Antioxidant activity of some (7-hydroxy-2-oxo-2*H*-chromen-4-yl) acetic acid derivatives

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Summary

A series of coumarin Schiff bases (7-(arylidenehydrazinocarbonylmethoxy)-2-oxo-2*H*-chromen-4-yl]acetic acid arylidenehydrazide) (**4a-k)** and *N*-(2-aryl-4-oxothiazolidin-3-yl)-2-{[4-(2-aryl-4-oxothiazolidin-3-yl)carbamoylmethyl]-2-oxo-2*H*-chromen-7-yloxy}-acetamides (**5a-k**) were synthesized and evaluated for their antioxidant activity (scavenging of 1,1-diphenyl-2-picryhydrazyl radical and phosphomolybdenum method). Compounds with 3,4-dihydroxyphenyl and 2,5-dihidroxyphenyl ring showed the best 1,1-diphenyl-2-picryhydrazyl (DPPH) scavenging activity, whereas the best activity investigated by phosphomolybdenum method was found for the compounds with 2,5-dihidroxydiphenyl ring.

Keywords: coumarin, antioxidant activity, Schiff base, 4-thiazolidinone

Introduction

Coumarins belong to a benzopyrone chemical class characterized with condensed benzene and pyrone ring. In nature, they can be found as free or in a combination with glycosides (Ojala, 2001). Coumarins are present in many dicotiledones, such as *Apiaceae* (*Ammi majus*), *Asteraceae* (*Trilisa odoratissima*), *Fabiaceae* (*Melilotus officinalis*), *Rosaceae* (*Prunus mahaleb* L.), *Rubiaceae* (*Asperula odorata*) and *Solanaceae* (*Atropa belladonna*) (Wienmann, 1997), especially *Rutaceae* i *Umbelliferae* (Lacy and O'Kennedy, 2004). Some coumarins were also isolated from microbial sources, like novobiocin and coumermycin from *Streptomyces*, aflatoxins from *Aspergillus* species (Lacy and O'Kennedy, 2004).
Moreover, it has been found that distribution of biologically active coumarins in plants correlates with their ability to act as phytoalexins. Coumarins are synthesized in plants as a response to traumatic injury, during the wilting process, by plant diseases or through drying. They accumulate on the surface of the leaves, fruits and seeds, and they inhibit the growth and sporulation of fungal plant pathogens and act as repellents against beetles and other terrestrial invertebrates (Weinmann, 1997). Also, presence of coumarins is often defence of the plants against various pathogenic fungi (Razavi, 2011; Kai et al., 2006) or responce to adverse conditions (Razavi, 2011).

Coumarins with styryl carbonyl group have been found to be very important in scavenging of reactive oxygen species (ROS), thus contributing to the prevention of oxidative damage caused by free radicals (Fylaktakidou et al., 2004; Manojkumar et al., 2009). Antioxidant activity of coumarins and their derivatives as well as their pharmacological and biochemical properties depend on their structure feature (Natella et al., 2010; Malhotra et al., 2008). The scavenging of ROS includes various mechanisms. By binding of Fe(III) coumarins can act as inhibitors of hydroxyl radicals formation and hydrogen peroxide formed in Fenton reaction. Coumarins with hydroxyl groups inhibit xantine oxidase (Vukovic et al., 2010; Traykova, Kostova, 2005) or lipid peroxidation (Roussaki et al., 2010; Bailly et al., 2004) and thus scavenge DPPH radicals (Bailly et al., 2004). It has been found that coumarins should contain at least one hydroxyl group to exhibit antioxidant activity (Traykova, Kostova, 2005), which is ascribed to H· donor ability of the hydroxyl groups for free radical acceptors (Sharma et al., 2005). Moreover, type and position of substituents are crucial for antioxidant activity (Natella et al., 2010). 4-Methylcoumarins possessing two hydroxy or acetoxy groups in *ortho* position are potent antioxidants and free radical scavengers (Malhotra et al., 2008; Morabito et al., 2010) which also proves that not only the type, but also the position of the substituents too, is important for antioxidant activity.
The aim of this work was the synthesis of coumarin compounds with potential antioxidant activity.

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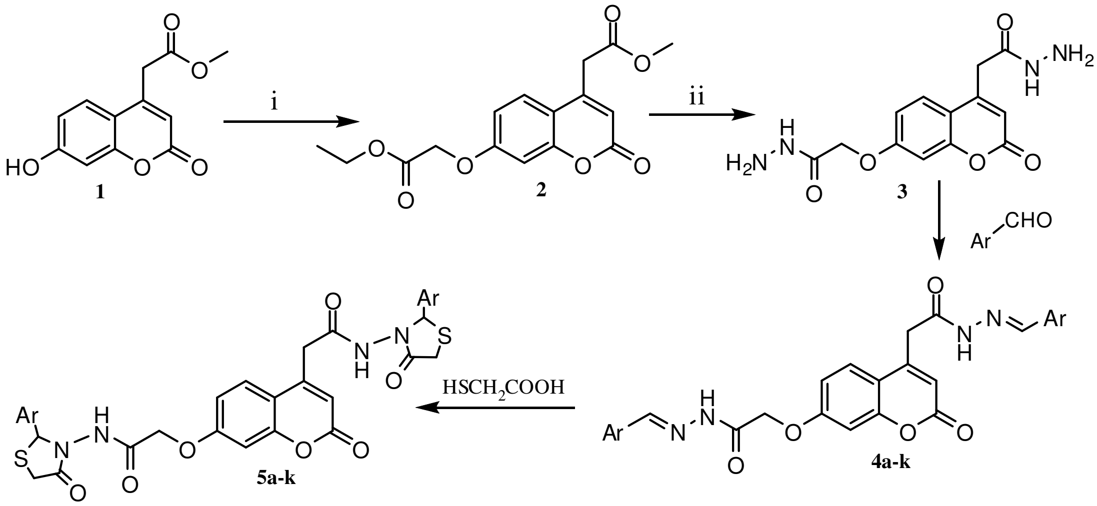
Materials and methods

All the chemicals were purchased from commercial suppliers.
The absorbance was measured on UV visible spectrophotometer Heliosγ, (Thermo Spectronic, Cambridge, UK).

Tested (7-hydroxy-2-oxo-2H-chromen-4-yl)acetic acid derivatives

A series of Schiff bases (7-(arylidene-hydrazinocarbonylmethoxy)-2-oxo-2*H*-chromen-4-yl)-acetic acid aryilidene hydrazides **(4a-k)** and *N*-(2-aryl-4-oxothiazolidin-3-yl)-2-{[4-(2-aryl-4-oxothiazolidin-3-yl)carbamoylmethyl]-2-oxo-2*H*-chromen-7-yloxy}-acetamides (**5a-k)** were prepared according to the procedure described by Cacic et al. (2009). Synthetic path and structures of the tested compounds, Schiff bases (**4a-k)** and thiazolidine-4-ones (**5a-k)** are shown in Fig. 1.



entry	Ar	entry	Ar
a	phenyl	**g**	4-hydroxy-3-methoxyphenyl
b	2-chlorophenyl	**h**	3-phenoxyphenyl
c	3-chlorophenyl	**i**	4-dimethylaminophenyl
d	2,4-dihydroxyphenyl	**j**	2-hydroxy-5-nitrophenyl
e	3,4-dihydroxyphenyl	**k**	styryl
f	2,5-dihidroxyphenyl		

Fig. 1. Synthetic path for Schiff bases (**4a-k**) and 4-thiazolidinones (**5a-k**) (i-$BrCH_2COOC_2H_5$, K_2CO_3; ii-H_2NNH_2)

Structures of all the compounds were elucidated and confirmed by various methods (Cacic et al., 2009).

