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ORIGINAL ARTICLE

Synthesis and evaluation of anticancer activity of 6-pyrazolinylcoumarin derivatives

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Abstract A series of novel 6-pyrazolinylcoumarins has been synthesized via multi-step protocol. The synthetic procedure was based on the acetylation of hydroxycoumarins; Fries rearrangement and Claisen–Schmidt condensation; the target 6-[5-aryl-4,5-dihydropyrazol-3-yl]-5-hydroxy-7-met hylcoumarins (**33–49**) were obtained under reactions of hydrazine and 2-aryl-5-methyl-2,3-dihydro pyrano[2,3-*f*]chromen-4,8-diones as the last phase of the protocol. Anticancer activity screening in NCI60-cell lines assay allowed identification of compound **47** with the highest level of antimitotic activity with mean GI_{50} value of 10.20 μM and certain sensitivity profile toward the Leukemia cell lines *CCRF-CEM* and *MOLT-4* (GI_{50}/TGI values 1.88/5.06 μM and 1.92/4.04 μM respectively).

1. Introduction

Coumarins of natural and synthetic origin constitute a large family of heterocyclic compounds bearing a benzopyran-2-one moiety. Coumarins occur as secondary metabolites in the seeds, roots and leaves of many plant species (Borges et al., 2005), bacteria, fungi, and marine sources (Vazquez-Rodriguez et al., 2015) and exhibit diverse biological activities (Riveiro et al., 2010; Barot et al., 2015). Coumarins are of scientific interest as anti-HIV agents (Kostova et al., 2006), antituberculosis agents (Keri et al., 2015), cholinesterase and monoamine oxidase inhibitors (Orhan and Gulcan, 2015), antioxidants and anti-inflammatories (Fylaktakidou et al., 2004; Najmanová et al., 2015; Figueroa-Guiñez et al., 2015; Torres et al., 2014). Despite numerous effects of coumarins in the search for bioactive compounds, they still remain as one of the most versatile class of compounds for anticancer drug design and discovery (Kostova, 2005; Musa et al., 2008; Thakur et al., 2015; Emami and Dadashpour, 2015).

In the recent years, the actual trend in the field of chemistry of coumarins is a modification of the benzopyran-2-one by directed introduction of heterocyclic substituent. Such studies are of interest for the theory of organic synthesis and purposeful search of new biologically active compounds based on coumarin core. In most cases heteroaryl substituent is introduced at position 3 or 4 of the coumarin ring. Thus, 3- and 4-heteroarylcoumarins are reported to exhibit significant biological activities such as anticancer (Ganina et al.,

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2008), antimicrobial (Arshad et al., 2011), antibacterial, anticancer (DNA cleavage) (Gali et al., 2015), human monoamine oxidase inhibitory (Delogu et al., 2011), antioxidant and anticholinesterase (Kurt et al., 2015). Much less works are devoted to the synthesis of coumarins containing heterocyclic moiety in the benzene ring of benzopyran-2-one.

On the other hand, pyrazoline-based heterocycles are interesting compounds due to their high chemotherapeutic potential (Kumar et al., 2009; Marella et al., 2013). Diversely substituted pyrazolines combined with coumarin system showed good cytotoxic and antiproliferative activities toward a wide range of human tumor cell lines. For example, coumarin derivatives bearing 4,5-dihydropyrazole moiety possess high antiproliferative activity (Liu et al., 2010; Wu et al., 2014). They belong to the inhibitors of telomerase and PI3K protein kinase (Amin et al., 2013) and act as the antiproliferative agents toward hepatocellular carcinoma cell line HepG2 (Amin et al., 2015).

In continuation of our work on the synthesis of 6-heteroarylcoumarins (Nagorichna et al., 2009b; Nikitina et al., 2015; Galayev et al., 2015), we have synthesized new 6-pyrazolinylcoumarin derivatives and studied their anticancer activity.

2. Experimental

2.1. Chemistry

All starting materials were purchased from Merck and used without purification. NMR spectra were determined with Varian Mercury 400 (400 MHz) spectrometer, in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. Elemental analysis (C, H, N, Cl) was performed at the Perkin–Elmer 2400 CHN analyzer and was within ±0.4% from the theoretical values. The purity of the compounds was checked by thin-layer chromatography performed with Merck Silica Gel 60 F254 aluminum sheets. Coumarins **1–5** (Nagorichna et al., 2009a) were synthesized as described previously.

2.2. General procedure for synthesis of 5-acetoxy-7-methylcoumarins *6–10*

A mixture of 5-hydroxy-7-methylcoumarin (**1–5**, 50 mmol), acetic anhydride (9.5 mL, 100 mmol), and freshly distilled pyridine (5 mL) was heated for 1 h and left overnight at room temperature. The resulting precipitate was filtered off and crystallized from propanol-2. Spectral and analytical data of synthesized **6–10** are described (Nagorichna et al., 2009a).

2.3. General procedure for synthesis of 6-acetyl-5-hydroxy-7-methylcoumarins *11–15*

A ground mixture of 5-acetoxy-7-methylcoumarin (**6–10**, 30 mmol) and anhydrous $AlCl_3$ (12.00 g, 90 mmol) was heated at 120–130 °C for 1 h, cooled, and diluted with HCl solution (100 mL, 1 N). The resulting precipitate was filtered off and crystallized from propanol-2. Spectral and analytical data of synthesized **11–15** are described (Nagorichna et al., 2009a).

2.4. General procedure for synthesis of 2-aryl-10-alkyl-5-methyl-2,3-dihydropyrano[2,3-f]chromen-4,8-diones *16–32*

A mixture of 6-acetyl-5-hydroxycoumarin (**11–15**, 4 mmol) and the appropriate aromatic aldehyde (4.8 mmol) in EtOH was refluxed for 5–6 h in the presence of catalytic amounts (1–2 drops) of pyrrolidine (end of reaction was determined by TLC). The reaction mixture was cooled. The resulting precipitate was filtered off and crystallized from EtOH.

2.4.1. 2-(2-Methoxyphenyl)-5,10-dimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (*16*)

Spectral and analytical data are described (Nikitina et al., 2015).

2.4.2. 2-(4-Methoxyphenyl)-5,10-dimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (*17*)

Spectral and analytical data are described (Nikitina et al., 2015).

2.4.3. 2-(2,4-Dimethoxyphenyl)-5,10-dimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (*18*)

Spectral and analytical data are described (Nikitina et al., 2015).

2.4.4. 2-(4-Dimethylaminophenyl)-5-methyl-10-propyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (*19*)

Spectral and analytical data are described (Nikitina et al., 2015).

2.4.5. 2-(3-Fluorophenyl)-5,9,10-trimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (*20*)

Yield 79%, mp 208–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ: 7.43–7.56 (m, 3H), 7.24–7.29 (m, 1H), 6.88 (s, 1H, H-6), 5.75 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.22 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 2.85 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.61 (s, 3H, CH_3-5), 2.40 (s, 3H, CH_3-10), 2.03 (s, 3H, CH_3-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.17 (C-4), 162.96, 161.18 (C-8), 152.58, 149.85, 146.95, 142.81, 142.11, 129.51, 123.69, 123.43, 121.74, 115.04, 114.78, 113.08, 107.55, 78.99 (C-2), 44.78 (C-3), 22.04, 16.51, 15.18. Anal. Calcd. for $C_{21}H_{17}FO_4$: C, 71.58; H, 4.86. Found: C, 71.36; H, 4.95.

