A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2023** (vii) 202312085

Synthesis of pyridocoumarin β-glycosides with possible biological activity¶

Christos Christou[a], Ioannis Pavlou[a](in part), Ioanna Sigala[b], Eleni Nikolakaki[b], Dimitra J. Hadjipavlou-Litina[c], and Konstantinos E. Litinas[a,*]

[a] *Laboratory of Organic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*

[b] *Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*

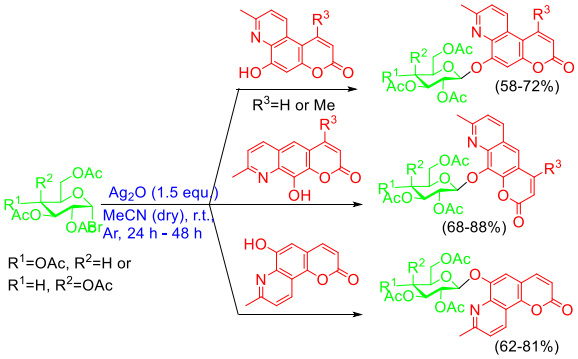
[c] *Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*

Email: klitinas@chem.auth.gr

Received 09-14-2023 **Accepted Manuscript** 11-24-2023 **Published on line** 12-06-2023

Abstract

New acetylated β-glycosides of fused pyridocoumarins are synthesized in good to very good yields from hydroxy-substituted fused pyridocoumarins and 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl bromide or 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide by the Koenigs and Knorr method using silver oxide. β-Glycosides of coumarins are also synthesized. The starting hydroxy derivatives of fused pyridocoumarins are prepared in excellent yield by the three-component reaction of amino hydroxycoumarin with *n*-butyl vinyl ether under iodine catalysis. The antioxidant ability of the title compounds, as well as their ability to inhibit soybean LOX and their antiproliferative activity against HeLa, MCF7 and MDA-MB-231 cancer cells have been examined.



Keywords: Dipyranoquinolinone's β-glycosides, coumarin's β-glycosides, fused pyridocoumarins, iodine catalysis, antioxidant activity, anti-inflammatory activity.

¶ Preliminary communication presented at 5th Chemistry Conference of Postgraduate and Undergraduate Students, Thessaloniki Greece, April 2-3, 2022.

Introduction

Coumarin derivatives, found in many natural products or synthetically prepared, present interesting biological activities such as anti-HIV, anti-inflammatory, antioxidant, antitumor, antimicrobial, antidiabetic, anti-Alzheimer, antihypertensive, antitubercular, antiproliferative, antimalarial, neuroprotective and, antibiotic activity.[1-9] A lot of coumarins are present in nature as β-glycosides and they have been isolated from diverse natural sources. Some β-glycosides of monosaccharides are depicted in Figure 1. Skimmin (**I**) is the main component of *Hydrangea Paniculata* extracts reducing the progression of diabetic nephropathy due to their antioxidant and anti-inflammatory activities.[10] Rhodonetin (**II**), a β-galactoside, is isolated from the aerial parts of *Rhododendron lepidotum*, which is used against headache.[11] Daphnin (**III**) is, also, isolated from the same plant and exhibited antibacterial activity.[12] Esculin (**IV**) is a main active component of *Cortex fraxini*, a traditional Chinese medicine with excellent anti-inflammatory and antioxidant activities.[13] Chichoriin (**V**) is isolated from *Taraxacum officinale* and the herb of *Hieracium pilosella* L. and is active against the key proteins of SARS-CoV-2 virus.[14] Scopolin (**VI**), a main constituent of the stems of *Erycibe obtusifolia* Benth, has presented anti-obesity effects by preventing adipocyte differentiation and weight gain in mice.[15] Fraxin (**VII**) was extracted from the leaves of *Weigela florida var. glabra* and it was found to protect cells from oxidative stress.[16] Isofraxetin-6-O-β-D-glucopyranoside (**VIII**) was isolated from the husks of *Xanthoceras sorbifolia* and it was reported to be a natural neuroinflammatory inhibitor.[17] Eleutheroside B1 (**IX**) is one of the major active components of *Acanthopanax senticosus* used as an adaptogenic substance.[18]

Interesting biological properties are also observed by fused heterocyclic coumarins.[3,5-7] Among these, fused pyridocoumarins are also present in nature.. Goniothaline A (**X**) and goniothaline B (**XI**) (Fig. 1) are isolated from *Goniothalamus Australis*, and evaluated for their antimalarial activity.[19] Polynemoraline C (**XII**) is extracted from the leaves and branches of *Polyalthia nemoralis* A DC and exhibited anti-inflammatory, antitumor, anticholinergic, and antimicrobial activities.[20] Ganocochliarine F (**XIII**) was found in *Ganoderma cochlear and* tested for its activity against renal fibrosis.[21] Santiagonamine (**XIV**) was isolated from the extracts of *Berberis darwinii* Hook exhibiting wound-healing activity.[22]

Fused pyridocoumarins are synthesized starting mainly from aminocoumarins under Skraup, Skraup-Doebner von Miller, Povarov, and Friedlander reaction conditions.[7,9] Multi-Component (MCR), and Metal-catalyzed reactions are, also, in use for the synthesis of these compounds. O-Glycosides are synthesized mostly from carbohydrate derivatives bearing a leaving group in the anomeric position.[23,24] Starting from the pioneering works of A. Michael,[25] and E. Fischer[26] for the glycosylation of phenols and alcohols using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride, and free glycose, respectively, there is a huge progress in the methods for the synthesis of glycosides. The Koenigs and Knorr method utilizes glycosyl bromides and Ag_2CO_3 or Ag_2O for the synthesis of β-glycosides.[27] In the modification of this method by Helferich, mercury salts are used.[28] In the Lemieux method, the glycosyl bromides in the presence of $Et_4N^+ Br^-$ led to α-glycosides.[29] The synthesis of glycosides is achieved, also, by replacing halides with other leaving groups such as imidate, trichloroacetimidate, trifluoroacetimidate, thioether, *o*-alkynyl benzoate, O-benzoxazolyl imidates or phosphate.[30-33]

