl-3-(3-coumarinyl)-2-pyrazolines by reaction of (3-coumarinyl)-chalcones and hydrazines. In view of these observations and in continuation of our previous research on the synthesis of various heterocyclic compounds,[24-26] we herein report a synthesis of some new 2-pyrazoline derivatives with two coumarins moieties, along with their antioxidant and iron chelating activity investigation.

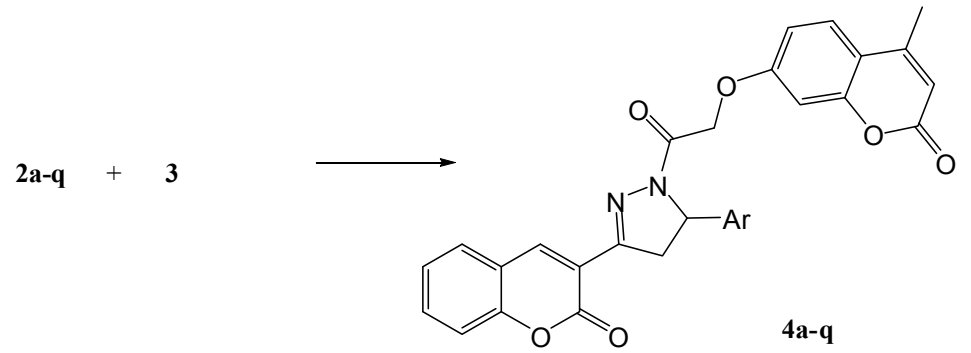
RESULTS AND DISCUSSION

In our work we have synthesized 5-aryl-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazolines (**4a-q**) by reaction of 3-aryl-1- (2-oxo-2*H*-chromen-3-yl)-2-propen-1-ones (**2a-q**) and 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy) acetohydrazide (**3**) in ethanol, with piperidine as catalyst (Scheme 2). Starting materials **1, 2a-q** and **3** were synthesized according to the known procedures (Scheme 1).[8-11, 25, 27, 28]

The structures of all the new compounds **4a-q** were elucidated by elemental analysis and spectroscopic technique data. In their IR spectra a characteristic lactone carbonyl band at around 1730 cm^{-1} proves the presence of the coumarin moiety. A C=N band at around 1600 cm^{-1} is in accordance with the 2-pyrazoline frame (skeleton). ^{1}H NMR spectra of compounds **4a-q** gave a characteristic ABX spin system for the protons on the C-4 and C-5 carbon atoms of the five-membered ring, which are typical for 2-pyrazoline structure.[18] Their mass spectra exhibit molecular ion peaks and contain fragments that confirm the pyrazoline ring structure.[29] Molecular ion peaks of compounds **4a-q** are of relatively low intensity.



Scheme 1. Synthetic route for preparation of 3-aryl-1-(2-oxo-2*H*-chromen-3-yl)2-propen-1-ones and 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide



Scheme 2. Synthetic route for preparation of 5-aryl-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazolines (**4a-q**)

2,4	**Ar**	**2,4**	**Ar**
a	phenyl	**j**	4-fluorophenyl
b	3-hydroxyphenyl	**k**	3-phenoxyphenyl
c	4-hydroxyphenyl	**l**	4-*N,N*-dimethylaminophenyl
d	2-methoxyphenyl	**m**	4-styryl
e	3-methoxyphenyl	**n**	4-hydroxy-3-methoxyphenyl
f	4-methoxyphenyl	**o**	2,4-dihydroxyphenyl
g	2-chlorophenyl	**p**	3,4-dihydroxyphenyl
h	3-bromophenyl	**q**	3,4,5-trimethoxyphenyl
i	4-bromophenyl		

Antioxidant activity

Antioxidant activity can be expressed in a several ways, by suppression of the formation of ROS by inhibiting the certain enzymes involved in its generation, by chelating the metal ions responsible for the production of free radicals, by scavenging the ROS and regulating and protecting antioxidant defence mechanism.[14] Antioxidant capacity of any compound is related to its hydrogen or electron donation capacity, its ability to stabilize and delocalize the unpaired electron and its potential to chelate transition metal ions.[14] In the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and phosphomolybdenum assay methods hydrogen and electron transfer from antioxidants to DPPH radical and Mo(VI) complex occurs. These transfers depend on the structure of antioxidants. The reason we used ascorbic acid as standard in both antioxidant investigations is that ascorbic acid has the ability to donate hydrogen and electrons and can be detected by both assay models.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl radical

The unpaired electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow when the unpaired electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The method used in this work applies to the antioxidant capacity of the sample after 30 minutes of incubation. DPPH scavenging activity was determined against 0.2 mM ascorbic acid as a standard compound.