*Synthesis of (7-ethoxycarbonylmethoxy-2-oxo-2H-chromen-4-yl)-acetic acid methylester (**2**)* (Cacic et al., 2009)

A mixture of (7-hydroxy-2-oxo-2*H*-chromen-4-yl)-acetic acid methylester (**1**, 25.74 g, 0.11 mole), anhydrous potassium carbonate (15.20 g, 0.11 mole) and ethyl bromoacetate (18.37 g, 0.11 mole) in dry acetone (200 mL) was refluxed with continuous stirring for 12 hours. After filtration, the solution was concentrated under reduced pressure, vacuum dried and the solid product was recrystallized from ethanol.
M.p.185-186 °C, yield 64 %; IR: ν_{max} 3429, 2986, 2941, 1753, 1724, 1619, 1439, 1393, 1341, 1221, 1198, 1089 cm^{-1}; ^{1}H-NMR: δ 7.76 (d, 1H, H-5), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.92 (s, 2H, -OCH_2), 4.19 (q, 2H, CH_2, -CH_2CH_3), 4.02 (s, 2H, CH_2), 3.65 (s, 3H, OCH_3), 1.22 (t, 3H, CH_3, -CH_2CH_3); ^{13}C-NMR: δ 14.2 (CH_2CH_3), 34.8 (CH_2CO), 52.1 (OCH_3), 61.3 (CH_2CH_3), 65.5 ($COCH_2O$), 109.6 (C-8), 112.8 (C-6), 113.8 (C-3), 114.8 (C-10), 128.3 (C-5), 151.2 (C-9), 155.2 (C-4), 160.3 (C-7), 160.9 (C-2), 168.9 (CO-O), 169.3 (C-CO-C); Anal. Calcd. For $C_{16}H_{16}O_7$: C, 60.00; H, 5.04; Found: C, 59.98; H, 5.01 %.

Synthesis of (7-hydrazinocarbonylmethoxy-2-oxo-2H-chromen-4-yl)-acetic acid hydrazide (3)

To a solution of methanol (120 mL) and 86 % hydrazine hydrate (12 mL) (7-ethoxycarbonylmethoxy-2-oxo-2*H*-chromen-4-yl)-acetic acid methylester (**2**, 3.2 g, 0.01 mole) was added, and the mixture was left to stand overnight at 5 °C. The product precipitated and was collected by suction filtration, washed with methanol (petrolether) and recrystallized from dil. acetic acid. M.p. >300 °C, yield 70 %; IR: ν_{max} 3461, 3325 (NH), (NH_2), 1707 (lactone C=O), 1623 (C=O, amide), 1516 (C=C, arom.), 1430, 1298, 1277 and 1153 cm^{-1}; ^{1}H-NMR: δ 9.41 (s, 1H, NH), 9.34 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.94 (s, 2H, -OCH_2), 4.34 (s, 2H, NH_2), 4.08 (s, 2H, CH_2), 3.38 (s, 2H, NH_2); ^{13}C-NMR: δ 45.8 (CH_2), 68.9 (CH_2O-), 108.0 (C-8), 111.8 (C-6), 112.9 (C-3), 114.1 (C-10), 128.3 (C-5), 152.2 (C-9), 155.2 (C-4), 160.4 (C-7), 160.9 (C-2), 166.8 ($COCH_2O$), 169.6 ($COCH_2$); Anal. Calcd. for $C_{13}H_{14}N_4O_5$: C, 50.98; H, 4.61; N, 18.29; Found: C, 51.02; H, 4.58; N, 18.25 %.

*Preparation of (7-(arylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl)-acetic acid aryilidene-hydrazides (**4a-k**)*

A mixture of (7-hydrazinocarbonylmethoxy-2-oxo-2*H*-chromen-4-yl)-acetic acid hydrazide (**3**, 3.06 g, 0.01 mole) and appropriate aromatic aldehyde (**Ar/a-k**, 0.01 mole) was refluxed in absolute ethanol (30 mL) in the presence of a catalytic amount of glacial acetic acid for 2 to 4 hours. The reaction mixture was cooled, the solid separated was filtered and recrystallized from methanol to give compounds **4a-k**.

[7-(Benzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid benzylidene hydrazide (**4a**)
M.p. 268-269 °C; yield 74 %; IR: ν_{max} 3418, 3313 (NH), 1712, 1682 (C=O, lactone), 1666 (C=O, amide), 1613 (C=C, arom., C=N, azomet.), 1550, 1378, 1269 and 1153 cm^{-1}; ^{1}H-NMR: δ 8.30 (s, 1H, HC=N-), 8.24 (s, 1H, HC=N-), 8.06 (s, 1H, NH), 8.02 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.72-7.31 (m, 10H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.83 (s, 2H, -OCH_2), 4.28 (s, 2H, CH_2); ^{13}C-NMR: δ 45.7 (CH_2), 69.2 (CH_2O-), 108.1 (C-8), 111.6 (C-6), 112.5 (C-3), 114.4 (C-10), 127.9 (C-5), 128.4 (C-3,5, Ar-), 129.0 (C-2,6, Ar-), 131.4 (C-4, Ar-), 133.9 (C-1, Ar-), 143.6 (N=CH-), 151.8 (C-9), 155.2 (C-4), 160.4 (C-7), 160.9 (C-2), 166.9 ($COCH_2O$), 170.0 (CONH-); Anal. Calcd. for $C_{27}H_{22}N_4O_5$: C, 67.21; H, 4.60; N, 11.61; Found: C, 67.19; H, 4.61; N, 11.58 %.

[7-(2-Chlorobenzylidenehydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid (2-chlorobenzylidene)-hydrazide (**4b**)
M.p. 225-226 °C, yield (76 %); IR: ν_{max} 3428, 3283 (NH), 1710, 1692 (C=O, lactone), 1656 (C=O, amide), 1612 (C=C, arom., C=N, azomet.), 1542, 1398, 1264 and 1155 cm^{-1}; ^{1}H-NMR: δ 8.73 (s, 1H, -HC=N-), δ 8.62 (s, 1H, -HC=N-), δ 8.48 (s, 1H, NH), 8.45 (s, 1H, NH), 7.72 (d, 1H, H-5), 7.60-7.30 (m, 8H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.84 (s, 2H, -OCH_2), 4.30 (s, 2H, CH_2); 13C-NMR: δ 45.6 (CH_2), 68.9 (CH_2O-), 107.8 (C-8), 111.5 (C-6), 112.7 (C-3), 113.9 (C-10), 127.8 (C-5), 129.4 (C-3, Ar-), 130.6 (C-6, Ar-), 132.7 (C-4, Ar-), 133.8 (C-1, Ar-), 134.3 (C-2, Ar-), 143.5 (N=CH-), 151.7 (C-9), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 ($COCH_2O$), 169.9 (CONH-); Anal. Calcd. For $C_{27}H_{20}C_{12}N_4O_5$: C, 58.81; H, 3.66; N, 10.16; Found: C, 58.79; H, 3.69; N, 10.12 %.