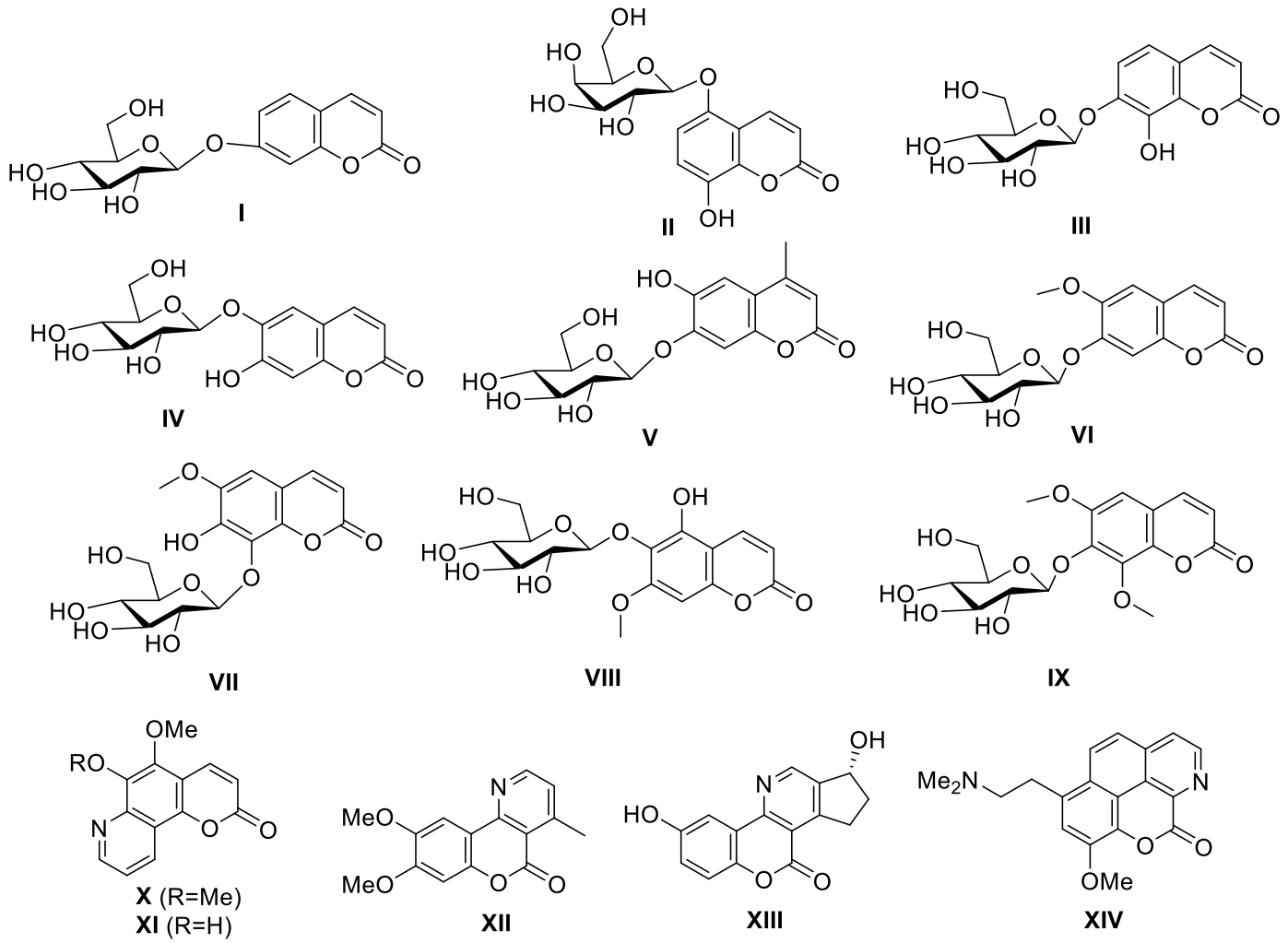


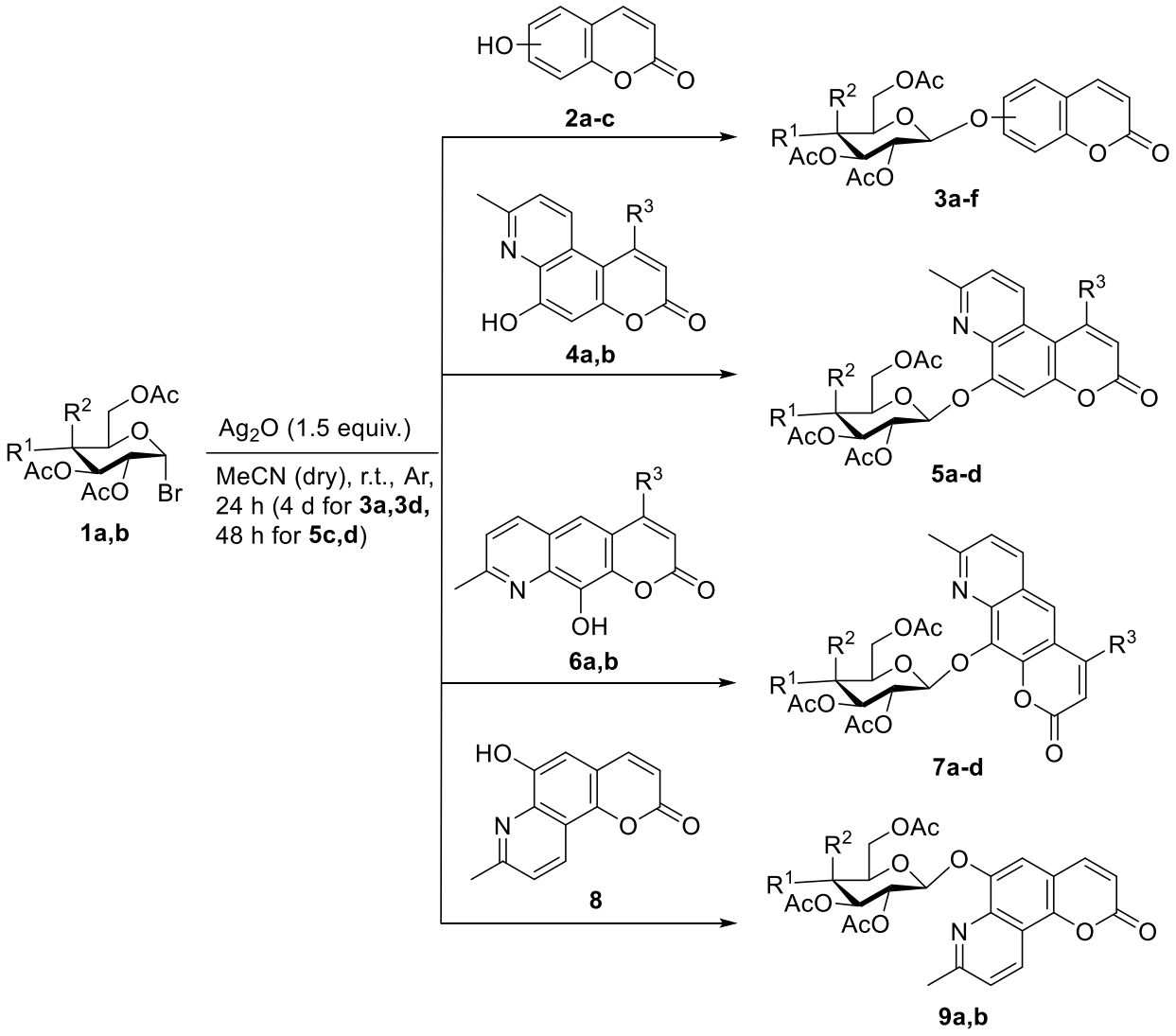
Figure 1. Natural coumarin β-glycosides and fused pyridocoumarins with biological activity.

The last decade, we have reported the synthesis and biological evaluation of fused pyridocoumarins,[34-36] as well as other coumarin derivatives, and hybrids.[37-41] As part of our ongoing interest in the synthesis and biological evaluation of new coumarin derivatives, we report herein the synthesis of new β-glycosides of fused pyridocoumarins following the above mentioned Koenigs and Knorr method. The reactions studied and the synthesized products are depicted in Scheme 1.

Results and Discussion

At first, we tested the synthesis of β-glycosides of coumarins as they are presented in Scheme 1. The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl bromide (**1a**)[42] with 6-hydroxycoumarin (**2a**) under treatment with Ag_2O in MeCN at rt in the absence of light, under argon atmosphere, for 4 d resulted in coumarin's β-glycoside **3a** in 45% yield. A similar reaction of **1a** with 7-hydroxycoumarin (**2b**) for 24 h led to the β-glycoside **3b** in 88% yield, while the reaction of **1a** with 8-hydroxycoumarin (**2c**)[43] gave **3c** in 84% yield. From these three compounds only **3b** was known, but here the yield of the synthesis is better using 1.5 equivalents of Ag_2O than 1.2 equivalent.[44] For the elucidation of the structure of **3a-c** ^{1}H-NMR, ^{13}C-NMR and gHSQC-NMR experiments were performed. The anomeric carbon for compounds **3a-c** is at 99.6, 98.3, and 101.1 ppm, while the

corresponding proton resonates at 5.08 (d, *J*=7.4 Hz, 1H), 5.17 (d, J=7.7 Hz, 1H), and 4.74 (d, *J*=8.0 Hz, 1H), respectively, revealing an axial-axial coupling of the protons and the β-substitution for the coumarin moiety.



1a: R^1=OAc, R^2=H
b: R^1=H, R^2=OAc

2a: 6-substituted
b: 7-substituted
c: 8-substituted

3a: R^1=OAc, R^2=H, 6-substituted
b: R^1=OAc, R^2=H, 7-substituted
c: R^1=OAc, R^2=H, 8-substituted
d: R^1=H, R^2=OAc, 6-substituted
e: R^1=H, R^2=OAc, 7-substituted
f: R^1=H, R^2=OAc, 8-substituted

4,6a: R=H
b: R=Me

5,7a: R^1=OAc, R^2=H, R^3=H
b: R^1=OAc, R^2=H, R^3=Me
c: R^1=H, R^2=OAc, R^3=H
d: R^1=H, R^2=OAc, R^3=Me

9a: R^1=OAc, R^2=H
b: R^1=H, R^2=OAc

Scheme 1. Synthesis of β-glycosides of coumarins and fused pyridocoumarins.

The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1b**)[42] with hydroxycoumarins **2a-c** under the similar Koenigs and Knorr conditions led to the new coumarin β-glycosides **3d-f**, in 53%, 65%, and 90% yield, respectively (Scheme 1). These glycosides have the β-conformation as the coupling constant of anomeric proton is 8.1, 8.1, and 6.7 Hz, respectively, revealing an axial-axial coupling with the nearby proton.