Table 1. DPPH scavenging activity of novel chalcones (**2a-q**) and pyrazolines (**4a-q**)[a]

compound	DPPH scavenging activity (%)	compound	DPPH scavenging activity (%)
AA[b]	88.2	**AA**[2]	88.2
2a	8.1	**4a**	13.2
2b	10.2	**4b**	20.8
2c	13.3	**4c**	7.4
2d	11.5	**4d**	19.0
2e	5.3	**4e**	55.0
2f	12.4	**4f**	14.4
2g	19.1	**4g**	22.0
2h	18.7	**4h**	42.7
2i	15.7	**4i**	20.3
2j	17.1	**4j**	15.2
2k	6.4	**4k**	5.5
2l	7.4	**4l**	48.3

2m	13.6	**4m**	6.0
2n	27.0	**4n**	33.9
2o	20.0	**4o**	20.5
2p	36.3	**4p**	14.0
2q	10.8	**4q**	19.8

[a]data are means of three replicates
[b]0.2 mM ascorbic acid was used as standard

According to the data in Table 1 the best DPPH scavengers are pyrazolines **4e**, **4l** and **4h** showing 55%, 48% and 43% scavenging activity, two of them **4e** and **4l** possessing electron donating groups and **4h** possessing electron withdrawing bromine substituent on a phenyl ring. Compounds possessing hydroxyl groups on phenyl ring showed less activity than expected, since the previous studies showed that the hydroxybenzoyl group has a key role as free radical scavenger in other classes of compounds.[30] This proves that the introduction of pyrazoline ring enhances the DPPH scavenging activity of the corresponding chalcones they were synthesized from.

In the series of chalcones the best scavenger of DPPH radicals was **2p**, possessing better activity than corresponding pyrazoline **4p** (14%), thus, with **2j** and **2k,** making an exception from the fact that the introduction of pyrazoline ring enhances scavenging activity of corresponding chalcones. Compounds **2n** and **2o** are the best DPPH scavengers, in a series of chalcones, after **2p**, both possessing two electron donating groups on a phenyl ring, which is in accordance with previous investigations.[27, 30]

Evaluation of antioxidant activity by phosphomolybdenum method

This method is based on the reduction of Mo(VI) to Mo(V) by the tested compounds followed by formation of green phosphate/Mo(V) complex at acid pH. The mechanism responsible for reduction of Mo(VI) to Mo(V) is dependent on the transfer of the single electron from the compound to molybdenum.

From the data in the Figure 1 it is evident that pyrazolines possess better antioxidant activity than the corresponding chalcones, as it is a case with the DPPH scavenging activity too. Pyrazoline **4m** showed even better antioxidant activity than ascorbic acid, which was used as standard. Other pyrazolines showing significant antioxidant activity, comparable to this of ascorbic acid are **4f**, **4c**, **4g**, **4o** and **4n**. All of these pyrazolines possess an electron donating substituent in *p*-position, except for **4g,** possessing an electron withdrawing -Cl substitutent in *o*-position of phenyl ring.

Among the chalcone series the one showing the highest activity was **2l**, but in comparison with pyrazolines, its activity is not significant. The introduction of a pyrazoline ring obviously increases the antioxidant activity in this assay as well.