[7-(3-Chlorobenzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid (3-chlorobenzylidene)-hydrazide (**4c**)
M.p. 259-261 °C, yield 72 %; IR: ν_{max} 3408, 3188 (NH), 1727, 1683 (C=O, lactone), 1616 (C=O, amide, C=N, azomet.), 1561 (C=C, arom.), 1394, 1262 and 1138 cm^{-1}; ^{1}H-NMR: δ 8.31 (s, 1H, -HC=N-), 8.22 (s, 1H, -HC=N-), 8.48 (s, 1H, NH), 8.45 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.67-7.30 (m, 8H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.84 (s, 2H, -OCH_2), 4.28 (s, 2H, CH_2); ^{13}C-NMR: δ 45.5 (CH_2), 69.1 (CH_2O-), 107.9 (C-8), 111.4 (C-6), 112.5 (C-3), 113.4 (C-10), 127.3 (C-6, Ar-), 127.9 (C-5), 129.3 (C-2, Ar-), 130.3 (C-5, Ar-), 131.2 (C-4, Ar-), 135.2 (C-1, Ar-), 134.4 (C-3, Ar-), 143.4 (N=CH-), 151.6 (C-9), 155.2 (C-4), 160.6 (C-7), 160.9 (C-2), 166.7 ($COCH_2O$), 169.8 (CONH-); Anal. Calcd. For $C_{27}H_{20}C_{12}N_4O_5$: C, 58.81; H, 3.66; N, 10.16; Found: C, 58.80; H, 3.68; N, 10.13 %.

[7-(2,4-Dihydroxy-benzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid (2,4-dihydroxybenzylidene)-hydrazide (**4d**)
M.p. 273-275 °C, yield 52 %; IR: ν_{max} 3434 (OH), 3366, 3092 (NH), 1712, 1672 (C=O, lactone), 1623 (C=O, amide, C=N, azomethine), 1612 (C=C, arom., C=N), 1559, 1509, 1395, 1265 and 1153 cm^{-1}; ^{1}H-NMR: δ 11.80 (s, 1H, OH), 11.17 (s, 1H, OH), 8.42 (s, 1H, -HC=N-), 8.30 (s, 1H, -HC=N-), 8.23 (s, 1H, NH), 8.19 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.61-7.30 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.82 (s, 2H, -OCH2), 4.28 (s, 2H, CH_2); ^{13}C-NMR: δ 45.6 (CH_2), 69.2 (CH_2O-), 103.8 (C-3, Ar-), 107.6 (C-8), 108.7 (C-5, Ar-), 111.3 (C-6), 112.7 (C-3), 113.4 (C-10), 127.2 (C-6, Ar-), 127.9

(C-5), 135.2 (C-1, Ar-), 143.0 (N=CH-), 151.2 (C-9), 155.1 (C-4), 160.5 (C-7), 160.9 (C-2), 162.4 (C-2, Ar-), 162.6 (C-4, Ar-), 166.5 ($COCH_2O$), 169.4 (CONH-); Anal. Calcd. For $C_{27}H_{22}N_4O_9$: C, 59.34; H, 4.06; N, 10.25; Found: C, 59.30; H, 4.07; N, 10.29 %.

[7-(3,4-Dihydroxy-benzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]- acetic acid (3,4-dihydroxybenzylidene)-hydrazide (**4e**)

M.p. 205 °C, yield 62 %; IR: ν_{max} 3408, 2922 (NH), 1725, 1664 (C=O, lactone), 1619 (C=O, amide, C=N, azomethine), 1593 (C=C, arom.), 1444, 1393, 1284 and 1152 cm^{-1}; ^{1}H-NMR: δ 11.98 (s, 1H, OH), 11.45 (s, 1H, OH), 8.41 (s, 1H, -HC=N-), 8.30 (s, 1H, -HC=N-), 8.12 (s, 1H, NH), 8.03 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.65-7.41 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.77 (s, 2H, -OCH_2), 4.22 (s, 2H, CH_2); ^{13}C-NMR: δ 45.5 (CH_2), 69.3 (CH_2O-), 107.6 (C-8), 111.4 (C-6), 112.5 (C-3), 113.5 (C-10), 116.4 (C-2, Ar-), 117.5 (C-5, Ar-), 123.3 (C-6, Ar-), 127.8 (C-5), 127.9 (C-1, Ar-), 143.1 (N=CH-), 147.4 (C-3, Ar-), 149.6 (C-4, Ar-), 151.3 (C-9), 155.0 (C-4), 160.4 (C-7), 160.9 (C-2), 166.6 ($COCH_2O$), 169.5 (CONH-); Anal. Calcd. For $C_{27}H_{22}N_4O_9$: C, 59.34; H, 4.06; N, 10.25; Found: C, 59.13; H, 4.03; N, 10.04 %.

[7-(2,5-Dihydroxybenzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]- acetic acid (2,5-dihydroxybenzylidene)-hydrazide (**4f**)

M.p. 275-276 °C, yield 76 %; IR: ν_{max} 3369, 3286 (NH), 1717, 1681, 1667 (C=O, lactone), 1624 (C=O, amide, C=N, azomethine), 1585 (C=C arom.), 1492, 1396, 1267 and 1156 cm^{-1}; ^{1}H-NMR: δ 11.95 (s, 1H,OH), 11.56 (s,1H,OH), 8.48 (s, 1H, -HC=N-), 8.34 (s, 1H, -HC=N-), 8.30 (s, 1H, NH), 8.25 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.68-7.30 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.82 (s, 2H, -OCH_2), 4.24 (s, 2H, CH_2); ^{13}C-NMR: δ 45.6 (CH_2), 69.3 (CH_2O-), 107.8 (C-8), 111.4 (C-6), 112.7 (C-3), 113.8 (C-10), 116.4 (C-6, Ar-), 117.4 (C-3, Ar-), 119.6 (C-4, Ar-), 119.9 (C-1, Ar-), 127.8 (C-5), 143.5 (N=CH-), 151.4 (C-9), 151.3 (C-5, Ar-), 153.7 (C-2, Ar-), 155.2 (C-4), 160.4 (C-7), 160.9 (C-2), 166.7 ($COCH_2O$), 169.4 (CONH-); Anal. Calcd. For $C_{27}H_{22}N_4O_9$: C, 59.34; H, 4.06; N, 10.25; Found: C, 59.32; H, 4.04; N, 10.20 %.