After the successful synthesis of β-glycosides of coumarins, we continued with the synthesis of β-glycosides of fused pyridocoumarins (Scheme 1). The reactions of glycopyranosyl bromide **1a** with 6-hydroxy-

8-methyl-3*H*-pyrano[3,2-*f*]quinolin-3-ones **4a,b**[45] in the presence of Ag_2O, in CH_3CN, in the absence of light, under argon atmosphere resulted in the synthesis of β-glycopyranosides **5a,b** in 60% and 58% yield, respectively (Table 1, entries 1,2). According to ^{1}H-NMR, ^{13}C-NMR and gHSQC-NMR experiments, the anomeric carbons of **5a,b** resonate at 100.1 and 101.6 ppm, respectively, while the corresponding proton shift is presented at 5.41 (d, J=8.8 Hz, 1H) and 5.37 (d, J=6.2 Hz, 1H) ppm, showing an axial-axial coupling of the protons and the β-conformation for these glycosides. The analogous reactions of galactopyranosyl bromide **1b** with hydroxy compounds **4a,b** gave β-glycosides **5c,d** in 71% and 72% yield, respectively (Table 1, entries 3,4). The coupling constants *J* for the anomeric protons were 7.9 Hz and 8.0 Hz, respectively.

The similar Koenigs and Knorr reactions of glycopyranosyl bromide **1a** with the new 10-hydroxy-2*H*-pyrano[3,2-g]quinoline-2-ones **6a,b** led to the synthesis of β-glycopyranosides **7a,b** in 71% and 68% yield, respectively (Table 1, entries 5,6). The β-glycopyranosides **7c,d** were synthesized from the reactions of bromide **1b** with quinolinones **6a,b** in 84% and 88% yield, respectively (Table 1, entries 7,8). The coupling constant *J* of anomeric protons of **7a-d** was 7.4-7.7 Hz, revealing the β-conformation of glycosides. The starting compounds 8-methyl-10-hydroxy-2*H*-pyrano[3,2-g]quinoline-2-one (**6a**) and 4,8-dimethyl-10-hydroxy-2*H*-pyrano[3,2-g]quinoline-2-one (**6b**) were prepared in 86% and 85% yield from 7-amino-8-hydroxycoumarin[46] and 7-amino-8-hydroxy-4-methylcoumarin,[47] respectively, by the three-component reaction with *n*-butyl vinyl ether in the presence of 10% iodine in CH_3CN under reflux, following our recent published procedure.[45] 7-Amino-8-hydroxycoumarin was prepared by the reduction of 8-hydroxy-7-nitrocoumarin (synthesized by treatment of 8-hydroxycoumarin[48] with KNO_3 in H_2SO_4 solution in an ice bath) with H_2 in the presence of 10% Pd/C in 98% yield.

We have examined, also, the reactions of 6-hydroxy-8-methyl-2H-pyrano[2,3-f]quinolin-2-one (**8**) with the glycosyl bromides **1a,b**, which resulted in the β-glycosides **11a,b** in 81% and 62% yield, respectively (Table 1, entries 9,10). In the above products the coupling constant *J* of anomeric protons was 7.0-7.9 Hz, revealing the β-conformation of glycosides.

In the case of β-glycosides of fused pyridocoumarins the yield is lower for the 7-substituted derivatives. This is possibly due to the diminished activity of 7-hydroxy group from the conjugation to the pyranone carbonyl.

Table 1. Synthesis of β-glycosides of fused pyridocoumarins **5a-d**, **7a-d**, **9a,b**

Entry	Reacting Compounds	Reaction Conditions	Product (Yield, %)
1	**1a**, **4a** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**5a** (60)
2	**1a**, **4b**(1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**5b** (58)
3	**1b**, **4a** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 48 h	**5c** (71)
4	**1b**, **4b** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 48 h	**5d** (72)
5	**1a**, **6a** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**7a** (71)
6	**1a**, **6b** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**7b** (68)
7	**1b**, **6a** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**7c** (84)
8	**1b**, **6b** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**7d** (88)
9	**1a**, **8** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**9a** (81)
10	**1b**, **8** (1.2 equiv.)	Ag_2O (1.5 equiv.), CH_3CN, r.t., Ar, 24 h	**9b** (62)

Since skimmin (I),[10] esculetin (IV),[13] fraxin (VII),[16] and isofraxetin-6-O-β-D-glucopyranoside (VIII)[17] are known for their antioxidant and antiinflammatory activities, we decided to test the synthesized compounds as antioxidants and antiinflammatory agents through their ability to inhibit soybean lipoxygenase (Table 2). We used the water soluble azo compound AAPH protocol to evaluate their antilipid peroxidation ability *in vitro*. The role of reactive oxygen species is well known in the induction of oxidative stress and inflammatory disorders. In addition, NDGA and Trolox were included in the study as standards for comparison.

Table 2. *In vitro* activities of compounds: Lipoxygenase inhibition (LOX%); inhibition of lipid peroxidation (ILP %)

Entry	Compounds	LOX inhibition (% at 100 μM)	ILP (% at 100 μM)
1	**3a**	na	26.8
2	**3b**	na	na
3	**3c**	na	47
4	**3d**	22	na
5	**3e**	na	10
6	**3f**	na	13
7	**5a**	na	49
8	**5b**	na	na
9	**5c**	70	70
10	**5d**	37	na
11	**7a**	na	68.6
12	**7b**	41	28.5
13	**7c**	5.5	48
14	**7d**	65	na
15	**9a**	na	23.5
16	**9b**	na	9.3
17	**NDGA**	88	nt
18	**Trolox**	nt	91

Values are means of three or four different determinations. na= no activity under the experimental conditions. Means within each column differ significantly ($p < 0.05$). nt = Not determined.

The water-soluble azo compound AAPH has been extensively used as a clean and controllable source to produce alkylperoxyl free radicals by heating. The % inhibition of lipid peroxidation, using the APPH assay, for the examined compounds is shown in Table 2. **3a**, **3e, 3f, 7b, 9a** and **9b** were found to be weak inhibitors of lipid peroxidation (9.3-28.5%), whereas **3b**, **5b**, **3d, 5d** and **7d** were found inactive under the reported experimental conditions. On the contrary, the **5c** followed by **7a** showed the highest antioxidant activities. **3c** and **7c** showed moderate equipotent activity. In general, it seems that among the coumarin-β-glycosides the position of glycosidation plays an important role. The 6-/7-derivatives are less potent antioxidants compared to the 8-derivatives. Among the pyridocoumarin-β-glycosides the 7- and 8-derivatives are more potent,

whereas the 6-analogues present moderate activity. The stereochemistry of molecules **5a** and **5b** significantly influences activity. No role for lipophilicity was defined.

The lipoxygenase (LOX) is one of the enzymes implicated in the first two steps in the metabolism of arachidonic acid to leukotrienes. The generation of LTB4 is important in the pathogenesis of neutrophil-mediated inflammatory diseases related to the severity of cardiovascular diseases, asthma, and cancer. Published results suggest the relationship between LOX inhibition and the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+}. However, alternative mechanisms suggest that most of the LOX inhibitors are antioxidants or free radical scavengers. Perusal of the % inhibition values (Table 2) show that **5c** and **7d** were found to be more potent. Between them it is important that **5c** presents the higher antilipid peroxidation activity.Thus, the most potent inhibitor is **5c** followed by **7d** and then the **7b** and **5d.** It seems that the galactosides of the pyridocoumarins **5c** and **7d** present better activities.

Next, the cytotoxic activity of the above more potent compounds **5c, 5d, 7b, 7d** was examined. The cervical cancer cell line HeLa and two breast cancer cell lines MCF7 and MDA-MB-231 were used to assess the cytotoxic activity of the compounds at a concentration range of 2.5−100 μM for 48 h by using the colorimetric method MTT. The results were expressed as EC_{50} (the concentration that results in 50% loss of cell viability). The data from the experiments are presented in Table 3. Treatment of both breast cancer cells with all tested compounds exhibited no cytotoxicity after 48 h, while **7b**, **5c**, **7d** had a rather limited effect against HeLa cells with EC_{50} values ranging from 72 to 86 μM.

Table 3. Half maximal effective concentration (EC_{50} Values) of **7b, 5c, 5d, 7d**in HeLa, MCF7 and MDA-MB-231 cancer cell lines. Results are presented as mean ± SE of three independent experiments

		EC_{50} *(*μM)		
Entry	Compound	HeLa	MCF7	MDA-MB-231
1	**7b**	76 ± 2.8	>100	>100
2	**5c**	72 ± 3.4	>100	>100
3	**5d**	>100	>100	>100
4	**7d**	86 ± 6.3	>100	>100

Conclusions

Acetylated β-glycosides of fused pyridocoumarins have been synthesized for a first time in good to very good yields from hydroxy-substituted fused pyridocoumarins and 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl bromide or 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide through the Koenigs and Knorr method. The starting hydroxy derivatives of fused pyridocoumarins are prepared in excellent yield by the three-component reaction of amino hydroxycoumarin with *n*-butyl vinyl ether under iodine catalysis. The antioxidant ability of the title compounds, as well as their ability to inhibit soybean LOX have been examined. The most potent inhibitor is **5c,** which also presents high antioxidant activity**..** It seems that the galactosides of the pyridocoumarins **5c** and **7d** presented better activities, pointed that the presence of OAc substituent in R^1 position influences activity. The tested compounds had a rather limited effect on HeLa, MCF7 and MDA-MB-231 cancer cells.