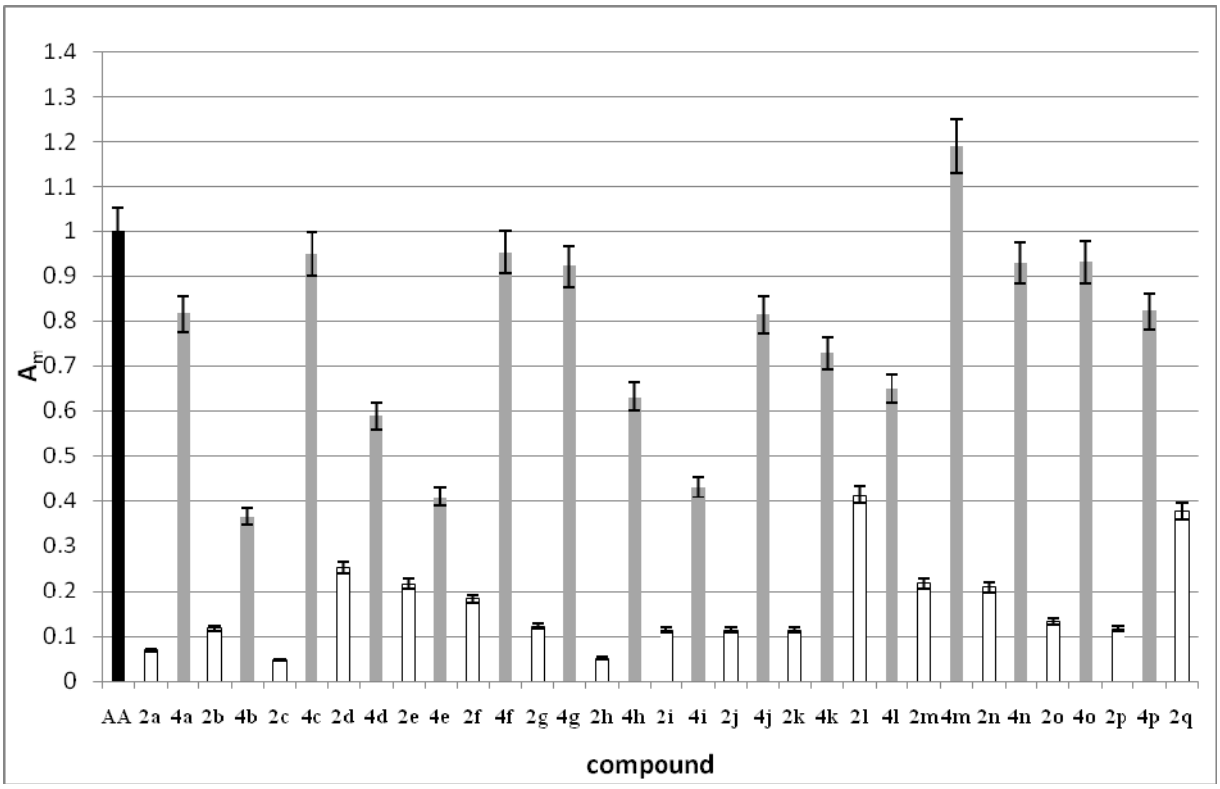


Figure 1. Antioxidant activities of chalcones and pyrazolines relative to ascorbic acid (A_m – activity relative to ascorbic acid (AA) on a molar basis). 2 mM ascorbic acid was used as standard.

Iron chelating activity

Various compounds can act as antioxidants by chelating the metal ion responsible for the production of free radical, Fe^{2+} being one of them. This investigation is based upon complex formation between ferrozine and Fe^{2+}, which is red in colour. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Therefore, the chelating activity of the coexisting chelator is estimated by measurement of colour reduction at 562 nm.

Table 2. Chelating activity of chalcones and pyrazolines[a,c,e]

compound	chelating activity (%)
EDTA[d]	93.46
2c	1.49
2l	5.43
4l	2.00
2o	61.13

[a]data are means of three replicates
[c]compounds not listed are inactive
[d]2 mM EDTA was used as standard
[e]compounds **4a**, **4c**, **4d**, **4f**, **2h**, **4j**, **4k**, **4m** are not completely soluble in the solvent used, thus not possible to determine chelating activity under these conditions

Of all the compounds investigated on iron chelating activity, only four of them showed some kind of activity, with **2o** being the best iron chelator of all, with 61% chelating activity, respectively. Compound **2o** is coumarinyl chalcone, bearing 2,4-dihydroxyphenyl moiety, and its ability to chelate metal ions is in accordance with inevestigation of Khatib *et al.*[31] who have proven an importance of resorcinol group (2,4 dihidroxy phenyl ring) for tyrosinase inhibition and copper chelating activity.[31] In general chalcones have proven to possess better chelating activity than corresponding pyrazolines.