[7-(4-Hydroxy-3-methoxybenzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4yl]-acetic acid(4-hydroxy-3-methoxybenzylidene)-hydrazide (**4g**)

M.p. 232-233 °C, yield 84 %; IR: ν_{max} 3430, 3224 (NH), 1711, 1671 (C=O, lactone), 1622 (C=O, amide, C=N, azomethine), 1605 (C=C, arom.), 1429, 1394, 1272 and 1164 cm^{-1}; ^{1}H-NMR: δ 11.96 (s, 1H, OH), δ 11.50 (s, 1H, OH), 8.19 (s, 1H, -HC=N-), 8.10 (s, 1H, -HC=N-), 7.99 (s, 1H, NH), 7.97 (s, 1H, NH), 7.77 (d, 1H, H-5), 7.40-7.21 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.78 (s, 2H, -OCH_2), 4.24 (s, 2H, CH_2), 3.80 (s, 6H, -OCH^3); ^{13}C-NMR: δ 45.6 (CH_2), 56.0 (OCH_3), 69.2 (CH_2O-), 107.6 (C-8), 111.2 (C-6), 112.5 (C-3), 113.6(C-10), 114.8 (C-2, Ar-), 117.0 (C-5, Ar-), 122.9 (C-6, Ar-), 127.4 (C-1,Ar-), 127.8 (C-5), 143.3 (N=CH-), 148.1 (C-4, Ar-), 151.4 (C-9), 151.5 (C-3, Ar-), 155.1 (C-4), 160.4 (C-7), 160.9 (C-2), 166.8 ($COCH_2O$), 169.8 (CONH-); Anal. Calcd. For $C_{29}H_{26}N_4O_9$: C, 60.62; H, 4.56; N, 9.75; Found: C, 60.59; H, 4.75; N, 9.70 %.

[7-(3-Phenoxybenzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid 3-phenoxybenzylidene)-hydrazide (**4h**)

M.p. 236-237 °C, yield 57 %; IR: ν_{max} 3409, 3071 (NH), 1726, 1685 (C=O, lactone), 1624 (C=O, lactone, C=N, azomethine), 1597 (C=C, arom.), 1490, 1394, 1261 and 1156 cm^{-1}; ^{1}H-NMR: δ 8.30 (s, 1H, -HC=N-), 8.21 (s, 1H, -HC=N-), 8.03 (s, 1H, NH), 7.99 (s, 1H, NH), 7.76 (d, 1H, H-5), 7.70-7.10 (m, 18H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.79 (s, 2H, -OCH_2), 4.18 (s, 2H, CH_2); ^{13}C-NMR: δ 45.5 (CH_2), 56.1 (OCH_3), 69.1 (CH_2O-), 107.8 (C-8), 111.4 (C-6), 112.7 (C-3), 113.5 (C-10), 116.6 (C-2, Ar-), 117.5 (C-2,6, Ar-PhO), 119.8 (C-4, Ar-), 121.9 (C-4, Ar- PhO), 122.3 (C-2, Ar-), 127.8 (C-5), 128.5 (C-3,5 Ar- PhO), 128.9 (C-5 Ar-), 133.5 (C-1 Ar-), 143.4 (N=CH-), 151.3 (C-9), 155.4 (C-4), 157.1 (C-1 Ar- PhO), 157.1 (C-3 Ar-), 160.4 (C-7), 160.9 (C-2), 166.8 ($COCH_2O$), 170.0 (CONH-); Anal. Calcd. For $C_{39}H_{30}N_4O_7$: C,70.26; H, 4.54; N, 8.40; Found: C, 70.23; H, 4.55; N, 8.37 %.

[7-(4-N,N-Dimethylaminobenzylidene-hydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid (4-N,N-dimethylaminobenzylidene)-hydrazide (**4i**)

M.p. 207-209 °C, yield 63 %; IR: ν_{max} 3408, 3082 (NH), 1724, 1679 (C=O, lactone), 1623 (C=O, amide, C=N, azomethine), 1604 (C=C, arom.), 1554, 1525, 1364, 1269 and 1181 cm^{-1}; ^{1}H-NMR: δ 8.49 (s, 1H, -HC=N-), 8.44 (s, 1H, -HC=N-), 8.17 (s, 1H, NH), 8.07 (s, 1H, NH), 7.66 (d, 1H, H-5), 7.24-7.52 (m, 8H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.74 (s, 2H, -OCH_2), 4.18 (s, 2H, CH_2), 3.32 (s, 6H, -$N(CH_3)_2$), 2.99 (s, 6H, -$N(CH_3)_2$); ^{13}C-NMR: δ 40.3 (CH_3N-), 45.6 (CH_2), 69.1 (CH_2O-), 107.6 (C-8), 111.3 (C-6), 112.7 (C-3), 113.8 (C-10), 114.4 (C-3,5 Ar-), 123.3 (C-1, Ar-), 127.8 (C-5), 130.2 (C-2,6 Ar-), 143.3 (N=CH-), 151.4 (C-9), 151.0 (C-4, Ar-), 155.5 (C-4), 160.6 (C-7), 160.9 (C-2), 166.7 ($COCH_2O$), 169.8 (CONH-); Anal. Calcd. For $C_{31}H_{32}N_6O_5$: C, 64.97; H, 5.45; N, 15.15; Found: C, 65.89; H, 5.58; N, 15.11 %.

[7-(2-Hydroxy-5-nitrobenzylidenehydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid (2-hydroxy-5-nitrobenzylidene)-hydrazide (**4j**)
M.p. 204 °C, yield 82 %; IR: ν_{max} 3367, 3272 (NH), 1706, 1689 (C=O, lactone), 1616 (C=O, C=N, azomethine), 1600 (C=C, arom.), 1577, 1517, 1481, 1342, 1287 and 1150 cm^{-1}; ^{1}H-NMR: δ 12.02 (s, 2H, OH), 8.71 (s, 1H, -HC=N-), 8.59 (s, 1H, -HC=N-), 8.36 (s, 1H, NH), 8.31 (s, 1H, NH), 7.67 (d, 1H, H-5), 7.32-7.54 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.84 (s, 2H, $-OCH_2$), 4.08 (s, 2H, CH_2); ^{13}C-NMR: δ 45.7 (CH_2), 69.2 (CH_2O-), 107.8 (C-8), 111.5 (C-6), 112.4 (C-3), 113.8 (C-10), 116.9 (C-3, Ar-), 119.4 (C-1, Ar-), 124.8 (C-4, Ar-), 125.5 (C-2, Ar-), 127.8 (C-5), 141.6 (C-5, Ar-), 143.4 (N=CH-), 151.4 (C-9), 155.4 (C-4), 160.8 (C-7), 160.9 (C-2), 166.2 (C-2, Ar-), 166.8 (COCH2O), 170.0 (CONH-); Anal. Calcd. For $C_{27}H_{20}N_6O_{11}$: C, 53.65; H, 3.33; N, 13.90; Found: C, 53.63; H, 3.35; N, 13.91 %.