2.4.6. 2-(4-Hydroxyphenyl)-5,9,10-trimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (*21*)

Yield 67%, mp 223–224 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 9.38 (s, 1H, OH-4″), 7.45 (d, J = 8.8 Hz, 2H, H-2″, H-6″), 6.83 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.85 (s, 1H, H-6), 5.65 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.28 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 2.79 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.61 (s, 3H, CH_3-5), 2.40 (s, 3H, CH_3-10), 2.05 (s, 3H, CH_3-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.54 (C-4), 161.29 (C-8), 157.63, 152.51, 150.88, 147.12, 142.89, 131.93, 128.12, 127.35, 123.69, 121.74, 115.86, 115.18, 114.78, 107.55, 77.63 (C-2), 44.71 (C-3), 22.09, 16.59, 15.12. Anal. Calcd. for $C_{21}H_{18}O_5$: C, 71.99; H, 5.18. Found: C, 72.12; H, 5.21.

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*2.4.7. 2-(4-Hydroxy-3-methoxyphenyl)-5,9,10-trimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (**22**)*

Yield 71%, mp 216–217 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ: 8.95 (1H, s, OH-4′), 6.97–7.01 (m, 3H, H-2′, 5′, 6′), 6.91 (s, 1H, H-6), 5.62 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.82 (s, 3H, OCH_3-3), 3.25 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 2.76 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.61 (s, 3H, CH_3-5), 2.41 (s, 3H, CH_3-10), 2.05 (s, 3H, CH_3-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 190.12 (C-4), 161.13 (C-8), 152.58, 150.77, 147.49, 147.16, 146.95, 142.89, 132.93, 123.61, 121.79, 118.17, 114.93, 114.13, 110.47, 107.32, 78.55 (C-2), 55.61, 44.96 (C-3), 22.04, 16.65, 15.39. Anal. calcd for $C_{22}H_{20}O_6$: C, 69.46; H, 5.30. Found: C, 69.56; H, 5.25.

*2.4.8. 2-(2,4-Dimethoxyphenyl)-5,9,10-trimethyl-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (**23**)*

Yield 81%, mp 225–226 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.47 (d, J = 8.8 Hz, 1H, H-6′), 6.86 (s, 1H, H-6), 6.66 (d, J = 2.4 Hz, 1H, H-3′), 6.62 (dd, J = 2.4 Hz, J = 8.8 Hz, 1H, H-5′), 5.79 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.26 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 2.73 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.63 (s, 3H, CH_3-5), 2.40 (s, 3H, CH_3-10), 2.04 (s, 3H, CH_3-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.95 (C-4), 160.91 (C-8), 159.79, 157.13, 152.91, 152.58, 146.88, 143.13, 130.38, 124.35, 121.74, 118.53, 114.14, 108.37, 107.92, 97.92, 75.37 (C-2), 56.39, 55.23, 44.29 (C-3), 22.35, 16.84, 15.42. Anal. Calcd. for $C_{23}H_{22}O_6$: C, 70.04; H, 5.62. Found: C, 70.12; H, 5.54.

*2.4.9. 5,9,10-Trimethyl-2-(2,4,5-trimethoxyphenyl)-2,3-dihydropyrano[2,3-f]chromene-4,8-dione (**24**)*

Yield 84%, mp 232–233 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.20 (1H, s, H-6′), 6.92 (s, 1H, H-6), 6.78 (s, 1H, H-3′), 5.83 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.24 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 2.68 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.65 (s, 3H, CH_3-5), 2.45 (s, 3H, CH_3-10), 2.06 (s, 3H, CH_3-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.82 (C-4), 160.95 (C-8), 152.89, 152.58, 151.01, 150.76, 146.95, 144.24, 142.89, 125.56, 123.57, 121.86, 121.68, 114.78, 107.43, 102.76, 76.22 (C-2), 56.59, 56.13, 55.90, 43.86 (C-3), 22.23, 16.81, 15.43. Anal. Calcd. for $C_{24}H_{24}O_7$: C, 67.91; H, 5.70. Found: C, 67.84; H, 5.61.

*2.4.10. 2-(2-Chlorophenyl)-5-methyl-10,11-dihydrocyclopenta[c]pyrano[2,3-f]chromene-4,8-dione (**25**)*

Yield 83%, mp 248–249 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.81 (d, J = 7.2 Hz, 1H, H-6′), 7.45–7.58 (m, 3H), 6.97 (s, 1H, H-6), 5.99 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.05–3.20 (m, 3H, H-3$_{ax}$, CH_2-9), 2.86 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.68 (s, 3H, CH_3-5), 2.63–2.75 (m, 2H, CH_2-11), 1.91–2.08 (m, 2H, CH_2-10). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 190.16 (C-4), 160.94 (C-8), 152.67, 152.26, 150.12, 142.63, 136.65, 131.33, 129.49, 128.78, 128.07, 126.51, 126.22, 123.16, 114.48, 111.78, 74.44 (C-2), 44.98 (C-3), 35.04, 31.98, 24.99, 22.04. Anal. Calcd. for $C_{22}H_{17}ClO_4$: C, 69.45; H, 4.50; Cl, 9.31. Found: C, 69.34; H, 4.58; Cl, 9.38.

*2.4.11. 2-(2-Methoxyphenyl)-5-methyl-10,11-dihydrocyclopenta[c]pyrano[2,3-f]chromene-4,8-dione (**26**)*

Yield 74%, mp 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.57 (d, J = 7.6 Hz, 1H, H-6′), 7.41 (t, J = 7.6 Hz, 1H), 7.06–7.12 (m, 2H), 6.89 (s, 1H, H-6), 5.85 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.84 (s, 3H, OCH_3-6′), 3.07–3.21 (m, 3H, H-3$_{ax}$, CH_2-9), 2.78 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.63 (s, 3H, CH_3-5), 2.58–2.71 (m, 2H, CH_2-11), 1.90–2.07 (m, 2H, CH_2-10). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 190.32 (C-4), 161.06 (C-8), 154.82, 153.01, 152.67, 152.26, 142.89, 129.30, 126.51, 125.34, 123.81, 123.27, 120.69, 114.69, 111.89, 110.52, 75.37 (C-2), 55.51, 43.86 (C-3), 35.23, 32.49, 25.86, 22.34. Anal. Calcd. for $C_{23}H_{20}O_5$: C, 73.39; H, 5.36. Found: C, 73.46; H, 5.34.