EXPERIMENTAL

General information

The melting points were taken on Electrothermal Capillary melting point apparatus and are uncorrected. Thin-layer chromatographies were performed using fluorescent silica gel plates HF_{254} Merck, which were examined under UV 254 and 365 nm light, using benzene:acetone:acetic acid (8:1:1) as eluent. The elemental analysis for C, H and N were done on a Perkin-Elmer Analyzer 2440. Infrared spectra (ν/cm^{-1}) were recorded on a Shimadzu FT-IR 8400 S, using KBr discs. ^{1}H NMR spectra were recorded on JEOL EX-270 MHz NMR Spectrometer at 293 K in DMSO-d_6. Spectra were internally referenced to TMS. Peaks are reported in ppm downfield of TMS. The mass spectra were recorded on the LC/MS/MS (API 2000) Applied Biosystems. The absorbance was measured on a UV/Vis spectrophotometer Helios γ, (Thermo Spectronic, Cambridge UK).

Synthesis of 3-acetyl-2-oxo-2*H*-chromen (1)

To a cold mixture of salicylaldehyde (0.2 mole) and ethyl acetoacetate (0.2 mole), 2 ml piperidine was added by rapid stirring. After 20-30 min the yellowish precipitate was filtered off and subsequently washed with EtOH. Recystallization from water: EtOH mp 118-120 ºC (mp 117-119 ºC),[32] yield 81%.

Procedure for synthesis of 3-aryl-1-(2-oxo-2*H*-chromen-3-yl)- 2-propen-1-ones (2a-q)

Mixture of 0.031 mole of 3-acetylcoumarin (**1**) and 0.03 mole of appropriate aromatic aldehyde is dissolved in 30 mL of $CHCl_3$. Piperidine (0.02 mole) is added and the solution is refluxed for 1.5 h. $CHCl_3$ is distilled off and the precipitate is washed with MeOH.[7-10]

Synthesis of 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetohydrazide (3)[27, 28]

Methyl 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetate (0.01 mole) in EtOH (20 mL) was stirred for 30 min at room temperature. Hydrazine hydrate (0.014 mole) was added to the solution. This mixture was stirred under reflux for 1 h, and the solid was filtered. The residue was dried and then desiccated to afford a crystalline powder. The powder was recrystallized from $CHCl_3$/MeOH to afford colourless needles with,

mp 198-200 ºC (lit., mp 202-204 ºC)[28] and yield 80-90%.

Synthesis of 5-aryl-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4a-q)

Chalcones (**2a-q**) (0.05 mole) and 0.2 mole of hydrazide (3) is dissolved in 30 mL of pyridine and refluxed for 6 h. Solution is poured into crushed ice and neutralized with 2N HCl. The precipitate is filtered off, dried and recrystallized from appropriate solvent.

5-Phenyl-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4a)

This compound was prepared as yellow crystals in 75% yield, mp 235-240 ºC; FT-IR (KBr), ν_{max} 3173, 3049, 1724, 1685, 1616, 1560, 1392, 1277, 1201, 1142, 1078, 842, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.41 (dd, $1H_{trans}$, CH_2, pyr.), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.56 (s, 1H, CH), 6.90-7.54 (m, 12H, coum. and arom.), 8.40 (s, 1H, H-4 coum); MS *m/z*: 507.0 [$M+H^+$], (M=506.51). Anal. Calcd for $C_{30}H_{22}N_2O_6$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.08; H, 4.40; N, 5.50.