[2-Oxo-7-(3-phenylallylidenehydrazinocarbonylmethoxy)-2-oxo-2H-chromen-4-yl]-acetic acid (3-phenylallylidene)-hydrazide (**4k**)
M.p. 290-292 °C, yield 68 %; IR: ν_{max} 3428, 3256 (NH), 1718 (C=O, lactone), 1624 (C=O, amide, C=N, azomethine), 1613 (C=C, arom.), 1560, 1509, 1393, 1266 and 1151 cm^{-1}; ^{1}H-NMR: δ 8.38 (s, 1H, -HC=N-), 8.24 (s, 1H, -HC=N-), 8.15 (s, 1H, NH), 8.08 (s, 1H, NH), 7.78 (2d, 4H, -HC=CH-), 7.64 (d, 1H, H-5), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 4.77 (s, 2H, $-OCH_2$), 4.08 (s, 2H, CH_2); ^{13}C-NMR: δ 45.6 (CH_2), 69.1 (CH_2O-), 107.8 (C-8), 111.4 (C-6), 112.8 (C-3), 113.4 (C-10), 126.3 (C-2, Ar-), 126.4 (C-2,6, Ar-), 127.8 (C-5), 128.0 (C-4, Ar-), 128.9 (C-3,5, Ar-), 135.1 (C-1, Ar-), 139.0 (C-3, Ar-), 143.3 (N=CH-), 151.2 (C-9), 155.4 (C-4), 160.5 (C-7), 160.9 (C-2), 166.7 ($COCH_2O$), 169.8,(CONH-); Anal. Calcd. For $C_{31}H_{26}N_4O_5$: C, 69.65; H, 4.90; N, 10.48; Found: C, 69.67; H, 4.88; N, 10.45 %.

*Preparation of N-(2-aryl-4-oxo-thiazolidin-3-yl)-2-(4-(2-aryl-4-oxothiazolidin-3-ylcarbamoyl)-methyl)-2-oxo-2H-chromen-7-yloxy)-acetamides (**5a-k**)*

A mixture of (7-(arylidene-hydrazinocarbonylmethoxy)-2-oxo-2*H*-chromen-4-yl)-acetic acid aryilidenehydrazide (**4a-k**, 0.01 mole) and mercaptoacetic acid (1.82 g, 0.02 mole) in DMF (30 mL) containing a pinch of anhydrous $ZnCl_2$ was refluxed 6-8 hours. The reaction mixture was cooled and poured onto crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from DMF yielding **5a-k**.

2-{2-Oxo-7-[(4-oxo-2-phenylthiazolidin-3-ylcarbamoyl)-methoxy]-2H-chromen-4-yl}-N-(4-oxo-2-phenylthiazolidin-3-yl)acetamide (**5a**)
M.p. 202-204 °C, yield 40 %; IR: ν_{max} 3418, 3313 (NH), 1712, 1682 (C=O, lactone), 1666 (C=O, amide), 1613 (C=C, arom.), 1550, 1378, 1269 and 1153 cm^{-1}; ^{1}H-NMR: δ 8.22 (s, 1H, -NH), 8.12 (s, 1H, -NH), 7.76 (d, 1H, H-5), 7.71-7.23 (m, 10H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, -SCHN-), 4.83 (s, 2H, $-OCH_2$), 4.28 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$-); ^{13}C-NMR: δ 35.8 ($COCH_2S$), 45.5 (CH_2), 57.4 (NCHS), 69.1 (CH2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 127.2 (C-4, Ar-), 127.8 (C-5), 128.7 (C-3,5, Ar-), 128.8 (C-2,6 Ar-), 139.2 (C-1, Ar-), 151.2 (C-9), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 ($COCH_2O$), 168.8 (SCH_2CO-N), 173.3 (CONH-); Anal. Calcd. For $C_{31}H_{26}N_4O_7S_2$: C, 59.94; H, 4.16; N, 8.88; S, 10.17; Found: C, 60.05; H, 4.14; N, 8.91; S, 10.14 %.

N-[2-(2-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-2-(7-{[2-(2-chlorophenyl)-4-oxo-thiazolidin-3-ylcarbamoyl]-methoxy}-2-oxo-2H-chromen-4-yl)-acetamide (**5b**)
M.p. 184 °C, yield 76 %; IR: ν_{max} 3425, 3283 (NH), 1692 (C=O, lactone), 1656 (C=O, amide), 1612 (C=C, arom.), 1542, 1398, 1264 and 1155 cm^{-1}; ^{1}H-NMR: δ 8.73 (s, 1H, NH-), 8.62 (s, 1H, NH-), 7.76 (d, 1H, H-5), 7.60-7.30 (m, 8H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.84 (s, 2H, $-OCH_2$), 4.30 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$); ^{13}C-NMR: δ 35.7 ($COCH_2S$), 45.5 (CH_2), 57.4 (NCHS), 69.10 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 127.0 (C-5), 129.0 (C-3, Ar-), 130.6 (C-6, Ar-), 132.5 (C-4, Ar-), 133.4 (C-1, Ar-), 134.0 (C-2, Ar-), 143.0 (N=CH-), 151.2 (C-9), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 ($COCH_2O$), 173.0 (CONH-); Anal. Calcd. For $C_{31}H_{24}C_{12}N_4O_7S_2$: C, 53.22; H, 3.46; N, 8.01; S, 9.17; Found: C, 53.18; H, 3.44; N, 7.89; S, 9.20 %.

N-[2-(3-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-2-(7-{[2-(3-chlorophenyl)-4-oxo-thiazolidin-3-ylcarbamoyl]-methoxy}-2-oxo-2H-chromen-4-yl)-acetamide (**5c**)
M.p. 240-241 °C, yield 72 %; IR: ν_{max} 3450, 3188 (NH), 1727, 1683 (CO, lactone), 1616 (C=O, amide), 1598 (C=C, arom.), 1394, 1262 and 1138 cm^{-1}; ^{1}H-NMR: δ 8.31 (s, 1H, NH-), 8.22,(s, 1H, NH-), 7.76 (d, 1H, H-5), 7.67-7.30 (m, 8H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.84 (s, 2H, $-OCH_2$), 4.28 (s, 2H, CH_2),

3.38 (s, 2H, $COCH_2S$); [13]C-NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 57.4 (NCHS), 69.10 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 127.3 (C-6, Ar-), 127.8 (C-5), 129.3 (C-2, Ar-), 130.3 (C-5, Ar-), 131.2 (C-4, Ar-), 135.2 (C-1, Ar-), 134.4 (C-3, Ar-), 143.0 (N=CH-), 151.2 (C-9), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 173.0 ($COCH_2O$), 173.0 (CONH-); Anal. Calcd. For $C_{31}H_{24}Cl_2N_4O_7S_2$: C, 53.22; H, 3.46; N, 8.01; S, 9.17; Found: C, 53.18; H, 3.44; N, 7.89; S, 9.20 %.