Experimental Section

General. All the chemicals were purchased from either Sigma–Aldrich Chemie GmbH (Eschenstr. 5, 82024 Taufkirchen, Germany) or Merck KGAA (Frankfurter Strasse 250, Darmstadt, 64293, Germany). Melting points were determined with a Kofler hotstage apparatus and are uncorrected. IR spectra were obtained with a Perkin–Elmer Spectrum BX spectrophotometer as KBr pellets. NMR spectra were recorded with an Agilent 500/54 (DD2) (500 MHz and 125 MHz for ^{1}H and ^{13}C, respectively) using TMS as an internal standard. *J* values are reported in Hz. Mass spectra were determined with an LCMS-2010 EV Instrument (Shimadzu) under electrospray ionization (ESI) conditions. HRMS (ESI-MS) were recorded with a ThermoFisher Scientific (168 Third Avenue, Waltham, MA USA 02451) model LTQ Orbitrap Discovery MS. Silica gel No. 60, Merck KGAA (Frankfurter Strasse 250, Darmstadt, 64293, Germany) was used for column chromatography. 6-hydroxycoumarin and 7-hydroxycoumarin are commercially available. 2,3,4,6-Tetra-*O*-acetyl-α-D-glycopyranosyl bromide (**1a**) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1b**) were prepared according to the literature.[42]

Biochemical reagents were of analytical grade and purchased from commercial sources (Merck, Merck KGaA, Darmstadt, Germany, Fluka Sigma-Aldrich, Laborchemikalien GmbH, Hannover, Germany, Alfa Aesar, Karlsruhe, Germany and Sigma, St. Louis, MO, USA). Soybean lipoxygenase, sodium linoleate, 2,2-azobis-(2-amidinopropane), dihydrochloride (AAPH), and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical, Co. (St. Louis, MO, USA).

8-Hydroxy-7-nitro-2*H*-chromen-2-one. A solution of 8-hydroxycoumarin (**2c**) (0.488 g, 3.0 mmol) in concentrated H_2SO_4 (2 mL) was placed in a ice-bath. KNO_3 (0.364 g, 3.6 mmol) was then added. The mixture was stirred for 15 min at 0°C and for 30 min at room temperature. Then, the mixture was poured in an ice solution of 10% Na_2CO_3 (10 mL). The precipitated solid was filtered, dried under vacuum, and separated by column chromatography (silica gel No 60, hexane/EtOAc 2:1 then 1:1 and 1:99) to give from the faster moving band 8-hydroxy-7-nitro-2*H*-chromen-2-one (0.342 g, 55%).Yellow solid, mp 238-240°C (EtOH) (lit.[48] mp 224°C), ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.68 (d, *J* 9.3 Hz, 1H), 7.28 (d, *J* 8.4 Hz, 1H), 7.81 (d, *J* 8.4 Hz, 1H), 8.09 (d, *J* 9.3 Hz, 1H) ; ^{13}C-NMR (125 MHz, DMSO- d_6) δ: 117.5, 119.0, 119.7, 122.9, 138.2, 140.8, 143.6, 144.1, 159.1.

7-Amino-8-hydroxy-2*H*-chromen-2-one. In a solution of 8-hydroxy-7-nitro-2*H*-chromen-2-one (90 mg, 0.44 mmol) in methanol (50 mL) 10% Pd/C (52 mg, 0.05 mmol) was added. The mixture was stirred under 1 atm of hydrogen at r. t. for 2 h and filtered. The precipitate was washed with hot methanol (3 x 10 mL) and the filtrate was concentrated under reduced pressure to give 7-amino-8-hydroxy-2*H*-chromen-2-one (76 mg, 98%). Brown solid, mp 202-203°C (hexane/EtOAc) (lit.[46] mp 199-200°C), ^{1}H-NMR (500 MHz, DMSO- d_6) δ: 5.61 (s, 2H), 5.97 (d, *J* 9.2 Hz, 1H), 6.61 (d, *J* 8.3 Hz, 1H), 6.93 (d, *J* 8.3 Hz, 1H), 7.79 (d, *J* 9.2 Hz, 1H), 9.22 (brs, 1H).

10-Hydroxy-8-methyl-2*H*-pyran[3,2-*g*]quinoline-2-one (6a). **Typical procedure.** 7-Amino-8-hydroxycoumarin (0.12 g, 0.68 mmol) was dissolved in acetonitrile (4 mL). *n*-Butyl vinyl ether (261.0 µL, 203.5 mg, 2.03 mmol) and iodine (17.2 mg, 0.068 mmol) were, then, added and the resulting mixture was refluxed for 2 h. After cooling, the solvent was evaporated and the residue was separated by column chromatography (silica gel No 60, hexane/EtOAc 1:2) to give **6a** (0.141 g, 86%). Brown solid, m.p.232-234°C (CH_3COOEt), ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.77 (s, 3H), 6.48 (d, *J* 9.5 Hz, 1H), 7.34 (d, *J* 8.3 Hz, 1H), 7.44 (s, 1H), 7.78 (d, *J* 9.5 Hz, 1H), 8.10 (d, *J* 8.3 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 24.7, 117.5, 117.6, 119.8, 123.1, 123.3, 123.5, 138.0, 139.1, 144.8, 144.9, 159.9, 160.0; MS (ESI): 228 $[M + H]^+$; HRMS (ESI): Calcd for $C_{13}H_{10}NO_3$ $[M + H]^+$ 228.0662. Found: 228.0671.

10-Hydroxy-4,8-dimethyl-2*H*-pyran[3,2-*g*]quinoline-2-one (6b). 81 mg, 85% (from 7-amino-8-hydroxy-4-methyl-2*H*-chromen-2-one, 80 mg, 0.42 mmol, *n*-butyl vinyl ether, 160.3 mL, 125 mg, 1.25 mmol, and I_2, 10.2 mg, 0.042 mmol), brown solid, mp 191-193°C (CH_3COOEt), ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.48 (s, 3H), 2.71 (s, 3H), 6.43 (s, 1H), 7.41 (d, *J* 8.5 Hz, 1H), 7.75 (s, 1H), 8.28 (d, *J* 8.5 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 18.8, 25.5, 114.0, 115.5, 120.2, 122.8, 123.0, 137.4, 138.3, 138.7, 138.9, 153.4, 159.7, 159.9; MS (ESI): 242 $[M + H]^+$; HRMS (ESI): Calcd for $C_{14}H_{12}NO_3$ $[M + H]^+$ 242.0818. Found: 242.0830.

***(2R,3R,4S,5R,6S)*-2-(Acetoxymethyl)-6-((2-oxo-2*H*-chromen-7-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3b)**. **Typical procedure.** Ag_2O (52 mg, 0.225 mmol) was added to a solution of **1a** (62 mg, 0.15 mmol) and **2b** (29 mg, 0.18 mmol in dry CH_3CN (5 mL) in the absence of light. The mixture was stirred for 24 h. Then, it was diluted with EtOAc (5 mL), filtered through a celite pad and washed with EtOAc (3 x 5 mL). The filtrate was washed with 10% $NaHCO_3$ (2 x 5 mL), brine (5 mL) and water (5 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was separated by column chromatography (silica gel No 60, hexane/EtOAc 1:1 to 1:2) to give **3b** (66 mg, 88%). White solid, mp 130-132°C (hexane), $[a]_D^{25.3}$ =-13.6 (c=0.8, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.04 (s, 3H), 2.06 (s, 6H), 2.11 (s, 3H), 3.90-3.95 (m, 1H), 4.19 (d, *J* 12.3 Hz, 1H), 4.29 (dd, J_1 = 5.7 Hz, J_2 = 12.3 Hz, 1H), 5.15 (d, *J* 10. 3 Hz, 1H), 5.17 (d, *J* 7.7 Hz, 1H), 5.26-5.37 (m, 2H), 6.31 (d, *J* 9.5 Hz, 1H), 6.91 (d, *J* 8.5 Hz, 1H), 6.96 (s, 1H), 7.40 (d, *J* 8.5 Hz, 1H), 7.65 (d, *J* 9.5 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.5, 20.56, 20.59, 20.7, 61.9, 68.1, 70.9, 72.4, 72.5, 98.3, 104.0, 114.2, 114.4, 114.5, 129.0, 143,1, 155.4, 159.3, 160.6, 169.2, 169.4, 170.1, 170.6 ; MS (ESI): 515 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{23}H_{25}O_{12}$ $[M + H]^+$ 493.1354. Found: 493.1352.