*2.4.12. 2-(2,4-Dimethoxyphenyl)-5-methyl-10,11-dihydrocyclopenta[c]pyrano[2,3-f]chromene-4,8-dione (**27**)*

Yield 69%, mp 207–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.47 (d, J = 8.4 Hz, 1H, H-6′), 6.96 (s, 1H, H-6), 6.66 (d, J = 2.4 Hz, 1H, H-3′), 6.63 (dd, J = 2.4 Hz, J = 8.4 Hz, 1H, H-5′), 5.81 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.23 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 3.12–3.16 (m, 2H, CH_2-9), 2.74 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.65 (s, 3H, CH_3-5), 2.64–2.69 (m, 2H, CH_2-11), 1.94–2.04 (m, 2H, CH_2-10). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 190.53 (C-4), 161.12 (C-8), 159.79, 157.13, 153.01, 152.79, 152.26, 143.26, 130.38, 126.92, 123.85, 118.56, 114.48, 112.03, 108.37, 97.92, 75.12 (C-2), 55.78, 55.13, 44.02 (C-3), 35.04, 32.11, 24.99, 22.18. Anal. Calcd. for $C_{24}H_{22}O_6$: C, 70.93; H, 5.46. Found: C, 71.02; H, 5.49.

*2.4.13. 5-Methyl-2-(2,3,4-trimethoxyphenyl)-10,11-dihydrocyclopenta[c]pyrano[2,3-f]chromene-4,8-dione (**28**)*

Yield 78%, mp 214–215 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ7.31 (d, J = 8.8 Hz, 1H, H-6′), 6.95 (s, 1H, H-6), 6.93 (d, J = 8.8 Hz, 1H, H-5′), 5.81 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.85 (s, 6H, OCH_3), 3.80 (s, 3H, OCH_3), 3.22 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 3.12–3.16 (m, 2H, CH_2-9), 2.71 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.67 (s, 3H, CH_3-5), 2.64–2.70 (m, 2H, CH_2-11), 1.94–2.04 (m, 2H, CH_2-10). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 190.12 (C-4), 161.11 (C-8), 155.31, 152.89, 152.67, 152.21, 150.03, 143.96, 142.85, 126.51, 124.25, 123.27, 121.71, 114.89, 111.89, 108.08, 76.22 (C-2), 60.05, 59.73, 56.81, 43.88 (C-3), 35.04, 32.06, 24.91, 22.08. Anal. Calcd. for $C_{25}H_{24}O_7$: C, 68.80; H, 5.54. Found: C, 68.73; H, 5.57.

*2.4.14. 5-Methyl-2-(3,4,5-trimethoxyphenyl)-10,11-dihydrocyclopenta[c]pyrano[2,3-f]chromene-4,8-dione (**29**)*

Yield 72%, mp 214–215 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.96 (s, 2H, H-2′,6′), 6.93 (s, 1H, H-6), 5.63 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.81 (s, 6H, OCH_3-3′, OCH_3-5′), 3.68 (s, 3H, OCH_3-4′), 3.46 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3$_{ax}$), 3.08–3.15 (m, 2H, CH_2-9), 2.71 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3$_{eq}$), 2.65 (s, 3H, CH_3-5), 2.65–2.72 (m, 2H, CH_2-11), 1.95–2.05 (m, 2H, CH_2-10). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.93 (C-4), 160.91 (C-8), 154.69, 154.43, 152.89, 152.18, 150.75, 142.89, 137.87, 133.59, 126.79, 123.38, 114.48, 112.01, 104.12,

103.91, 76.11 (C-2), 59.90, 59.18, 56.09, 44.58 (C-3), 35.12, 32.08, 24.85, 22.01. Anal. Calcd. for $C_{25}H_{24}O_7$: C, 68.80; H, 5.54. Found: C, 68.89; H, 5.48.

*2.4.15. 2-(4-Hydroxy-3-methoxyphenyl)-5-methyl-2,3,9,10,11,12-hexahydrobenzo[c]pyrano[2,3-f]chromene-4,8-dione (**30**)*

Yield 69%, mp 223–224 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 9.22 (s, 1H, OH-4′), 7.17 (s, 1H, H-6), 6.93 (dd, J = 2.0 Hz, J = 8.0 Hz, 1H, H-6′), 6.85 (d, J = 2.0 Hz, 1H, H-2′), 6.85 (d, J = 8.0 Hz, 1H, H-5′), 5.56 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.80 (s, 3H, OCH_3-3′), 3.22 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3_{ax}), 2.88–2.96 (m, 2H, CH_2-9), 2.75 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3_{eq}), 2.61 (s, 3H, CH_3-5), 2.34–2.41 (m, 2H, CH_2-12), 1.52–1.66 (m, 4H, CH_2-10, CH_2-11). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.54 (C-4), 161.57 (C-8), 152.33, 150.51, 149.12, 147.46, 147.16, 142.89, 132.93, 123.37, 122.96, 118.17, 114.72, 114.47, 113.77, 110.47, 79.29 (C-2), 55.63, 44.85 (C-3), 25.76, 24.36, 22.04, 21.65, 21.36. Anal. Calcd. for $C_{24}H_{22}O_6$: C, 70.93; H, 5.46. Found: C, 71.02; H, 5.49.

*2.4.16. 2-(3,5-Dimethoxyphenyl)-5-methyl-2,3,9,10,11,12-hexahydrobenzo[c]pyrano[2,3-f]chromene-4,8-dione (**31**)*

Yield 73%, mp 209–210 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.88 (s, 1H, H-6), 6.74 (d, J = 2.4 Hz, 2H, H-2′, H-6′), 6.52 (dd, J = 2.4 Hz, J = 2.4 Hz, 1H, H-4′), 5.64 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.79 (s, 6H, OCH_3-3′, OCH_3-5′), 3.16 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3_{ax}), 2.96–3.07 (m, 2H, CH_2-9), 2.84 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3_{eq}), 2.63 (s, 3H, CH_3-5), 2.38–2.44 (m, 2H, CH_2-12), 1.56–1.71 (m, 4H, CH_2-10, CH_2-11). ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 192.56 (C-4), 161.76 (C-8), 161.20, 155.77, 148.89, 144.24, 141.45, 122.48, 116.68, 113.60, 113.55, 109.12, 104.96, 104.83, 100.78, 80.16 (C-2), 55.09, 55.91, 45.33 (C-3), 30.43, 25.02, 23.38, 22.28, 20.97. Anal. Calcd. for $C_{25}H_{24}O_6$: C, 71.42; H, 5.75. Found:C, 71.50; H, 5.78.

*2.4.17. 5-Methyl-2-(2,4,5-trimethoxyphenyl)-2,3,9,10,11,12-hexahydrobenzo[c]pyrano[2,3-f]chromene-4,8-dione (**32**)*

Yield 82%, mp 229–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.20 (s, 1H, H-6′), 6.92 (s, 1H, H-6),6.78 (s, 2H, H-3′), 5.66 (dd, J = 2.4 Hz, J = 13.6 Hz, 1H, H-2), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.18 (dd, J = 13.6 Hz, J = 16.8 Hz, 1H, H-3_{ax}), 2.95–3.05 (m, 2H, CH_2-9), 2.82 (dd, J = 2.4 Hz, J = 16.8 Hz, 1H, H-3_{eq}), 2.65 (s, 3H, CH_3-5), 2.35–2.45 (m, 2H, CH_2-12), 1.55–1.70 (m, 4H, CH_2-10, CH_2-11). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 189.95 (C-4), 161.52 (C-8), 152.54, 152.44, 151.01, 150.84, 149.12, 144.24, 142.89, 125.56, 123.98, 122.96, 121.71, 114.47, 113.66, 102.76, 78.11 (C-2), 56.59, 56.13, 55.96, 43.86 (C-3), 25.76, 24.36, 22.08, 21.65, 21.37. Anal. Calcd. for $C_{26}H_{26}O_7$:C, 69.32; H, 5.82. Found: C, 69.27; H, 5.76.