N-[2-(2,4-Dihydroxyphenyl)-4-oxo-thiazolidin-3-yl]-2-(4-{[2-(2,4-dihydroxyphenyl)-4-oxo-thiazolidin-3-ylcarbamoyl]-methyl}-2-oxo-2H-chromen-7-yloxy)-acetamide (**5d**)

M.p. 239-241 °C, yield 52 %; IR: ν_{max} 3266 (OH), 3092 (NH), 1712, 1672 (C=O, lactone), 1624 (C=O, amide), 1612 (C=C, arom.), 1559, 1509, 1395, 1265 and 1153 cm^{-1}; [1]H-NMR: δ 11.80 (s, 1H, OH), 11.17 (s, 1H, OH), 8.42 (s, 1H, NH-), 8.30 (s, 1H, NH-), 7.76 (d, 1H, H-5), 7.61-7.30 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.82 (s, 2H, -OCH_2), 4.28 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$); [13]C-NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 47.4 (NCHS), 69.10 (CH_2O-), 103.7 (C-3, Ar-), 107.6 (C-8), 108.4 (C-5, Ar-), 110.1 (C-1, Ar-), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 127.8 (C-5), 131.3 (C-6, Ar-), 151.2 (C-9), 157.2 (C-2, Ar-), 158.2 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 (CONH), 168.8 ($NCOCH_2$), 173.3 (CH_2CONH); Anal. Calcd. For $C_{31}H_{26}N_4O_{11}S_2$: C, 53.60; H, 3.77; N, 8.07; S, 9.23; Found: C, 53.58; H, 3.79; N, 7.98; S, 9.20 %.

N-[2-(3,4-Dihydroxyphenyl)-4-oxo-thiazolidin-3-yl]-2-(7-{[2-(3,4-dihydroxyphenyl)-4-oxo-thiazolidin-3-ylcarbamoyl]-methoxy}-2-oxo-2H-chromen-4-yl)-acetamide (**5e**)

M.p. 198-200 °C, yield 47 %; IR: ν_{max} 3388 (NH), 2922 (OH), 1725, 1694 (C=O, lactone), 1619 (C=O, amide), 1523, 1444, 1393, 1284 and 1152 cm^{-1}; [1]H-NMR: δ 11.98 (s, 1H, OH), 11.45 (s, 1H, OH), 8.41 (s, 1H, NH), 8.30 (s, 1H, NH-), 7.76 (d, 1H, H-5), 7.65-7.41 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.77 (s, 2H, -OCH_2), 4.22 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$); [13]C-NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 57.4 (NCHS), 69.10 (CH_2O-), 103.7 (C-3, from Ph), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 115.4 (C-2, Ar-), 117.4 (C-5, Ar-), 122.2 (C-6, Ar-), 117.8 (C-5), 133.8 (C-1, Ar-), 143.0 (N=CH-), 147.4 (C-3, Ar-), 145.6 (C-4, Ar-), 151.2 (C-9), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 (CONH-), 168.8 ($COCH_2S$), 173.3 (CH_2CONH-); Anal. Calcd. For $C_{31}H_{26}N_4O_{11}S_2$: C, 53.60; H, 3.77; N, 8.07; S, 9.23; Found: C, 53.58; H, 3.79; N, 7.98; S, 9.20 %.

N-[2-(2,5-Dihydroxyphenyl)-4-oxo-thiazolidin-3-yl]-2-(7-{[2-(2,5-dihydroxyphenyl)-4-oxo-thiazolidin-3-ylcarbamoyl]-methoxy}-2-oxo-2H-chromen-4-yl)-acetamide (**5f**)

M.p. 221-223 °C, yield 46 %; IR: ν_{max} 3369 (OH), 3286 (NH), 1717, 1681 (C=O, lactone), 1667 (C=O, amide), 1624 (C=C, arom.), 1585, 1492, 1396, 1267 and 1156 cm^{-1}; [1]H-NMR: δ 11.95 (s, 1H, OH), 11.56 (s, 1H, OH), 8.48 (s, 1H, NH-), 8.34 (s, 1H, NH-), 7.76 (d, 1H, H-5), 7.68-7.30 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.82 (s, 2H, -OCH_2), 4.24 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$); [13]C-NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 47.4 (NCHS), 69.1 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 115.4 (C-6, Ar-), 117.4 (C-3, Ar-), 115.6 (C-4, Ar-), 119.6 (C-1, Ar-), 127.8 (C-5), 143.0 (N=CH-), 148.7 (C-2, Ar-), 151.2 (C-9), 151.2 (C-5, Ar-), 155.0 (C-4), 160.2 (C-7), 160.9 (C-2), 166.4 (CONH-), 168.8 ($COCH_2S$), 173.3 (CONH-); Anal. Calcd. For $C_{31}H_{26}N_4O_{11}S_2$: C, 53.60; H, 3.77; N, 8.07; S, 9.23; Found: C, 53.58; H, 3.79; N, 7.98; S, 9.20 %.

N-[2-(4-Hydroxy-3-methoxyphenyl)-4-oxo-thiazolidin-3-yl]-2-(7-{[2-(4-hydroxy-3-methoxyphenyl-4-oxo-thiazolidin-3-ylcarbamoyl]-methoxy}-2-oxo-2H-chromen-4-yl)-acetamide (**5g**)

M.p. 217-218 °C, yield 84 %; IR: ν_{max} 3434, 3224 (NH), 1711, 1671 (C=O, lactone), 1632 (C=O, amide), 1603 (C=C, arom.), 1529, 1394, 1272 and 1164 cm^{-1}; [1]H-NMR: δ 11.96 (s, 1H, OH), 8.19 (s, 1H, NH-), 8.10 (s, 1H, NH-), 7.77 (d, 1H, H-5), 7.40-7.21 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.78 (s, 2H, -OCH_2), 4.24 (s, 2H, CH_2), 3.80 (s, 6H, -OCH_3), 3.38 (s, 2H, $COCH_2S$); [13]C-NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 56.2 (OCH_3), 57.8 (NCHS), 69.1 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 114.8 (C-2, Ar-), 117.0 (C-5, Ar-), 122.9 (C-6, Ar-), 132.4 (C-1, Ar-), 144.1 (C-4, Ar-), 151.2 (C-9), 151.5 (C-3, Ar-), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 (CONH-), 168.8 ($COCH_2S$), 173.0 (CONH-), Anal. Calcd. For $C_{33}H_{30}N_4O_{11}S_2$: C, 54.84; H, 4.18; N, 7.75; S, 8.87; Found: C, 54.79; H, 4.19; N, 7.71; S, 8.82 %.