(2R,3R,4S,5R,6S)-2-(Acetoxymethyl)-6-((2-oxo-2*H*-chromen-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3a). 33 mg, 45% (from **1a**, 62 mg, 0.15 mmol and **2a**, 29 mg, 0.18 mmol, stirring for 4 d), yellow oil, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.04 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 3.86 (ddd, , J_1 = 2.4 Hz, J_2 = 5.2 Hz, J_3 = 9.9 Hz, 1H), 4.19 (dd, J_1 = 2.4 Hz, J_2 = 12.3 Hz, 1H), 4.29 (dd, J_1 = 5.2 Hz, J_2 = 12.3 Hz, 1H), 5.08 (d, *J* 7.4 Hz, 1H), 5.18 (t, *J* 9.4 Hz, 1H), 5.26-5.32 (m, 2H), 6.45 (d, *J* 9.6 Hz, 1H), 7.10 (d, *J* 2.7 Hz, 1H), 7.19 (dd, J_1 = 2.7 Hz, J_2 = 9.0 Hz, 1H), 7.28 (d, *J* 9.0 Hz, 1H), 7.63 (d, *J* 9.6 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.57, 20.58, 20.6, 20.7, 61.9, 68.1, 71.1, 72.2, 72.5, 99.6, 115.4, 117.6, 118.0, 119.3, 121.6, 142.7, 150.1, 152.9, 160.5, 169.2, 169.3, 170.2, 170.4 ; MS (ESI): 515 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{23}H_{25}O_{12}$ 493.1354; $[M + H]^{+\cdot}$. Found: 493.1353.

(2R,3R,4S,5R,6S)-2-(Acetoxymethyl)-6-((2-oxo-2*H*-chromen-8-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3c). 62 mg, 84% (from **1a**, 62 mg, 0.15 mmol and **2c**, 29 mg, 0.18 mmol), yellow oil, $[a]_D^{25.4}$ =17.46 (c=1.3, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.01 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 3.72-3.81 (m, 1H), 4.20-4.31 (m, 2H), 4.74 (d, *J* 8.0 Hz, 1H), 4.89 (dd, J_1 = 3.5 Hz, J_2 = 10.3 Hz, 1H), 5.45 (d, *J* 3.5 Hz, 1H), 5.53 (t, *J* 9.8 Hz, 1H), 6.42 (d, *J* 9.6 Hz, 1H), 7.18 (t, *J* 8.0 Hz, 1H), 7.25 (d, *J* 8.0 Hz, 1H), 7.41 (d, *J* 8.0 Hz, 1H), 7.68 (d, *J* 9.6 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.6, 20.65, 20.67, 20.7, 61.8, 68.3, 70.8, 72.1, 73.1, 101.1, 117.1, 120.1, 123.5, 123.6, 124.2, 143.3, 143.6, 145.4, 159.7, 169.7, 170.2 (2 x C), 170.9. MS (ESI): 515 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{23}H_{25}O_{12}$ $[M + H]^+$ 493.1354. Found: 493.1355.

(*2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((2-oxo-2*H*-chromen-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3d). 22 mg, 53% (from **1b**, 82 mg, 0.2 mmol and **2a**, 39 mg, 0.24 mmol, stirring for 4 d), yellow oil, ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.00 (s, 3H), 2.06 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 4.07-4.19 (m, 2H), 4.48 (t, *J* 6.6 Hz, 1H), 5.04 (d, *J* 8.1 Hz, 1H), 5.08 (d, *J* 4.6 Hz, 1H), 5.10-5.14 (m, 1H), 5.18 (d, *J* 7.8 Hz, 1H), 6.46 (d, *J* 8.3 Hz, 1H), 7.11 (d, *J* 2.4 Hz, 1H), 7.21 (d, J_1 = 2.4 Hz, J_2 = 8.0 Hz, 1H), 7.29 (d, *J* 8.0 Hz, 1H), 7.64 (d, *J* 8.3 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.59, 20.61, 20.66, 20.7, 60.8, 66.5, 68.0, 70.6, 70.7, 100.1, 115.4, 117.6, 118.1, 119.3, 121.7, 142.4, 150.3, 153.0, 160.6, 170.12, 170.14, 170.3, 170.4 ; MS (ESI): 515 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{23}H_{25}O_{12}$ $[M + H]^+$ 493.1354. Found: 493.1356.

(*2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((2-oxo-2*H*-chromen-7-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3e). 93 mg, 65% (from **1b**, 0.12 g, 0.29 mmol and **2b**, 57 mg, 0.35 mmol), yellow oil, $[a]_D^{23.5}$ =10.1 (c=1.8, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.01 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.17 (s, 3H), 4.10-4.14 (m, 1H), 4.16-4.21 (m, 2H), 5.13 (d, *J* 8.1 Hz, 1H), 5.14 (t, *J* 5.2 Hz, 1H), 5.47 (d, *J* 2.2 Hz, 1H), 5.50 (t, J_1 = 9.8 Hz, J_2 = 10.2 Hz, 1H), 6.30 (d, *J* 9.5 Hz, 1H), 6.91 (dd, J_1 = 2.2 Hz, J_2 = 8.5 Hz, 1H), 6.96 (s, 1H), 7.40 (d, *J* 8.5 Hz, 1H), 7.64 (d, *J* 9.5 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.5, 20.60, 20.64, 20.7, 61.4, 66.8, 68.3, 70.6, 71.5, 98.9, 104.1, 114.1, 114.4, 114.5, 128.9, 124.2, 143.0, 155.4, 159.4, 160.6, 169.3, 170.0, 170.1, 170.4 ; MS (ESI): 515 $[M + Na]^+$, HRMS (ESI): Calcd for $C_{23}H_{25}O_{12}$ $[M + H]^+$ 493.1354. Found: 493.1358.

(*2R,3S,4S,5R,6S)*-2-(Acetoxymethyl)-6-((2-oxo-2*H*-chromen-8-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3f). 53 mg, 90% (from **1b**, 49 mg, 0.12 mmol and **2c**, 24 mg, 0.15 mmol), yellow oil, $[a]_D^{25.7}$ =-5.1 (c=0.5, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.02 (s, 3H), 2.03 (s, 3H), 2.20 (s, 3H), 2.26 (s, 3H), 3.98 (t, *J* 6.7 Hz, 1H), 4.12-4.20 (m, 1H), 4.25 (dd, J_1 = 6.7 Hz, J_2 = 11.3 Hz, 1H), 4.97 (d, *J* 6.7 Hz, 1H), 5.12 (dd, J_1 = 3.3 Hz, J_2 = 10.5 Hz, 1H), 5.42 (dd, J_1 = 3.3 Hz, J_2 = 10.5 Hz, 1H), 5.46 (d, *J* 3.3 Hz 1H), 6.43 (d, *J* 9.6 Hz, 1H), 7.18 (t, *J* 7.9 Hz, 1H), 7.26 (d, *J* 8.2 Hz, 1H), 7.44 (d, *J* 7.9 Hz, 1H), 7.69 (d, *J* 9.6 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.6, 20.63, 20.7, 20.8, 61.2, 66.8, 68.3, 70.6, 71.1, 101.8, 117.2, 120.1, 123.6, 123.9, 124.1, 143.2, 143.7, 145.6, 159.5, 170.10, 170.11, 170.2, 170.3 ; MS (ESI): 515 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{23}H_{25}O_{12}$ $[M + H]^+$ 493.1354. Found: 493.1369.

(*2R,3R,4S,5R,6S*)-2-(Acetoxymethyl)-6-((8-methyl-3-oxo-3*H*-pyrano[3,2-*f*]quinolin-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (5a). 50 mg, 60% (from **1a**, 62 mg, 0.15 mmol and **4a**, 41 mg, 0.18 mmol), yellow oil, $[a]_D^{23}$ =-2.8 (c=1.2, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.06 (s, 3H), 2.07 (s, 6H), 2.14 (s, 3H), 2.76 (s, 3H), 3.92-4.01 (m, 1H), 4.21-4.35 (m, 2H), 5.21 (t, *J* 9.5 Hz, 1H), 5.37 (d, *J* 9.5 Hz, 1H), 5.41 (d, *J* 8.8 Hz, 1H), 5.53-5.60 (m, 1H), 6.49 (d, *J* 9.8 Hz 1H), 7.36 (s, 1H), 7.49 (d, *J* 8.6 Hz, 1H), 8.30 (d, *J* 9.8 Hz, 1H), 8.37 (d, *J* 8.6 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.59, 20.64, 20.70, 20.72, 25.2, 61.9, 66.8, 68.3, 70.9, 72.4, 72.6, 100.1, 105.9, 108.6, 114.3, 123.0, 124.1, 129.7, 137.8, 138.1, 153.6, 155.7, 158.4, 160.6, 169.2, 169.4, 170.2, 170.6 ; MS (ESI): 580 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{27}H_{28}NO_{12}$ $[M + H]^+$ 558.1622. Found: 558.1622.