*2.5. General procedure for synthesis of 6-[5-aryl-4,5-dihydropyrazol-3-yl]-4-alkyl-5-hydroxy-7-methylchromen-2-ones **33–49***

A mixture of **20**–**32** (2 mmol) and hydrazine monohydrate (0.50 mL, 10 mmol) in EtOH was refluxed for 2–3 h (end of reaction was determined by TLC). The reaction mixture was cooled. The resulting precipitate was filtered off and crystallized from EtOH.

*2.5.1. 6-[5-(2-Methoxyphenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-4,7-dimethylchromen-2-one (**33**)*

Spectral and analytical data are described (Nikitina et al., 2015).

*2.5.2. 6-[5-(4-Methoxyphenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-4,7-dimethylchromen-2-one (**34**)*

Spectral and analytical data are described (Nikitina et al., 2015).

*2.5.3. 6-[5-(2,4-Dimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-4,7-dimethylchromen-2-one (**35**)*

Spectral and analytical data are described (Nikitina et al., 2015).

2.5.4. 6-[5-(4-Dimethylaminophenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-4-propyl-7-methylchromen-2-one (36)

Spectral and analytical data are described (Nikitina et al., 2015).

*2.5.5. 6-[5-(3-Fluorophenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-3,4,7-trimethylchromen-2-one (**37**)*

Yield 85%, mp 219–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.73 (s, 1H, OH-5), 7.88 (d, J = 3.6 Hz, 1H, NH), 7.43 (q, J = 7.2 Hz, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.12–7.16 (m, 1H), 6.68 (s, 1H, H-8), 4.88 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.82 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.31 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.61 (s, 3H, CH_3-4), 2.48 (s, 3H, CH_3-7), 2.07 (s, 3H, CH_3-3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 164.12, 161.69 (C-2), 160.09, 156.51, 156.09 (C-3′), 146.31, 143.52, 140.96, 132.69, 125.75, 123.19, 115.26, 114.88, 113.56, 108.68, 107.95, 59.82 (C-5′), 46.45 (C-4′), 21.38, 16.51, 15.16. Anal. Calcd. for $C_{21}H_{19}FN_2O_3$: C, 68.84; H, 5.23; N, 7.65. Found: C 68.92; H, 5.19; N, 7.69.

*2.5.6. 5-Hydroxy-6-[5-(4-hydroxyphenyl)-4,5-dihydropyrazol-3-yl]-3,4,7-trimethylchromen-2-one (**38**)*

Yield 71%, mp 236–237 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.94 (s, 1H, OH-5), 9.38 (s, 1H, OH-4″), 7.72 (d, J = 3.6 Hz, 1H, NH), 7.23 (d, J = 8.8 Hz, 2H, H-2″, H-6″), 6.75 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.68 (s, 1H, H-8), 4.75 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.72 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.13 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.61 (s, 3H, CH_3-4), 2.48 (s, 3H, CH_3-7), 2.07 (s, 3H, CH_3-3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 161.13 (C-2), 160.28, 156.95, 156.51, 155.91 (C-3′), 146.48, 142.15, 130.99, 128.63, 128.14, 123.18, 117.69, 117.25, 115.18, 108.96, 108.13, 59.06 (C-5′), 46.88 (C-4′), 21.37, 16.59, 15.09. Anal. Calcd. for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.31; H, 5.48; N, 7.73.

*2.5.7. 6-[5-(4-Hydroxy-3-methoxyphenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-3,4,7-trimethyl-2H-chromen-2-one (**39**)*

Yield 83%, mp 229–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.94 (s, 1H, OH-5), 8.94 (s, 1H, OH-4″), 7.74 (d, J = 3.6 Hz, 1H, NH), 7.23 (d, J = 2.4 Hz, 1H, H-2″), 6.81

(dd, J = 2.4 Hz, J = 8.8 Hz, 1H, H-6″), 6.75 (d, J = 8.8 Hz, 1H, H-5″), 6.68 (s, 1H, H-8), 4.76 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.78 (s, 3H, OCH_3-3″), 3.72 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.17 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.61 (s, 3H, CH_3-4), 2.48 (s, 3H, CH_3-7), 2.07 (s, 3H, CH_3-3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 161.26 (C-2), 160.09, 156.66, 156.24 (C-3′), 149.15, 146.31, 144.56, 140.96, 132.98, 123.29, 122.06, 121.34, 115.26, 109.63, 108.91, 108.13, 63.38, 59.26 (C-5′), 46.48 (C-4′), 21.38, 16.51, 15.10. Anal. Calcd. for $C_{22}H_{22}N_2O_5$: C, 66.99; H, 5.62; N, 7.10. Found: C, 67.08; H, 5.66; N, 7.02.

*2.5.8. 6-[5-(2,4-Dimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-5-hydroxy-3,4,7-trimethylchromen-2-one (**40**)*

Yield 74%, mp 218–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.87 (s, 1H, OH-5), 7.53 (d, J = 3.6 Hz, 1H, NH), 7.30 (d, J = 8.0 Hz, 1H, H-6″), 6.62 (s, 1H, H-8), 6.59 (d, J = 2.4 Hz, 1H, H-3″), 7.30 (dd, J = 2.4 Hz, J = 8.0 Hz, 1H, H-5″), 4.99 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.69 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.07 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.59 (s, 3H, CH_3-4), 2.45 (s, 3H, CH_3-7), 2.06 (s, 3H, CH_3-3). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 161.25 (C-2), 160.09, 159.56, 158.25, 157.02, 156.28 (C-3′), 146.42, 140.96, 128.56, 123.20, 117.28, 115.64, 109.61, 109.12, 108.45, 102.53, 59.46 (C-5′), 55.87, 55.27, 46.62 (C-4′), 21.36, 16.46, 15.14. Anal. Calcd. for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.71; H, 5.95; N, 6.81.

*2.5.9. 5-Hydroxy-3,4,7-trimethyl-6-[5-(2,4,5-trimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-chromen-2-one (**41**)*

Yield 69%, mp 214–215 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.90 (s, 1H, OH-5), 7.63 (d, J = 3.6 Hz, 1H, NH), 7.06 (s, 1H, H-6″), 6.72 (s, 1H, H-3″), 6.67 (s, 1H, H-8), 5.02 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.79 (s, 6H, OCH_3), 3.71 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.70 (s, 3H, OCH_3), 3.06 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.61 (s, 3H, CH_3-4), 2.46 (s, 3H, CH_3-7), 2.07 (s, 3H, CH_3-3). ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 161.06 (C-2), 157.98 (C-3′), 153.57, 152.36, 151.74, 149.49, 148.88, 143.13, 140.19, 121.26, 119.41, 113.37, 112.39, 110.25, 108.19, 99.07, 57.44 (C-5′), 56.96, 56.93, 56.51, 44.40 (C-4′), 23.22, 20.05, 13.46. Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.65; H, 5.91; N, 6.42.