2-(2-Oxo-7-{[4-oxo-2-(3-phenoxyphenyl)-thiazolidin-3-ylcarbamoyl]-methoxy}-2H-chromen-4-yl)-N-[4-oxo-2-(3-phenoxyphenyl)-thiazolidin-3-yl]-acetamide (**5h**)

M.p. 221-222 °C, yield 57 %; IR: ν_{max} 3389, 3071 (NH), 1726, 1685 (C=O, lactone), 1628 (C=O, amide), 1614 (C=C, arom.), 1577, 1490, 1394, 1261 and 1156 cm^{-1}; [1]H-NMR: δ 8.30 (s, 1H, NH-), 8.21 (s, 1H, NH-), 7.76 (d, 1H, H-5), 7.70-7.10 (m, 18H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.79 (s, 2H, -OCH_2), 4.18 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$); ^{13}C-NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 56.2 (OCH_3), 57.6 (NCHS), 69.10 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 115.4 (C-4, Ar-), 116.1 (C-2, Ar-), 117.5 (C -2,6, Ar- PhO), 121.4 (C-4, Ar-PhO), 121.9 (C-6, Ar-), 127.8 (C-5), 128.5 (C-3,5 Ar- PhO), 128.6 (C-5 Ar-), 139.1 (C-1 Ar-), 151.2 (C-9), 155.0 (C-4), 156.8 (C-3, Ar-), 157.6 (C-1, Ar-PhO), 160.3 (C-7), 160.9 (C-2), 166.4 (CONH-), 168.9 ($COCH_2S$), 173.3 (CH_2CONH-); Anal. Calcd. For $C_{43}H_{34}N_4O_9S_2$: C, 63.38; H, 4.21; N, 6.88; S, 7.87; Found: C, 63.34; H, 4.19; N, 6.86; S, 7.84 %.

N-[2-(4-N,N-Dimethylaminophenyl)-4-oxo-thiazolidin-3-yl]-2-(7-{[2-(4-N,N-dimethylaminophenyl)-4-oxo-thiazolidin-3-ylcarbamoyl]-methoxy}-2-oxo-2H-chromen-4-yl)-acetamide (**5i**)

M.p. 198-201 °C, yield 71 %; IR: ν_{max} 3398, 3082 (NH), 1724, 1679 (C=O, lactone), 1623 (C=O, amide), 1604 (C=C, arom.), 1554, 1525, 1364, 1269 and 1181 cm^{-1}; ^{1}H-NMR: δ 8.49 (s, 1H, NH-), 8.44 (s, 1H, NH-), 7.66 (d, 1H, H-5), 7.24-7.52 (m, 8H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.74 (s, 2H, -OCH_2), 4.18 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$), 3.32 (s, 6H, -$N(CH_3)_2$), 2.99 (s, 6H, -$N(CH_3)_2$); ^{13}C-NMR: δ 35.7 ($COCH_2S$), δ 40.3 (CH_3N-), 45.5 (CH_2), 57.6 (NCHS), 69.10 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 114.4 (C-3,5, Ar-), 127.8 (C-5), 128.9 (C-1, Ar-), 130.1 (C-2,6 Ar-), 148.2 (C-4, Ar-), 151.2 (C-9), 155.0 (C-4), 160.3 (C-7), 160.9 (C-2), 166.4 (CONH-), 168.9 ($COCH_2S$), 173.3 (CH_2CONH-); Anal. Calcd. For $C_{35}H_{36}N_6O_7S_2$: C, 58.64; H, 5.06; N, 11.72; S, 8.95; Found: C, 58.60; H, 4.98; N, 11.70; S, 8.90 %.

N-[2-(2-Hydroxy-5-nitrophenyl)-4-oxo-thiazolidin-3-yl]-2-(4-{[2-(2-hydroxy-5-nitrophenyl)-4-oxothiazolidin-3-ylcarbamoyl]-methyl}-2-oxo-2H-chromen-7-yloxy)-acetamide (**5j**)

M.p. 240-242 °C, yield 82 %; IR: ν_{max} 3367, 3272 (NH), 1689 (C=O), 1618 (C=O, amide), 1598 (C=C, arom.), 1577, 1517, 1481, 1342, 1287 and 1150 cm^{-1}; ^{1}H-NMR: δ 12.02 (s, 2H, OH), 8.71 (s, 1H, NH-), 8.59 (s, 1H, NH-), 7.67 (d, 1H, H-5), 7.32-7.54 (m, 6H, arom.), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.84 (s, 2H, -OCH_2), 4.08 (s, 2H, CH_2), 3.38 (s, 2H, $COCH_2S$); ^{13}C NMR: δ 35.7 ($COCH_2S$), δ 45.5 (CH_2), 47.6 (NCHS), 69.10 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10), 116.9 (C-3, Ar-), 119.4 (C-1, Ar-), 121.8 (C-4, Ar-), 125.5 (C-6, Ar-), 127.8 (C-5), 141.1 (C-5, Ar-), 151.2 (C-9), 155.0 (C-4) 160.3 (C-7), 160.9 (C-2), 163.2 (C-2 Ar-), 166.4 (CONH-), 168.9 ($COCH_2S$), 173.3 (CH_2CONH-); Anal. Calcd. For $C_{31}H_{24}N_6O_{13}S_2$: C, 49.47; H, 3.21; N, 11.17; S, 8.52; Found: C, 49.45; H, 3.19; N, 11.12; S, 8.50 %.

2-{2-Oxo-7-[(4-oxo-2-styrylthiazolidin-3-ylcarbamoyl)-methoxy]-2H-chromen-4-yl}-N-(4-oxo-2-styrylthiazolidin-3-yl)-acetamide (**5k**)

M.p. 221-224 °C, yield 48 %; IR: ν_{max} 3424, 3276 (NH), 1718 (C=O, lactone), 1628 (C=O, amide), 1613 (C=C, arom.), 1560, 1509, 1393, 1266 and 1151 cm^{-1}; ^{1}H-NMR: δ 8.38 (s, 1H, NH-), 8.24 (s, 1H, NH-), 7.78 (2d, 4H, -HC=CH-), 7.64 (d, 1H, H-5), 7.04 (d, 1H, H-6), 7.02 (s, 1H, H-8), 6.34 (s, 1H, H-3), 5.92 (s, 1H, NCHS), 4.77 (s, 2H, -OCH2), 4.08 (s, 2H, CH2), 3.38 (s, 2H, COCH2S); 13C-NMR: δ 36.3 (COCH2S), δ 45.5 (CH2), 56.9 (NCHS), 69.10 (CH_2O-), 107.6 (C-8), 111.0 (C-6), 112.5 (C-3), 113.4 (C-10) 123.8 (C-1, ethenyl-Ar), 126.4 (C-2,6, Ar-), 128.0 (C-4, Ar-), 128.7 (C-3,5, Ar-), 129.6 (C-2, ethenyl-Ar), 135.2 (C-1, Ar-), 137.3 (N=CH-), 139.0 (C-3 Ar-), 151.2 (C-9), 155.0 (C-4), 160.2 (C-7), 160.9 (C-2), 166.4 (CONH-), 168.9 ($COCH_2S$), 173.3 (CH_2CONH-); Anal. Calcd. For $C_{35}H_{30}N_4O_7S_2$: C, 61.57; H, 4.43; N, 8.21; S, 9.39;Found: C, 61.56; H, 4.41; N, 8.19; S, 9.40 %.

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

Determination of antioxidant activity was performed according to the previously published procedure (Cacic and Molnar, 2011). The DPPH free radical, bearing an odd electron, gives a strong absorption maximum at λ = 517 nm (being purple in color). When the odd electron of the DPPH radical pairs with a hydrogen atom from an antioxidant, the reduced form DPPH-H is created, and the color turns from purple to yellow.