(*2R,3R,4S,5R,6S*)-2-(Acetoxymethyl)-6-((1,8-dimethyl-3-oxo-3*H*-pyrano [3,2-*f*]quinolin-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (5b). 50 mg, 58% (from **1a**, 62 mg, 0.15 mmol and **4b**, 43 mg, 0.18 mmol), yellow oil, $[a]_D^{25.2}$=2.75 (c=0.55, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.03 (s, 3H), 2.05 (s, 3H), 2.13 (s, 3H), 2.18 (s, 3H), 2.73 (s, 3H), 2.84 (s, 3H), 3.86-3.98 (m, 1H), 4.19-4.29 (m, 2H), 5.03-5.10 (m, 1H), 5.37 (d, *J* 6.2 Hz, 1H), 5.45-5.48 (m, 1H), 5.51-5.58 (m, 1H), 6.28 (s, 1H), 7.34 (s, 1H), 7.41 (d, *J* 9.0 Hz, 1H), 8.77 (d, *J* 9.0 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.57, 20.61, 20.69, 20.71, 21.1, 25.3, 62.5, 68.9, 71.2, 72.7, 72.9, 101.6, 106.1, 108.9, 114.6, 123.3, 124.4, 130.0, 138.1, 138.5, 154.0, 156.0, 158.6, 160.9, 169.4, 169.7, 170.3, 170.8 ; MS (ESI): 594 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{28}H_{30}NO_{12}$ $[M + H]^+$ 572.1784. Found: 572.1785.

(*2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((8-methyl-3-oxo-3H-pyrano[3,2-*f*]quinolin-6-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5c). 40 mg, 71% (from **1b**, 42 mg, 0.1 mmol and **4a**, 28 mg, 0.12 mmol, stirring for 48 h), yellow oil, $[a]_D^{24.1}$=-1.89 (c=0.81, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.06 (s, 3H), 2.07 (s, 6H), 2.13 (s, 3H), 2.76 (s, 3H), 3.93-3.98 (m, 1H), 4.23-4.29 (m, 2H), 5.21 (t, *J* 9.6 Hz, 1H), 5.36 (d, *J* 9.4 Hz, 1H), 5.41 (d, *J* 7.9 Hz, 1H), 5.52-5.59 (m, 1H), 6.48 (d, *J* 9.7 Hz 1H), 7.35 (s, 1H), 7.48 (d, *J* 8.7 Hz, 1H), 8.29 (d, *J* 9.7 Hz, 1H), 8.36 (d, *J* 8.7 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.74, 20.79, 20.70, 20.85, 20.87, 25.4, 62.0, 68.4, 71.0, 72.5, 72.7, 100.1, 106.0, 108.7, 114.4, 123.2, 124.3, 129.9, 138.0, 138.3, 153.8, 155.8, 158.5, 160.7, 169.4, 169.5, 170.4, 170.8 ; MS (ESI): 580 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{27}H_{28}NO_{12}$ $[M + H]^+$ 558.1622. Found: 558.1620.

(*2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((1,8-dimethyl-3-oxo-3*H*-pyrano[3,2-*f*]quinolin-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (5d). 50 mg, 72% (from **1b**, 50 mg, 0.12 mmol and **4b**, 35 mg, 0.145 mmol, stirring for 48 h), yellow oil, $[a]_D^{22.5}$=30.70 (c=0.93, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.03 (s, 3H), 2.05 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 2.76 (s, 3H), 2.85 (s, 3H), 4.10-4.12 (m, 1H), 4.15-4.24 (m, 2H), 5.19 (dt, J_1 = 5.5 Hz, J_2 = 8.9 Hz, 1H), 5.31 (d, *J* 8.0 Hz, 1H), 5.49-5.52 (m, 1H), 5.80-5.87 (m, 1H), 6.29 (s, 1H), 7.38 (s, 1H), 7.43 (d, *J* 9.4 Hz, 1H), 8.78 (d, *J* 9.4 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.79, 20.82, 20.88, 20.95, 21.0, 25.8, 62.8, 68.8, 71.2, 72.7, 72.9, 101.0, 106.7, 109.3, 115.0, 123.8, 124.9, 130.3, 138.8, 139.0, 154.5, 156.3, 158.9, 161.1, 169.9, 170.8, 171.4, 171.6 ; MS (ESI): 594 [M + Na]$^+$; HRMS (ESI): Calcd for $C_{28}H_{30}NO_{12}$ [M + H]$^+$ 572.1784. Found: 572.1787.

(*2R,3R,4S,5R,6S*)-2-(Acetoxymethyl)-6-((8-methyl-2-oxo-2*H*-pyrano[3,2-*g*]quinolin-10-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (7a). 22 mg, 71% (from **1a**, 23 mg, 0.056 mmol and **6a**, 14 mg, 0.062 mmol), yellow oil, $[a]_D^{23.3}$=-1.44 (c=0.62, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.97 (s, 3H), 2.03 (s, 6H), 2.05 (s, 3H), 2.80 (s, 3H), 3.72-3.81 (m, 1H), 4.09-4.24 (m, 2H), 5.27-5.39 (m, 2H), 5.58 (t, *J* 7.4 Hz, 1H), 5.87 (d, *J* 7.4 Hz, 1H), 6.47 (d, *J* 9.4 Hz 1H), 7.32 (d, *J* 8.1 Hz, 1H), 7.72 (s, 1H), 7.78 (d, *J* 9.4 Hz, 1H), 8.10 (d, *J* 8.1 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.60, 20.62, 20.7, 20.8, 25.7, 61.9, 68.5, 72.0, 72.2, 73.1, 101.3, 117.7, 119.0, 122.3, 122.6, 123.6, 136.6, 136.7, 141.9, 142.8, 144.8, 159.0, 161.2, 169.3, 169.4, 170.5, 170.6 ; MS (ESI): 580 [M + Na]$^+$; HRMS (ESI): Calcd for $C_{27}H_{28}NO_{12}$ [M + H]$^+$ 558.1622. Found: 558.1634.

***(2R,3R,4S,5R,6S*)-2-(Acetoxymethyl)-6-((4,8-dimethyl-2-oxo-2*H*-pyrano[3,2-*g*] quinolin-10-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (7b)**. 39 mg, 68% (from **1a**, 41 mg, 0.11 mmol and **6b**, 29 mg, 0.12 mmol), yellow oil, $[a]_D^{25}$=-2.55 (c=0.85, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.97 (s, 3H), 2.02 (s, 6H), 2.05 (s, 3H), 2.52 (s, 3H), 2.77 (s, 3H), 3.72-3.78 (m, 1H), 4.15-4.19 (m, 2H), 5.32-5.38 (m, 2H), 5.58 (t, *J* 8.0 Hz, 1H), 5.85 (d, *J* 7.4 Hz, 1H), 6.34 (s, 1H), 7.30 (d, *J* 8.5 Hz, 1H), 7.79 (s, 1H), 8.08 (d, *J* 8.5 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 18.85, 20.59, 20.63, 20.71, 20.75, 25.8, 61.9, 68.5, 71.9, 72.3, 73.2, 101.3, 116.1, 119.4, 120.1, 122.1, 123.2, 136.5, 136.9, 142.1, 144.6, 151.2, 159.2, 161.2, 169.35, 169.43, 170.5, 170.7 ; MS (ESI): 594 [M + Na]$^+$; HRMS (ESI): Calcd for $C_{28}H_{30}NO_{12}$ [M + H]$^+$ 572.1784. Found: 572.1796.