*2.5.10. 8-[5-(2-Chlorophenyl)-4,5-dihydropyrazol-3-yl]-9-hydroxy-7-methyl-2,3-dihydrocyclopenta[c]chromen-4-one (**42**)*

Yield 79%, mp 231–232 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.29 (s, 1H, OH-9), 7.84 (d, J = 3.6 Hz, 1H, NH), 7.66 (d, J = 7.6 Hz, 1H, H-6″), 7.48 (d, J = 7.6 Hz, 1H), 7.32–7.41 (m, 2H), 6.69 (s, 1H, H-8), 5.16 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.90 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.08 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 3.28–3.40 (m, 2H, CH_2-3), 2.62–2.70 (m, 2H, CH_2-1), 2.45 (s, 3H, CH_3-7), 2.00–2.09 (m, 2H, CH_2-2). Anal. Calcd. for $C_{22}H_{19}ClN_2O_3$: C, 66.92; H, 4.85; Cl, 8.98; N 7.09. Found: C, 66.68; H, 4.80; Cl, 9.02; N, 7.03.

*2.5.11. 9-Hydroxy-8-[5-(2-methoxyphenyl)-4,5-dihydropyrazol-3-yl]-7-methyl-2,3-dihydrocyclopenta[c]chromen-4-one (**43**)*

Yield 75%, mp 205–206 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.54 (s, 1H, OH-9), 7.66 (d, J = 3.6 Hz, 1H, NH), 7.66 (d, J = 7.2 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.96 (t, J = 7.6 Hz, 1H), 6.72 (s, 1H, H-8), 5.06 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.79 (s, 3H, OCH_3-2″), 3.76 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.29–3.42 (m, 2H, CH_2-3), 3.08 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.63–2.69 (m, 2H, CH_2-1), 2.48 (s, 3H, CH_3-7), 2.01–2.11 (m, 2H, CH_2-2). Anal. Calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.81; H, 5.62; N, 7.19.

*2.5.12. 8-[5-(2,4-Dimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-9-hydroxy-7-methyl-2,3-dihydrocyclopenta[c]chromen-4-one (**44**)*

Yield 86%, mp 219–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.57 (s, 1H, OH-9), 7.58 (d, J = 3.6 Hz, 1H, NH), 7.29 (d, J = 8.4 Hz, 1H, H-6″), 6.72 (s, 1H, H-8), 6.58 (d, J = 2.4 Hz, 1H, H-3″), 6.52 (dd, J = 2.4 Hz, J = 8.4 Hz, 1H, H-5″), 5.00 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.80 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.71 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.30–3.41 (m, 2H, CH_2-3), 3.07 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.62–2.71 (m, 2H, CH_2-1), 2.48 (s, 3H, CH_3-7), 2.01–2.09 (m, 2H, CH_2-2). Anal. Calcd. for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.51; H, 5.79; N, 6.71.

*2.5.13. 9-Hydroxy-7-methyl-8-[5-(2,3,4-trimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-2,3-dihydrocyclopenta[c]chromen-4-one (**45**)*

Yield 72%, mp 227–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.57 (s, 1H, OH-9), 7.62 (d, J = 3.6 Hz, 1H, NH), 7.14 (d, J = 8.4 Hz, 1H, H-6″), 6.80 (d, J = 8.4 Hz, 1H, H-5″), 6.69 (s, 1H, H-8), 4.98 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.72 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.25–3.42 (m, 2H, CH_2-3), 3.11 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.62–2.70 (m, 2H, CH_2-1), 2.48 (s, 3H, CH_3-7), 1.99–2.11 (m, 2H, CH_2-2). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 160.94 (C-4), 160.19, 156.79, 156.18 (C-3′), 153.03, 152.46, 151.62, 145.60, 140.97, 127.97, 122.65, 118.19, 114.85, 109.63, 108.19, 104.74, 60.88, 59.22 (C-5′), 57.51, 55.97, 46.63 (C-4′), 35.04, 31.98, 24.89, 21.38. Anal. Calcd. for $C_{25}H_{26}N_2O_6$: C, 66.66; H, 5.82; N, 6.22. Found: C, 66.72; H, 5.86; N, 6.29.

*2.5.14. 9-Hydroxy-7-methyl-8-[5-(3,4,5-trimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-2,3-dihydrocyclopenta[c]chromen-4-one (**46**)*

Yield 84%, mp 231–232 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.49 (s, 1H, OH-9), 7.80 (d, J = 3.6 Hz, 1H, NH), 6.77 (s, 2H, H-2″, H-6″), 6.72 (s, 1H, H-8), 4.81 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.79 (s, 6H, OCH_3), 3.76 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.65 (s, 3H, OCH_3), 3.28–3.42 (m, 2H, CH_2-3), 3.19 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.61–2.70 (m, 2H, CH_2-1), 2.47 (s, 3H, CH_3-7), 2.00–2.11 (m, 2H, CH_2-2). ^{13}C NMR

(100 MHz, DMSO-d_6, TMS): δ 161.08 (C-4), 160.36, 156.60, 156.09 (C-3′), 154.28, 153.88, 151.48, 140.83, 137.65, 135.53, 127.69, 114.96, 108.29, 104.68, 104.12, 103.98, 59.69 (C-5′), 57.63, 55.85, 55.81, 46.45 (C-4′), 35.06, 32.04, 24.92, 21.37. Anal. Calcd. for $C_{25}H_{26}N_2O_6$: C, 66.66; H, 5.82; N, 6.22. Found: C, 66.63; H, 5.88; N, 6.17.

2.5.15. 1-Hydroxy-2-[5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydropyrazol-3-yl]-3-methyl-7,8,9,10-tetrahydrobenzo[c]chromen-6-one (47)

Yield 78%, mp 238–239 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.88 (s, 1H, OH-1), 8.93 (s, 1H, OH-4″), 7.73 (d, J = 3.6 Hz, 1H, NH), 7.02 (d, J = 2.0 Hz, 1H, H-2″), 6.81 (dd, J = 2.0 Hz, J = 8.0 Hz, 1H, H-6″), 6.75 (d, J = 8.0 Hz, 1H, H-5″), 6.68 (s, 1H, H-4), 4.75 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.78 (s, 3H, OCH_3-3″), 3.78 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.19 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 3.11–3.17 (m, 2H, CH_2-7), 2.48 (s, 3H, CH_3-3), 2.38–2.43 (m, 2H, CH_2-10), 1.62–1.72 (m, 4H, CH_2-8, CH_2-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 161.37 (C-4), 159.84, 156.55, 156.02 (C-3′), 149.15, 148.62, 144.57, 140.96, 132.98, 124.42, 122.06, 121.34, 114.95, 109.68, 108.36, 106.48, 59.85 (C-5′), 55.89, 46.57 (C-4′), 25.76, 24.36, 21.95, 21.69, 21.37. Anal. Calcd. for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.61; H, 5.73; N, 6.62.