A solution of corresponding coumarin derivative in DMSO (0.75 mL 0.2 mM solution) was added to a DMSO solution of DPPH radical (0.75 mL 0.2 mM solution), so that the final concentration of DPPH radical and the synthesized compound in a solution was 0.1 mM. The mixture was shaken and left at room temperature. After 30 min the absorbance at 517 nm was determined and the scavenging activity was calculated according to the Eq. (1). Ascorbic acid was used as a reference compound.

$$scavenging\ activity\ (\%) = \left[\frac{A_b + A_s - A_m}{A_b}\right] \times 100 \qquad (1)$$

A_b – absorbance of 0.1 mM DMSO solution of DPPH radical at 517 nm
A_s – absorbance of 0.1 mM DMSO solution of test compound at 517 nm
A_m – absorbance of DMSO mixture of test compound and DPPH radical at 517 nm

Phosphomolybdenum method

The antioxidant activity was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). 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 in an acid medium. An aliquot of 100 μL of sample solution (2 mM 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 are incubated in a water bath at 95 °C for 90 minutes. 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 ascorbic acid with a same concentration.

Results and Discussion

According to Fig. 2. the best DPPH scavengers were found to be compounds **4e**, **4f**, **5e** and **5f**, two Schiff bases and two 4-thiazolidinones, possessing 66 %, 63 %, 65 % and 55 % DPPH radical scavenging activity. These compounds contain 3,4-dihydroxyphenyl and 2,5-dihidroxyphenyl ring. Compounds with these substituents are expected to possess antioxidant activity (Roussaki et al., 2010) since hydrogen donation leads to formation of a stable quinoid structure. Namely, it has been reported that two hydroxyl groups in *ortho* position are important for antioxidant activity (Roussaki et al., 2010; Foti et al., 1996; Pedersen et al., 2007). Our results indicate that dihydroxyphenyl ring contributes also to the scavenging activity more than thiazolidinone moiety, since both Schiff bases and the corresponding 4-thiazolidinones showed similar activity.

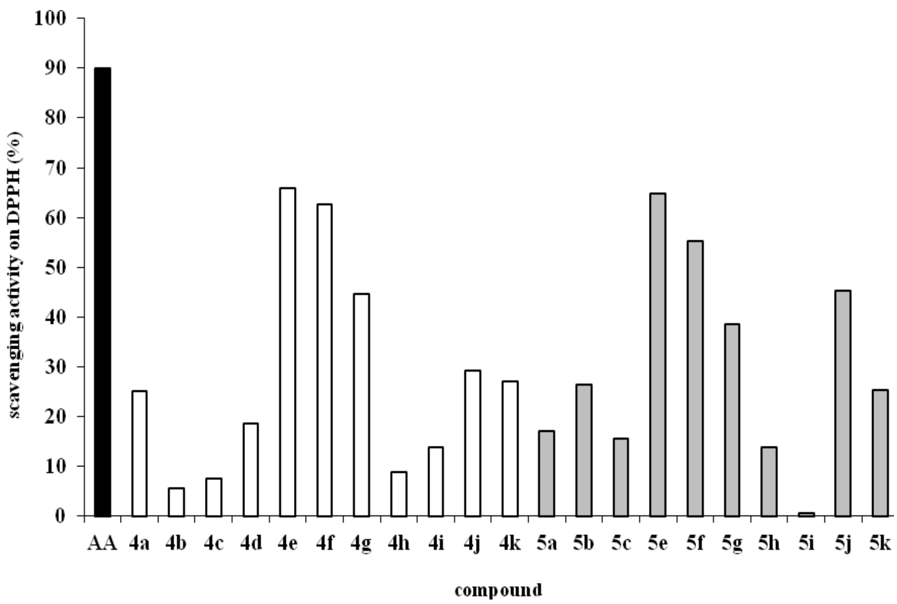


Fig. 2. DPPH radical scavenging activity of coumarin derivatives

According to the data presented in Fig. 3. the best antioxidants were compounds **4f**, **5f**, **4k**, **4j**, **4d**, **5j** and **5e**. These compounds showed better antioxidant activity than ascorbic acid itself, which was used as standard compound. The **4f** and **5f** are found to be the best ones; possessing 2,5-dihidroxyphenyl ring. All other compounds, with the exception of **4k** and **4c**, possess at least one hydroxyl group on phenyl ring.

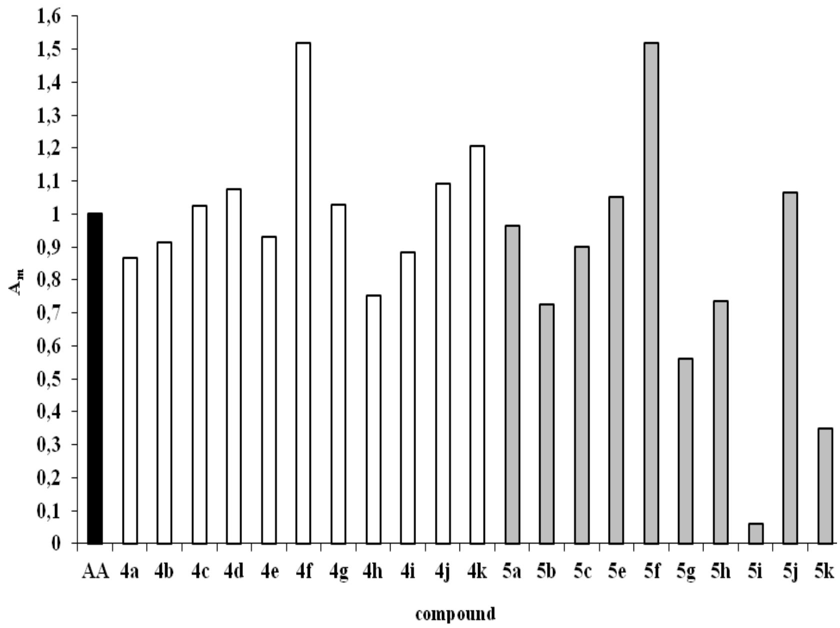


Fig. 3. Antioxidant activities of coumarin derivatives relative to ascorbic acid (A_m – activity relative to ascorbic acid (AA) on molar basis)

There is no correlation between the results obtained for DPPH scavenging activity and phosphomolybdenum method. This is understandable, since completely different mechanisms are involved in these two antioxidant activity determination methods.

As ROS high levels can increase mycotoxin biosynthesis (Reverberi, Ricelli, Zjalic, Fabbri & Fanelli, 2010; Narasaiah, Sashidhar & Subramanyam, 2006) application of antioxidants could affect it. So, it would be of interest to find new active compounds which exhibit both antioxidant and antifungal properties. The compounds **4e**, **4f**, **5e**, **5f** are promising candidates for further antifungal testing.

Conclusions

In our work we performed an antioxidant activity assay of several synthetic coumarin compounds by two different methods. The coumarin Schiff bases and 4-thiazolidinones showed significant antioxidant activity. The coumarins possessing hydroxyl groups at the 3,4- and 2,5-position have been proven to enhance antioxidant activity recommending these compounds for further detailed investigation.

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