(*2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((8-methyl-2-oxo-2*H*-pyrano[3,2-*g*]quinolin-10-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (7c). 30 mg, 84% (from **1b**, 26.5 mg, 0.065 mmol and **6a**, 17.5 mg, 0.077 mmol), yellow oil, $[a]_D^{22.8}$=43.16 (c=1.17, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.90 (s, 3H), 2.06 (s, 3H), 2.16 (s, 3H), 2.30 (s, 3H), 2.72 (s, 3H), 3.95 (dd, J_1 = 6.6 Hz, J_2 = 10.9 Hz, 1H), 4.10 (dd, J_1 = 6.6 Hz, J_2 = 10.9 Hz, 1H), 5.48-5.52 (m, 1H), 5.72-5.75 (m, 1H), 5.85 (t, *J* 6.6 Hz, 1H), 5.88 (d, *J* 8.0 Hz, 1H), 6.18 (d, *J* 7.7 Hz, 1H), 6.44 (d, *J* 9.6 Hz 1H), 7.30 (d, *J* 8.5 Hz, 1H), 7.73 (s, 1H), 7.79 (d, *J* 9.6 Hz, 1H), 8.07 (d, *J* 8.5 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.6, 20.75, 20.8, 21.3, 25.4, 61.7, 67.4, 67.6, 68.0, 68.4, 99.3, 117.3, 118.7, 122.4, 123.3, 123.8, 136.5, 137.1, 143.0, 143.1, 145.5, 158.9, 161.4, 170.0, 170.4 (2 x C), 171.0 ; MS (ESI): 580 [M + Na]$^+$ HRMS (ESI): Calcd for $C_{27}H_{28}NO_{12}$ [M + H]$^+$ 558.1622. Found: 558.1633.

(*2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((4,8-dimethyl-2-oxo-2*H*-pyrano[3,2-*g*]quinolin-10-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (7d). 50 mg, 88% (from **1b**, 41 mg, 0.1 mmol and **6b**, 29 mg, 0.12 mmol), yellow oil, $[a]_D^{25.6}$=102.63 (c=1.33, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.90 (s, 3H), 2.06 (s, 3H), 2.16 (s, 3H), 2.31 (s, 3H), 2.53 (s, 3H), 2.73 (s, 3H), 3.95 (dd, J_1 = 7.1 Hz, J_2 = 10.9 Hz, 1H), 4.11 (dd, J_1 = 7.1 Hz, J_2 = 10.9 Hz, 1H), 5.51 (d, *J* 11.1 Hz, 1H), 5.72-5.76 (m, 1H), 5.85-5.92 (m, 1H), 6.16 (d, *J* 7.6 Hz, 1H), 6.34 (s, 1H), 7.30 (d, *J* 8.5 Hz, 1H), 7.83 (s, 1H), 8.09 (d, *J* 8.5 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 19.0, 20.7, 20.87, 20.90, 21.4, 25.5, 61.8, 67.6, 67.8, 68.2, 68.6, 99.5, 116.0, 119.0, 120.2, 122.5, 123.6, 136.9, 137.4, 143.0, 145.5, 151.6, 159.0, 161.5,

170.1, 170.5 (2 x C), 171.1 ; MS (ESI): 594 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{28}H_{30}NO_{12}$ $[M + H]^+$ 572.1784. Found: 572.1794.

(*2R,3R,4S,5R,6S*)-2-(Acetoxymethyl)-6-((8-methyl-2-oxo-2*H*-pyrano[2,3-*f*]quinolin-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (9a). mg, 81% (from **1a**, 11 mg, 0.027 mmol and **8**, 7.3 mg, 0.032 mmol), yellow oil, $[a]_D^{24.3}$ =-0.76 (c=0.76, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.04 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.17 (s, 3H), 2.81 (s, 3H), 3.77-3.85 (m, 1H), 4.20 (d, *J* 11.8 Hz, 1H), 4.27 (dd, J_1 = 4.5 Hz, J_2 = 12.5 Hz, 1H), 5.22 (t, *J* 9.5 Hz, 1H), 5.36 (t, *J* 8.6 Hz, 1H), 5.43-5.45 (m, 1H), 5.47 (d, *J* 7.0 Hz 1H), 6.53 (d, *J* 9.4 Hz, 1H), 7.43 (s, 1H), 7.48 (d, *J* 8.5 Hz, 1H), 7.78 (d, *J* 9.4 Hz, 1H), 8.72 (d, *J* 8.5 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.60, 20.65, 20.7, 20.9, 25.7, 61.9, 68.5, 71.2, 72.1, 72.6, 100.8, 113.6, 115.4, 116.7, 117.8, 123.3, 130.9, 141.8, 143.6, 147.2, 148.1, 160.3, 161.2, 169.34 169.5, 170.3, 170.5 ; MS (ESI): 580 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{27}H_{28}NO_{12}$ $[M + H]^+$ 558.1622. Found: 558.1632.

***2R,3S,4S,5R,6S*)-2-(Acetoxymethyl)-6-((8-methyl-2-oxo-2*H*-pyrano[2,3-*f*]quinolin-6-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (9b)**. mg, 62% (from **1b**, 13.7 mg, 0.033 mmol and **8**, 9.1 mg, 0.04 mmol), yellow oil, $[a]_D^{24.8.}$ =-0.523 3 (c=0.53, $CHCl_3$) ; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.02 (s, 3H), 2.04 (s, 3H), 2.11 (s, 3H), 2.19 (s, 3H), 2.81 (s, 3H), 4.03 (t, *J* 6.7 Hz, 1H), 4.16 (dd, J_1 = 6.7 Hz, J_2 = 11.3 Hz, 1H), 4.26 (dd, J_1 = 6.7 Hz, J_2 = 11.3 Hz, 1H), 5.17 (dd, J_1 = 3.2 Hz, J_2 = 10.4 Hz, 1H), 5.39 (d, *J* 7.9 Hz, 1H), 5.48 (d, *J* 3.2 Hz, 1H), 5.71 (dd, J_1 = 7.9 Hz, J_2 = 10.4 Hz, 1H), 6.54 (d, *J* 9.5 Hz, 1H), 7.43 (s, 1H), 7.48 (d, *J* 8.6 Hz, 1H), 7.77 (d, *J* 9.5 Hz, 1H), 8.71 (d, *J* 8.6 Hz, 1H) ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.80, 20.83, 20.84 (2 x C), 25.8, 61.5, 67.1, 68.8, 70.9, 71.3, 101.6, 113.7, 114.8, 116.8, 117.8, 123.4, 131.1, 143.5, 147.1, 148.2, 160.24, 160.28, 161.2, 169.8, 170.37, 170.39, 170.5 ; MS (ESI): 580 $[M + Na]^+$; HRMS (ESI): Calcd for $C_{27}H_{28}NO_{12}$ $[M + H]^+$ 558.1622. Found: 558.1628.

In vitro experiments

In the *in vitro* experiments assays of each experiment was performed at a concentration of 100 µM (a 10 mM stock solution in DMSO was used, from which several dilutions were made) in triplicate and the standard deviation of absorbance was less than 10% of the mean. Statistical comparisons were made using the Student T-test. A statistically significant diference was defined as $p < 0.05$.

Inhibition of linoleic acid lipid peroxidation. The in vitro study was evaluated as reported previously by our group.[35] 10 µL of the 16 mM sodium linoleate solution was added to the UV cuvette containing 0.93 mL of a 0.05 M phosphate buffer, pH 7.4, pre-thermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 µL of a 40 mM AAPH solution, which was used as a free-radical initiator. Oxidation was carried out in the presence of the samples (10 µL from the stock solution of each compound) in the assay without antioxidants and monitored at 234 nm. Lipid oxidation was recorded in the presence of the same level of DMSO and served as a negative control. Trolox was used as the appropriate standard (positive control).

Soybean lipoxygenase inhibition study *in vitro*. The in vitro study was evaluated as reported previously by our group.[35] The tested compounds were incubated in a tris buffer pH 9, at room temperature, with sodium linoleate (0.1 mM) and 0.2 mL of enzyme solution ($1/9 \times 10^{-4}$ *w*/*v* in saline, 1000 U/mL) for 5 min, and after that the inhibition was measured. EC 1.13.11.12 from soybean was used. The method was based on the conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm by the appearance of the conjugated diene. Nor-dihydroguaeretic acid NDGA (IC_{50} = 0.45 µM) was used as a standard (positive control). Different concentrations were used to determine the IC_{50} values. A blank determination was used first to serve as a negative control.

Biochemical experiments

Cell Culture. HeLa (cervical cancer), MCF7 and MDA-MB-231 (breast cancer) cell lines were provided by ATCC (American Type Culture Collection) and were grown in medium supplemented with 10% fetal bovine serum (FBS) and antibiotics/antimycotics as monolayers at 37 ^{o}C in a 5% CO_2 incubator.

MTT Assays. HeLa ($2x10^3$ cells per well), MCF7 and MDA-MB-231 ($6x10^3$ cells per well) cells were seeded in 96-well plates and grown in a medium supplemented with 10% FBS. One day after seeding, cells were exposed to increasing concentrations (2.5-100) of compounds **10b**, **9c**, **9d**, **10d** for 48 h. The viability of the cells was estimated by an (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT metabolic colorimetric assay as desctribed previously.[49] The absorbance values were normalized to the untreated cells, which was set as 100% viability. Values shown represent the means ± SE of three independent experiments

Acknowledgements

We are grateful to 'Health and Exposome Research: Assessing Contributors to Lifetime Exposure and State of health (HERACLES)', KEDEK, Aristotle University of Thessaloniki, Thessaloniki, Greece for obtaining the HRMS spectra.