2.5.16. 2-[5-(3,5-Dimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-1-hydroxy-3-methyl-7,8,9,10-tetrahydrobenzo[c]chromen-6-one (48)

Yield 81%, mp 231–231 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.75 (s, 1H, OH-1), 7.80 (d, J = 3.6 Hz, 1H, NH), 6.67 (s, 1H, H-4), 6.60 (d, J = 2.4 Hz, 2H, H-2″, H-6″), 6.43 (dd, J = 2.4 Hz, J = 2.4 Hz, 1H, H-4″), 4.78 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.79 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.75 (s, 6H, OCH_3-3″, OCH_3-5″), 3.20 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 3.11–3.16 (m, 2H, CH_2-7), 2.47 (s, 3H, CH_3-3), 2.36–2.42 (m, 2H, CH_2-10), 1.64–1.74 (m, 4H, CH_2-8, CH_2-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 162.57, 162.39, 161.57 (C-4), 160.13, 157.03, 156.18 (C-3′), 148.48, 142.65, 141.23, 124.19, 114.88, 108.19, 106.37, 106.11, 105.68, 97.96, 59.23 (C-5′), 55.89, 55.41, 46.45 (C-4′), 25.77, 24.35, 21.97, 21.69, 21.37. Anal. Calcd. for $C_{25}H_{26}N_2O_5$: C, 69.11; H, 6.03; N, 6.45. Found: C, 69.04; H, 5.95; N, 6.49.

2.5.17. 1-Hydroxy-3-methyl-2-[5-(2,4,5-trimethoxyphenyl)-4,5-dihydropyrazol-3-yl]-7,8,9,10-tetrahydrobenzo[c]chromen-6-one (49)

Yield 73%, mp 217–218 °C. ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 13.87 (s, 1H, OH-1), 7.63 (d, J = 3.6 Hz, 1H, NH), 7.06 (s, 1H, H-6″), 6.72 (s, 1H, H-3″), 6.66 (s, 1H, H-4), 5.00 (ddd, J = 3.6 Hz, J = 10.4 Hz, J = 11.2 Hz, H-5′), 3.79 (s, 6H, OCH_3), 3.74 (dd, J = 10.4 Hz, J = 16.4 Hz, H-4′b), 3.70 (s, 6H, OCH_3), 3.11–3.16 (m, 2H, CH_2-7), 3.07 (dd, J = 11.2 Hz, J = 16.4 Hz, H-4′a), 2.46 (s, 3H, CH_3-3), 2.38–2.44 (m, 2H, CH_2-10), 1.62–1.73 (m, 4H, CH_2-8, CH_2-9). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ 161.30 (C-4), 159.84, 156.75, 156.69 (C-3′), 153.36, 149.90, 148.12, 145.96, 140.89, 124.41, 118.19, 114.89, 113.56, 108.36, 106.40, 103.24, 59.69 (C-5′), 56.94, 56.59, 55.87, 46.79 (C-4′), 25.76, 24.35, 21.98, 21.69, 21.37. Anal. Calcd. for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.19; H, 6.09; N, 6.07.

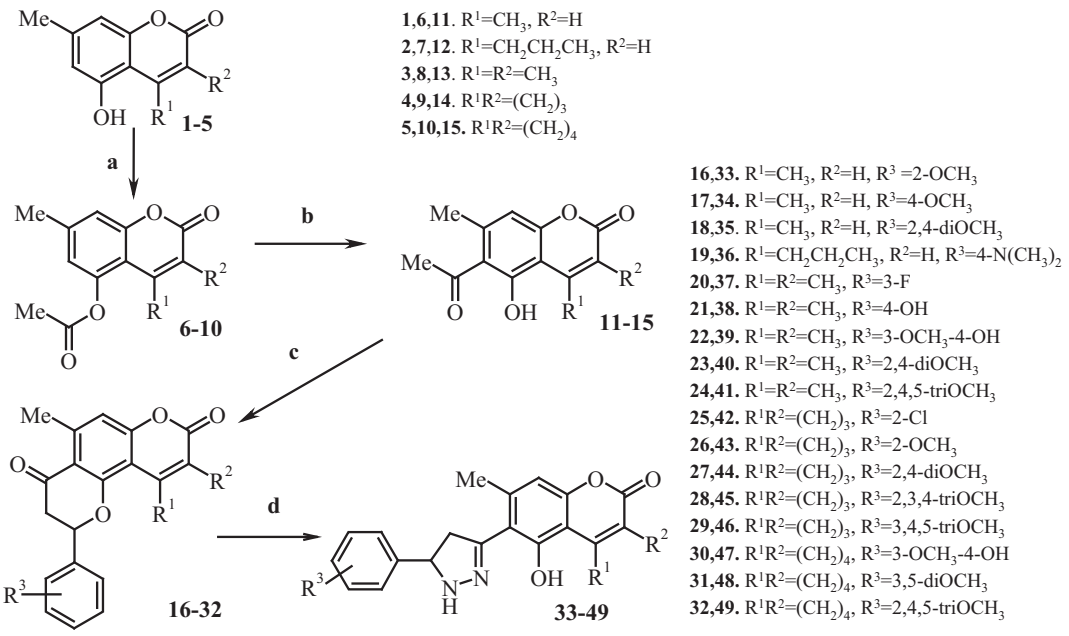
3. Results and discussion

3.1. Chemistry

The starting 5-hydroxy-7-methylcoumarins **1**–**5** were synthesized via a Pechmann reaction of orcinol and the appropriate ethylacylacetates in the presence of a condensing agent (conc. H_2SO_4) (Confalone and Confalone, 1983; Nagorichna et al., 2009a). Acetylation of hydroxycoumarins **1**–**5** by acetic anhydride in pyridine led to 5-acetoxycoumarins **6**–**10**, Fries rearrangement of which in the presence of anhydrous $AlCl_3$ at 120–130 °C afforded to 6-acetylcoumarins **11**–**15** in high yields (Confalone and Confalone, 1983; Nagorichna et al., 2009a). Claisen–Schmidt condensation of **11**–**15** and aromatic aldehydes in EtOH in the presence of catalytic amounts of pyrrolidine led to annelation of a 2-aryltetrahydropyran-4-one and formation of 2-aryl-5-methyl-2,3-dihydropyrano-[2,3-*f*]chromen-4,8-diones **16**–**32** (Nikitina et al., 2015; Khan and Bawa, 2001). Obviously, the angular pyronoflavanones were formed through the corresponding intermediate chalcones, which heterocyclized under the reaction conditions (see Scheme 1).

^{1}H NMR spectra of **16**–**32** showed resonances for H-2 (5.56–5.99 ppm, dd, J = 2.4 and 13.6 Hz), equatorial H-3 (2.68–2.86 ppm, dd, J = 2.4 and 16.8 Hz), and axial H-3 (3.16–3.46 ppm, dd, J = 13.6 and 16.8 Hz), which are characteristic for flavanone protons (Batterham and Highet, 1964). The annelation of 2-aryltetrahydropyran-4-one core also was confirmed by ^{13}C NMR spectral data, for compounds **16**–**32,** which are presented by the characteristic signals for flavanone cycle (189–190, 74–79 and 44–45 ppm) and the signal of carbonyl group (161 ppm).

