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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-methylcoumarins (**33–49**) were obtained under reactions of hydrazine and 2-aryl-5-methyl-2,3-dihydropyrano[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.