5-(3-Hydroxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4b)

This substance was prepared as yellow crystals in 85% yield, mp 268-269 ºC, FT- IR (KBr), ν_{max} 3283, 3084, 1720, 1612, 1538, 1391, 1276, 1253, 1151, 1078, 841, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.44 (dd, $1H_{trans}$, CH_2, pyr.), 3.95 (dd, $1H_{cis}$, CH_2, pyr.), 4.85 (s, 2H, CH_2), 5.59 (s, 1H, CH, H-5), 7.14-7.54 (m, 12H, coum. and arom.), 8.45 (s, 1H, H-4 coum), 10.12 (s, 1H, OH); MS *m/z*: 521.3 [$M-H^+$], (M=522.50). Anal. Calcd for $C_{30}H_{22}N_2O_7$: C, 68.96; H, 4.24; N, 5.36. Found: C, 68.90; H, 4.26; N, 5.32.

5-(4-Hydroxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4c)

This substance was prepared as pale yellow crystals in 85% yield, mp 289 ºC; FT- IR (KBr), ν_{max} 3286, 3074, 1720, 1693, 1612, 1558, 1512, 1390, 1269, 1153, 1078, 841, 750 cm^{-1}. ^1H NMR(DMSO-d_6) δ 2.43 (s, 3H, CH_3), 3.45 (dd, $1H_{trans}$, CH_2, pyr.), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.82 (s, 2H, CH_2), 5.59 (s, 1H, CH, H-5), 7.18-7.57 (m, 12H, coum. and arom.), 8.45 (s, 1H, H-4 coum), 10.05 (s, 1H, OH); MS *m/z*: 521.0 [$M-H^+$], (M=522.50). Anal. Calcd for $C_{30}H_{22}N_2O_7$: C, 68.96; H, 4.24; N, 5.36. Found: C, 68.92; H, 4.22; N, 5.30.

5-(2-Methoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-

pyrazoline (4d)

This compound was prepared as yellow crystals in 65% yield, mp 273 ºC, FT- IR (KBr), ν_{max} 3285, 3068, 1724, 1701, 1614, 1539, 1489, 1391, 1276, 1151, 1078, 842, 750 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.45 (dd, $1H_{trans}$, CH_2, pyr.), 3.95 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.59 (s, 1H, CH, H-5), 7.12-7.56 (m, 12H, coum. and arom.), 8.42 (s, 1H, H-4 coum); MS *m/z*: 537.3 [M+H^+], (M=536.53). Anal. Calcd for $C_{30}H_{22}N_2O_7$: C, 68.96; H, 4.24; N, 5.36. Found: C, 68.97; H, 4.20; N, 5.36.

5-(3-Methoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4e)

This substance was obtained as orange crystals in 80% yield, mp 213 ºC; FT- IR (KBr), ν_{max} 3311, 3097, 1720, 1664, 1606, 1541, 1483, 1450, 1291, 1184, 1049, 978, 760 cm^{-1}; ^1H NMR(DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.46 (dd, $1H_{trans}$, CH_2, pyr.), 3.73 (s, 3H, OCH_3), 3.94 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.59 (s, 1H, CH, H-5), 7.18-7.60 (m, 12H, coum. and arom.), 8.44 (s, 1H, H-4 coum); MS *m/z*: 537.0 [M+H^+], (M=536.53). Anal. Calcd for $C_{31}H_{24}N_2O_7$: C, 69.40; H, 4.51; N, 5.22. Found: C, 69.38; H, 4.49; N, 5.24.

5-(4-Methoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4f)

This substance was prepared as pale yellow in 78% yield, mp 267 ºC; FT- IR (KBr), ν_{max} 3282, 3174, 1724, 1685, 1620, 1537, 1429, 1392, 1300, 1153, 1078, 841, 748 cm^{-1}; ^1H NMR(DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.42 (dd, $1H_{trans}$, CH_2, pyr.), 3.71 (s, 3H, OCH_3), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.59 (s, 1H, CH, H-5), 7.16-7.64 (m, 12H, coum. and arom.), 8.44 (s, 1H, H-4 coum); MS *m/z*: 537.2 [M+H^+], (M=536.53). Anal. Calcd for $C_{31}H_{24}N_2O_7$: C, 69.40; H, 4.51; N, 5.22. Found: C, 69.41; H, 4.48; N, 5.20.