Hydrazine is known to react with flavanones to give various compounds, depending on the reaction conditions. In particular, the principal products can be hydrazones of flavanones, 3-(2-hydroxyphenyl)-5-phenylpyrazolines, or azines of flavanones (Kálly et al., 1965a, 1965b). We found that the flavanone core recyclized upon heating EtOH solutions of **16**–**32** with a fivefold excess of hydrazine hydrate and formed 6-[5-aryl-4,5-dihydropyrazol-3-yl]-5-hydroxy-7-methylcoumarins **33**–**49**. In the ^{1}H NMR spectra of the latters the resonances characteristic of the coumarin and pyrazoline moieties (Nikitina et al., 2015) are presented. In particular, the methylene diastereotopic protons are resonated at 3.06–3.31 (dd, J = 11.2 and 16.4 Hz) and 3.69–3.90 ppm (dd, J = 10.4 and 16.4 Hz) whereas pyrazoline H-5 was observed as a multiplet (ddd, J = 3.6, 10.4 and 11.2 Hz) at 4.75–5.16 ppm. A characteristic feature of the ^{1}H NMR spectra of **33**–**49** was the separation of the NH and OH proton signals. The NH proton appeared as a doublet with J = 3.6 Hz at 7.53–7.88 ppm. The presence of the hydroxyl proton at weak field (13.29–13.94 ppm) was indicative of an intramolecular interaction between of the latter and the pyrazoline N atom. Recyclization of pyronoflavanones and formation of substituted pyrazolines also were confirmed by ^{13}C NMR spectra data of the compounds **33**–**49**. In ^{13}C NMR spectra the characteristic signals of pyrazoline core (156, 59–60 and 46–47 ppm) and the signal of carbon atom of the carbonyl group of coumarin core (161 ppm) are observed.



a) Ac_2O, pyridine, reflux, 1 h; **b**) 1. $AlCl_3$, reflux, 1 h; 2. 1N HCl;
c) ArCHO, EtOH, pyrrolidine (cat), reflux, 5-6 h, 67-84%;
d) N_2H_4*H_2O, EtOH, reflux, 2-3 h, 69-86%

Scheme 1 Synthesis of new 6-pyrazolinylcoumarin derivatives.

Table 1 Anticancer screening data at the concentration of 10 μM.

Comp	Mean growth %	Range of growth %	The most sensitive cell lines	GP % of the most sensitive cell lines	Positive cytostatic effect[a]
33	87.06	58.56–132.97	LOX IMVI (Melanoma)	58.56	0/54
34	92.06	57.13–126.03	CCRF-CEM (Leukemia)	57.13	0/55
35	87.12	48.11–118.14	SNB-75 (CNS Cancer)	48.11	1/56
36	85.90	54.55–116.54	HL-60(TB) (Leukemia)	54.55	0/58
37	85.09	45.38–118.29	HL-60(TB) (Leukemia)	49.57	2/56
			RXF 393 (Renal Cancer)	45.38	
38	77.50	36.66–117.26	NCI-H226 (Non-Small Cell Lung Cancer)	36.66	4/55
			ACHN (Renal Cancer)	47.88	
			MDA-MB-231/ATCC (Breast Cancer)	42.56	
			T-47D (Breast Cancer)	42.17	
39	94.13	49.45–146.45	MDA-MB-231/ATCC (Breast Cancer)	49.45	1/55
40	89.72	52.34–115.97	MDA-MB-231/ATCC (Breast Cancer)	52.34	0/56
41	87.44	51.78–138.22	RXF 393 (Renal Cancer)	51.78	0/54
42	93.24	58.27–112.72	CCRF-CEM (Leukemia)	58.27	0/58
43	89.29	49.08–110.64	CCRF-CEM (Leukemia)	49.08	1/58
44	81.65	7.88–105.92	HL-60(TB) (Leukemia)	7.88	1/56
45	91.40	36.95–148.57	RXF 393 (Renal Cancer)	36.95	1/56
46	99.04	55.89–137.85	SNB-75 (CNS Cancer)	55.89	0/54
47	60.64	−44.56 to 107.74	CCRF-CEM (Leukemia)	16.09	17/58
			HL-60(TB) (Leukemia)	35.62	
			MOLT-4 (Leukemia)	−44.56	
			SR (Leukemia)	28.44	
			NCI-H460 (Non-Small Cell Lung Cancer)	37.66	
			HCT-15 (Colon Cancer)	39.85	
			LOX IMVI (Melanoma)	37.15	
			IGROV1 (Ovarian Cancer)	36.66	
			CAKI-1 (Renal Cancer)	39.10	
48	100.34	61.93–121.35	SNB-75 (CNS Cancer)	61.93	0/58
49	97.25	51.08–127.04	CCRF-CEM (Leukemia)	51.08	0/56

[a] Ratio between number of cell lines with percent growth from 0 to 50 and total number of cell lines.

3.2. In vitro evaluation of the anticancer activity

Synthesized derivatives **33–49** were selected by National Cancer Institute (NCI, Bethesda USA) Developmental Therapeutic Program (DTP) and evaluated for anticancer activity at the concentration of 10^{-5} M toward a panel of approximately sixty cancer cell lines (http://dtp.nci.nih.gov). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, central nervous system, ovarian, renal, prostate and breast cancers. Primary anticancer assays were performed according to the NCI protocol as described elsewhere (Boyd and Paull, 1995; Boyd, 1997; Shoemaker, 2006; Monks et al., 1991; Alley et al., 1988). The compounds were added at a single concentration and the cell cultures were incubated for 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each compound are reported as the percent growth (GP%) of treated cells when compared to untreated control cells (Table 1). The range of percent growth shows the lowest and the highest percent growth found among the different cancer cell lines.

The most active compound **47** was found to be effective against 17 cell lines with the average cell growth percents (GP_{mean}) of 60.64%. Moreover, this derivative demonstrated cytotoxic effect on Leukemia line *MOLT-4* (GP = −44.56%) and significant cytostatic action toward *CCRF-CEM* (Leukemia), SR (Leukemia), NCI-H460 (Non-Small Cell Lung Cancer), HCT-15 (Colon Cancer), LOX IMVI (Melanoma) and CAKI-1 (Renal Cancer) with range of GP = 16.09–39.85%. Compound **38** was found to be moderately effective against NCI-H226 (Non-Small Cell Lung Cancer), ACHN (Renal Cancer), T-47D (Breast Cancer) and MDA-MB-468 (Breast Cancer) with GP = 36.66–47.88%. For the compounds **35**, **37**, **39**, **43**, **44** and **45** the average percents of cell growth (GP_{mean}) were 81.65–94.13%. However, it should be noted the selectivity toward *SNB-75* (CNS Cancer) – GP = 48.11% (**35**), *RXF 393* (Renal Cancer) – GP = 45.38% (**37**) and 36.08% (**45**), *MDA-MB-231/ATCC* (Breast Cancer) – GP = 49.45% (**39**), *CCRF-CEM* (Leukemia) – GP = 49.08% (**43**), and *HL-60(TB)* (Leukemia) – GP = 7.88% (**44**) (Table 1).