5-(2-Chlorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4g)

This substance was prepared as orange crystals in 81% yield, mp 205 ºC; FT- IR (KBr), ν_{max} 3284, 3157, 1724, 1691, 1618, 1560, 1508, 1489, 1390, 1277, 1153, 1078, 841, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.46 (dd, $1H_{trans}$, CH_2, pyr.), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.82 (s, 2H, CH_2), 5.58 (s, 1H, CH, H-5), 7.16-7.64 (m, 12H, coum. and arom.), 8.45 (s, 1H, H-4 coum); MS *m/z*: 542.2 [M+H^+], (M=540.95). Anal. Calcd for $C_{30}H_{21}ClN_2O_6$: C, 66. 61; H, 3.91; N, 5.18. Found: C, 66.59; H, 3.90; N, 5.21.

5-(3-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4h)

This substance was prepared as orange crystals in 70% yield, mp 199 ºC; FT-IR (KBr), ν_{max} 3287, 3064,

1724, 1701, 1616, 1558, 1390, 1276, 1203, 1153, 1078, 843, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.42 (dd, $1H_{trans}$, CH_2, pyr.), 3.97 (dd, $1H_{cis}$, CH_2, pyr.), 4.84 (s,2H, CH_2), 5.56 (s, 1H, CH, H-5), 7.16-7.64 (m, 12H, coum. and arom.), 8.45 (s,1H, H-4 coum); MS *m/z*: 587.0 [M+H^+], (M=585.40). Anal. Calcd for $C_{30}H_{21}BrN_2O_6$: C, 61.55; H, 3.62; N, 4.79. Found: C, 61.50; H, 3.64; N, 4.82.

5-(4-Bromophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4i)

This substance was prepared as yellow-orange crystals in 68% yield, mp 289 ºC; FT- IR (KBr), ν_{max} 3288, 3080, 1724, 1614, 1557, 1487, 1390, 1277, 1201, 1151, 1076, 849, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.46 (dd, $1H_{trans}$, CH_2, pyr.), 3.97 (dd, $1H_{cis}$, CH_2, pyr.), 4.82 (s,2H, CH_2), 5.56 (s, 1H, CH, H-5), 7.16-7.64 (m, 12H, coum. and arom.), 8.44 (s,1H, H-4 coum); MS *m/z*: 587.4 [M+H^+], (M=585.40). Anal. Calcd for $C_{30}H_{21}BrN_2O_6$: C, 61.55; H, 3.62; N, 4.79. Found: C, 61.57; H, 3.61; N, 4.80.

5-(4-Fluorophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4j)

This substance was prepared as pale yellow crystals in 64% yield, mp 260 ºC; FT-IR (KBr), ν_{max} 3285, 3084, 1724, 1685, 1620, 1537, 1489, 1392, 1300, 1277, 1201, 1153, 1078, 841, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.45 (dd, $1H_{trans}$, CH_2, pyr.), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.54 (s, 1H, CH, H-5), 7.16-7.68 (m, 12H, coum. and arom.), 8.45(s,1H, H-4 coum); MS *m/z*: 525.60 [M+H^+], (M=524.50). Anal. Calcd for $C_{30}H_{21}FN_2O_6$: C, 68.70; H, 4.04; N, 5.34. Found: C, 68.71; H, 4.07; N, 5.32.

5-(3-Phenoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4k)

This substance was prepared as brown crystals in 70% yield, mp 225 ºC; FT-IR (KBr), ν_{max} 3282, 3063, 1724, 1689, 1612, 1560, 1532, 1489, 1300, 1300, 1274, 1202, 1153, 1078,748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.46 (dd, $1H_{trans}$, CH_2, pyr.), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.82 (s, 2H, CH_2), 5.55 (s, 1H, CH, H-5), 7.06-7.68 (m, 18H, coum. and arom.), 8.45 (s, 1H, H-4 coum); MS *m/z*: 599.80 [M+H^+], (M=598.60). Anal. Calcd for $C_{36}H_{26}N_2O_7$: C, 72.23; H, 4.38; N, 4.68. Found: C, 72.20; H, 4.40; N, 4.66.

5-(4-Dimethylaminophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxy-acetyl)-2-pyrazoline (4l)

This substance was prepared as red crystals in 84% yield, mp 173 ºC; FT-IR (KBr), ν_{max} 3433, 3288, 1732, 1608, 1570, 1532, 1371, 1276, 1176, 1078, 987, 812, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 2.85 (s, 6H, $N(CH_3)_2$), 3.46 (dd, $1H_{trans}$, CH_2, pyr.), 3.97 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.55 (s,

1H, CH, H-5), 7.16-7.68 (m, 13H, coum. and arom.), 8.44 (s, 1H, H-4 coum); MS *m/z*: 550.10 [M+H$^+$], (M=549.57). Anal. Calcd for $C_{32}H_{27}N_3O_6$: C, 69.93; H, 4.95; N, 7.65. Found: C, 69.90; H, 4.97; N, 7.61.

5-Styryl-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4m)

This substance was prepared as pale yellow crystals in 84% yield, mp 280-281 ºC; FT-IR (KBr), ν_{max} 3284, 3057, 1720, 1612, 1558, 1448, 1389, 1265, 1149, 1076, 972, 848, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 3.46 (dd, 1H$_{trans}$, CH_2, pyr.), 3.97 (dd, 1H$_{cis}$, CH_2, pyr.), 4.82 (s, 2H, CH_2), 5.54 (s, 1H, CH, H-5), 7.06-7.66 (m, 16H, HC=CH, coum. and arom.), 8.44 (s, 1H, H-4 coum); MS *m/z*: 531.40 [M-H$^+$], (M=532.54). Anal. Calcd for $C_{32}H_{24}N_2O_6$: C, 72.17; H, 4.54; N, 5.26. Found: C, 72.10; H, 4.51; N, 5.23.

5-(4-Hydroxy-3-methoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4n)

This substance was prepared as brown crystals in 84% yield, mp 167 ºC; FT-IR (KBr), ν_{max} 3288, 3078, 1724, 1691, 1618, 1559, 1512, 1392, 1274, 1201, 1155, 1080, 841, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 3.44 (dd, 1H$_{trans}$, CH_2, pyr.), 3.73 (s, 3H, OCH_3), 3.96 (dd, 1H$_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.56 (s, 1H, CH, H-5), 7.16-7.66 (m, 12H, coum. and arom.), 8.44 (s, 1H, H-4 coum), 9.95 (s, 1H, OH); MS *m/z*: 551.10 [M-H$^+$], (M=552.53). Anal. Calcd for $C_{31}H_{24}N_2O_8$: C, 67.39; H, 4.38; N, 5.07. Found: C, 67.34; H, 4.40; N, 5.00.

5-(2,4-Dihydroxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4o)

This substance was prepared as brown crystals in 65% yield, mp 184-185 ºC; FT-IR (KBr), ν_{max} 3269, 3068, 1685, 1612, 1538, 1508, 1391, 1265, 1203, 1151, 1078, 842, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.46 (dd, 1H$_{trans}$, CH_2, pyr.), 3.97 (dd, 1H$_{cis}$, CH_2, pyr.), 4.83 (s, 2H, CH_2), 5.52 (s, 1H, CH, H-5), 7.14-7.68 (m, 11H, coum. and arom.), 8.46 (s, 1H, H-4 coum), 9.95 (s, 1H, OH), 10.05 (s, 1H, OH); MS *m/z*: 537.20 [M-H$^+$], (M=538.50). Anal. Calcd for $C_{30}H_{22}N_2O_8$: C, 66.91; H, 4.12; N, 5.20. Found C, 66.88; H, 4.13; N, 5.10.