Finally, compound **47** was selected for an advanced assay against a panel of approximately sixty tumor cell lines at

Table 2 Influence of compound **47** on the growth of tumor cell lines.

Disease	Cell lime	GI_{50}, μM	Disease	Cell lime	GI_{50}, μM
Leukemia	CCRF-CEM	1.88	Melanoma	LOX IMVI	3.79
	HL-60(TB)	2.10		MALME-3M	30.7
	MOLT-4	1.92		M14	8.26
	RPMI-8226	5.10		MDA-MB-435	5.83
	SR	4.52		SK-MEL-2	32.5
				SK-MEL-28	12.1
				SK-MEL-5	3.47
				UACC-257	14.8
				UACC-62	4.96
	MG_MID	3.10		MG_MID	12.93
NSC lung cancer	A549/ATCC	6.56	Ovarian cancer	IGROV1	4.57
	EKVX	9.55		OVCAR-3	5.23
	HOP-62	7.75		OVCAR-4	7.51
	HOP-92	3.78		OVCAR-5	13.4
	NCI-H226	33.0		OVCAR-8	6.02
	NCI-H23	6.01		NCI/ADR-RES	4.24
	NCI-H322M	7.61		SK-OV-3	8.24
	NCI-H522	3.91			
	NCI-H460	5.41			
	MG_MID	9.29		MG_MID	7.03
Colon cancer	COLO 205	13.4	Renal cancer	786-0	7.24
	HCC-2998	6.97		A498	>100.0
	HCT-116	5.63		ACHN	5.14
	HCT-15	4.82		CAKI-1	4.20
	HT29	11.0		SN12C	8.14
	KM12	5.99		TK-10	13.4
	SW-620	5.93		UO-31	4.93
	MG_MID	7.68		MG_MID	20.44
CNS cancer	SF-268	6.18	Breast cancer	MCF7	3.44
	SF-295	4.91		MDA-MB-231/ATCC	4.22
	SF-539	6.60		HS 578T	6.87
	SNB-19	8.76		BT-549	44.7
	SNB-75	3.57		T-47D	3.25
	U251	6.37		MDA-MB-468	4.19
	MG_MID	6.07		MG_MID	11.11
Prostate Cancer	PC-3	12.7			
	DU-145	19.5			
	MG_MID	16.1			

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Table 3 COMPARE analysis results for compound **47** at GI_{50} level.

Rank	PCC[a]	Target	Target vector NSC	Target mechanism of action[b]
1	0.711	Fluorodopan	S73754	Alkylating agent
2	0.655	Melphalan	S8806	Nitrogen mustard alkylating agent
3	0.624	Hepsulfam	S329680	Alkylsulfonate alkylating agents, which induced DNA interstrand cross-links
4	0.623	Chlorambucil	S3088	Nitrogen mustard alkylating agent
5	0.609	Menogaril	S269148	Inhibition of initial rate of tubulin polymerization
6	0.605	Dichloroallyl lawsone	S126771	Inhibitor of pyrimidine nucleothides biosynthesis
7	0.605	m-AMSA (amsacrine)	S249992	Inhibitor of topoisomerase II

[a] Only correlations with PCC $\geqslant$ 0.60 were selected, as significant.
[b] Putative mechanisms of action were identified with the use of literature sources.

10-fold dilutions of five concentrations (100 μM, 10 μM, 1.0 μM, 0.1 μM and 0.01 μM) (Boyd and Paull, 1995; Boyd, 1997; Shoemaker, 2006; Monks et al., 1991; Alley et al., 1988). The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents after 48-h exposure and using SRB protein assay to estimate cell viability or growth. Dose–response parameters were calculated for each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to the total inhibition; and LC_{50} – molar concentration of the compound leading to 50% net cell death. Furthermore, a mean graph midpoints (MG_MID) were calculated for GI_{50}, giving an average activity parameter over all cell lines for the tested compound. For the MG_MID calculation, insensitive cell lines were included with the highest concentration tested.

The most active compound **47** showed inhibition activity (GI_{50} < 10 μM) against 45 of 58 human tumor cell lines with average GI_{50} values of 10.29. Moreover, the mentioned derivative demonstrated a certain sensitivity profile toward the Leukemia cell lines *CCRF-CEM*, *HL-60(TB)* and *MOLT-4* with the range of GI_{50} values 1.88–2.10 μM (Table 2). Values of TGI and LC_{50} were above the 100 μM except data of TGI for Leukemia cell lines *CCRF-CEM* (TGI = 5.06 μM), *HL-60(TB)* (TGI = 59.6 μM) and *MOLT-4* (TGI = 4.04 μM), as well Breast Cancer cell line MDA-MB-468 (TGI = 81.3 μM).

The SAR study revealed that the level of antitumor activity of synthesized compounds depends on substituents at 3,4-positions of coumarin core. The presence of the cyclohexyl fragment (**47**) improved the antiproliferative activity in comparison with cyclopentyl residue or methyl groups. The same trend was observed for other 6-heteroarylcoumarins described in our previous paper (Galayev et al., 2015). Moreover, we noticed that compounds bearing 3-methoxy-4-hydroxyphenyl (**47**) and 4-hydroxyphenyl (**38**) substituents at position 5 of the pyrazoline fragment were more active than other analogues (**40**, **41**, **48**, **49**).

3.3. COMPARE analysis

NCI's COMPARE algorithm (Boyd and Paull, 1995; Boyd, 1997; Shoemaker, 2006; Monks et al., 1991) allows to assume biochemical mechanisms of action of the novel compounds on the basis of their *in vitro* activity profiles when comparing with those of standard agents. We performed COMPARE computations for the compound **47** against the NCI "Standard Agents" database at the GI_{50} level (Table 3). However, the obtained Pearson correlation coefficients (PCC) did not allow to distinguish cytotoxicity mechanism of tested compounds with high probability. The compound **47** showed the highest correlation at the GI_{50} level with menogaril – tubulin polymerization inhibitor (PCC = 0.609); dichloroallyl lawsone – pyrimidine biosynthesis inhibitor (PCC = 0.605); amsacrine – inhibitor of topoisomerase II (PCC = 0.605), as well as some alkylating agents – flurodopan, melphalan, hepsulfam and chlorambucil (PCC = 0.623–0.711).

4. Conclusions

In the presented paper new 6-pyrazolinylcoumarin derivatives are described. Antitumor activity assay of seventeen synthesized compounds allowed to identify 1-hydroxy-2-[5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydropyrazol-3-yl]-3-methyl-7,8,9,10-tetrahydrobenzo[*c*]chromen-6-one **47** (GI_{50mean} = 10.20 μM in the NCI 60-cell-line assay) with certain sensitivity profile toward the Leukemia cell lines *CCRF-CEM* and *MOLT-4* (GI_{50}/TGI values 1.88/5.06 μM and 1.92/4.04 μM respectively). Further investigations of the 6-heteroarylcoumarins derivatives could lead to more potent compounds as promising candidates for the development of new anticancer chemotherapy.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsps.2016.05.005.

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