5-(3,4-Dihydroxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxyacetyl)-2-pyrazoline (4p)

This substance was prepared as oker crystals in 60% yield, mp 264 ºC; FT-IR (KBr), ν_{max} 3286, 3159, 1724, 1691, 1618, 1539, 1489, 1390, 1276, 1203, 1155, 1074, 843, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 3.46 (dd, 1H$_{trans}$, CH_2, pyr.), 3.96 (dd, 1H$_{cis}$, CH_2, pyr.), 4.83 (s,2H, CH_2), 5.54 (s, 1H, CH,

H-5), 7.14-7.68 (m, 11H, coum. and arom.), 8.44 (s,1H, H-4 coum.), 9.85 (s, 1H, OH), 10.08 (s, 1H, OH); MS *m/z*: 537.10 [M-H^+], (M=538.50). Anal. Calcd for $C_{30}H_{22}N_2O_8$: C, 66.91; H, 4.12; N, 5.20. Found: C, 66.89; H, 4.15; N, 5.23.

5-(3,4,5-Trimethoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1-(4-methyl-2-oxo-2*H*-chromen-7-yloxy-acetyl)-2-pyrazoline (4q)

This substance was prepared as yellow crystals in 78% yield, mp 277 ºC; FT-IR (KBr) ν_{max} 3287, 3067, 2941, 1720, 1693, 1612, 1560, 1508, 1421, 1390, 1274, 1202, 1155, 1074, 846, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.46 (dd, $1H_{trans}$, CH_2, pyr.), 3.73 (s, 9H, OCH_3), 3.96 (dd, $1H_{cis}$, CH_2, pyr.), 4.83 (s,2H, CH_2), 5.54 (s, 1H, CH, H-5), 7.14-7.68 (m, 10H, coum. and arom.), 8.44 (s,1H, H-4 coum.); MS *m/z*: 597.10 [M+H^+], (M=596.58). Anal. Calcd for $C_{33}H_{28}N_2O_9$: C, 66.44; H, 4.73; N, 4.70. Found: C, 66.44; H, 4.73; N, 4.70.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl radical

Determination of antioxidant activity was performed according to the procedure described in the literature[33,34] with some modifications. DMSO was used as a solvent,[35] due to the low solubility of synthesized compounds in EtOH and MeOH.

0.75 mL of DMSO solution of the corresponding synthesized compound (0.2 mM) was added to a DMSO solution of DPPH radical (0.2 mM), so that the final concentration of DPPH radical and the synthesized compound in a solution was 0.1 mM. The mixture was shaken and allowed to stand at room temperature. After 30 min the absorbance at 517 nm was determined and the scavenging activity was calculated according to the formula below. Ascorbic acid (AA) was used as a reference compound.

$$\text{scavenging actvitiy (\%)} = ((Ab + As - Am)/Ab)*100$$

Ab – absorbance of 0.1 mM DMSO solution of DPPH radical at 517 nm

As – absorbance of 0.1 mM DMSO solution of test compound at 517 nm

Am – absorbance of DMSO mixture of test compound and DPPH radical at 517 nm

Evaluation of antioxidant activity by phosphomolybdenum method

The antioxidant activity of tested coumarin derivatives was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*[36] An aliquot of 100 μL of sample solution (2mM in DMSO) is mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated in a water bath at 95 °C for 90 min. Samples are cooled to room temperature and the absorbance was measured at 695 nm. The antioxidant activity was expressed relative to the antioxidant activity of the same concentration of ascorbic acid.

Iron chelating activity

The chelating activity of dipicolinic acid derivatives for ferrous ions Fe^{2+} was measured according to the literature method[37] with some modifications. 0.025 mL of $FeCl_2$ (2 mM) was added to 1 mL 2mM MeOH/DMSO solution (4:1) of the compound investigated. After 30 s, 0.05 mL of ferrozine (5 mM) was added. Samples were incubated at room temperature for 10 min and the absorbance of the complex formed between Fe^{2+} and ferrozine was measured at 562 nm. Metal chelating efficiency of samples was compared to the chelating activtiy of EDTA disodium salt. The chelating activity of the extract for Fe^{2+} was calculated as:

$$\text{Chelating rate} = [(A_0 - A_1)/ A_0] \cdot 100\%$$

A_0 - absorbance of the control (blank, without samples) at 562 nm

A_1 - absorbance in the presence of the MeOH/DMSO sample solution at 562 nm

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