Supplementary Material

Proton and carbon-13 NMR spectra are presented in the Supplementary file associated with this article.

References

1. O'Kennedy, R.; Thornes, R. D. *Coumarins, Biology, Applications and Mode of Action*: Wiley, Chichester, 1997, p 1.
2. Fylaktakidou, K.; Hadjipavlou-Litina, D.; Litinas, K.; Nicolaides, D. *Curr. Pharm. Des*. **2004**, *30*, 3813–3833. https://doi.org/10.2174/1381612043382710
3. Medina, F. G.; Marrero, J. G.; Alonso, M. M.; González, M. C.; Córdova-Guerrero, I.; García, A. G. T.; Osegueda-Robles, S. *Nat. Prod. Rep*. **2015**, *32*, 1472-1507. https://doi.org/10.1039/C4NP00162A
4. Stefanachi, A.; Leonetti, F.; Pisani, L.; Catto, M.; Carotti, A. *Molecules* **2018**, *23*, 250. https://doi.org/10.3390/molecules23020250
5. Vlachou, E.-E.N.; Litinas, K. E. *Curr. Org. Chem*. **2019**, *23*, 2679-2721. https://doi.org/10.2174/1385272823666191025151236 .
6. Salehian, F.; Nadri, H.; Jalili-Baleh, L.; Youseftabar-Miri, L.; Abbas Bukhari, S. N.; Foroumadi, A.; Küçükkilinç, T. T.; Sharifzadeh, M.; Khoobi, M. *Eur. J. Med. Chem*. **2021**, 212, 113034. https://doi.org/10.1016/j.ejmech.2020.113034
7. Douka, M. D.; Litinas, K. E. *Molecules* **2022**, *27*, 7256. https://doi.org/10.3390/molecules27217256
8. Vlachou, E.-E.N.; Fotopoulos, I.; Gabriel, C.; Pontiki, E.; Hadjipavlou-Litina, D. J.; Litinas, K. E. *Eur. J. Med. Chem. Reports* **2022**, *5*, 100063.

https://doi.org/10.1016/j.ejmcr.2022.100063
9. Patra, P.; Patra, S. *Heterocycles* **2023**, *106*,241-269. https://doi.org/10.3987/REV-22.990
10. Zhang, S.; Wang, W.; Ma, J.; Sheng, L.; Zhang, D.; Chen, X. *Phytomedicine* **2019**, *57*, 385–395. https://doi.org/10.1016/j.phymed.2018.12.045
11. Khan, R.; Shawl, A. S.; Tantray, M.; Alam, M. S. *Fitoterapia* **2008,** *79*, 232–233. https://doi.org/10.1016/j.fitote.2007.11.009.
12. Rehman, S.; Khan, R.; Bhat, K. A.; Raja, A. F.; Shawl, A. S.; Alam, M. S. *Braz. J. Pharmacogn*. **2010**, *20*, 886-890. https://doi.org/10.1590/S0102-695X2010005000037
13. Li, C.-X.; Li, J.-C.; Lai, J.; Liu, Y. *Phytother. Res*. **2022**, *36*, 2434–2448. https://doi.org/10.1002/ptr.7470
14. Rivero-Segura, N. A.; Gomez-Verjan, J. C. *Biomolecules* **2021**, *11*, 216. https://doi.org/10.3390/biom11020216
15. Park, E.; Lee, C. G.; Kim, J.; Lim, E.; Yeo, S.; Jeong, S.-Y. *Int. J. Mol. Sci.* **2020**, *21*, 8699 https://doi.org/10.3390/ijms21228699.
16. Whang, W. K.; Park, H. S.; Ham, I.; Oh, M.; Namkoong, H.; Kim, H. K.; Hwang, D. W.; Hur, S. Y.; Kim, T. E.; Park, Y. G.; Kim, J.-R.; Kim, J. W. *Exp. Mol. Med*. **2005**, *37*, 436-446. https://doi.org/10.1038/emm.2005.54
17. Zhou, D.; Zhang, Y.; Jiang, Z.; Hou, Y.; Jiao, K.; Yan, C.; Li, N. *Bioorg. Med. Chem. Lett*. **2017**, *27*, 248–253. https://doi.org/10.1016/j.bmcl.2016.11.069
18. Sun, H.; Lv, H.; Zhang, Y.; Wang, X.; Bi, K.; Hongxin Cao, H. *Chem. Pharm. Bull.* **2007**, *55*, 1291—1295. https://doi.org/10.1248/cpb.55.1291
19. Ahn, S.; Yoon, J. A.; Han, Y. T. Total Synthesis of the Natural Pyridocoumarins Goniothaline A and B. *Synthesis* **2019**, *51*, 552-556. https://doi.org/10.1055/s-0037-1610909.
20. Pang, M.; Lee, J.; Jeon, J.-H.; Song, I.-S.; Han, Y.T.; Choi, M.-K. *Mass Spectrom. Lett*. **2021**, *12*, 200–205. https://doi.org/10.5478/MSL.2021.12.4.200
21. Wang, X.-L.; Dou, M.; Luo, Q.; Cheng, L.-Z.; Yan, Y.-M.; Li, R.-T.; Cheng, Y.-X. *Fitoterapia* **2017**, *116*, 93-98. https://doi.org/10.1016/j.fitote.2016.11.011
22. Markey, M. D.; Fu, Y.; Kelly, T. R. *Org. Lett*. **2007,** *9*, 3255-3257. https://doi.org/10.1021/ol0711974
23. Das, R.; Mukhopadhyay, B. *ChemistryOpen* **2016**, *5*, 401 – 433. https://doi.org/10.1002/open.201600043
24. Demchenko, A. V. General Aspects of the Glycosidiv Bond Formation, In "Handbook of Chemical Glycosylation, Advances in Stereoselectivity and Therapeutic Relevance"; Editor Demchenko, A. V.; Wiley-VCH: Weinheim, Germany, 2008; pp. 1-27. https://doi.org/10.1002/9783527621644.ch1
25. Michael, A. *Compt. rend*. **1879**, *89*, 355-357.
26. Fischer, E. *Chem. Ber*. **1893**, *26*, 2400-2412. https://doi.org/10.1002/cber.18930260327.
27. Koenigs, W.; Knorr, E. *Chem. Ber*. **1901**, *34*, 957-981. https://doi.org/10.1002/cber.190103401162.
28. Helferich, B.; Wedemeyer, K. F. *Justus Liebigs Ann. Chem.*, **1949**, *563*, 139-145.

https://doi.org/10.1002/jlac.19495630115
29. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am.Chem. Soc.*, **1975**, *97*, 4056-4062. https://doi.org/10.1021/ja00847a032
30. Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed*. **2009**, *48*, 1900 – 1934. https://doi.org/10.1002/anie.200802036
31. Yasomanee, J.; Demchenko, A. *J. Am. Chem. Soc.* **2012**, *134*, 20097–20102. https://doi.org/10.1021/ja307355n
32. Dutta, S.; Sarkar, S.; Gupta, S. J.; Sen, A. K. *Tetrahedron Lett*. **2013**, *54*, 865–870. https://doi.org/10.1016/j.tetlet.2012.11.101.
33. Nigudkar, S. S.; Parameswar, A. R.; Pornsuriyasak, P.; Stine, K. J.; Demchenko, A. *Org. Biomol. Chem*., **2013**, ***11***, 4068-4076. https://doi.org/10.1039/C3OB40667
34. Vlachou, E.-E.N.; Fotopoulos, I.; Gabriel, C.; Pontiki, E.; Hadjipavlou-Litina, D. J.; Litinas, K. E. *Eur. J. Med. Chem. Reports* **2022**, *5*, 100063. https://doi.org/10.1016/j.ejmcr.2022.100063.
35. Symeonidis, T. S.; Hadjipavlou-Litina, D. J.; Litinas, K. E. *J. Heterocyclic Chem.,* **2014**, *51*, 642-647. https://doi.org/10.1002/jhet.1791
36. Symeonidis, T. S.; Litinas, K. E. *Tetrahedron Lett*. **2013**, *54*, 6517–6519. https://doi.org/10.1016/j.tetlet.2013.09.089
37. Balalas, T. D.: Kanelli, M. G.; Gabriel, C.; Pontiki, E.; Hadjipavlou-Litina, D. J.; Litinas, K. E. *Synthesis* **2022**, *54*, 2894–2906. https://doi.org/10.1055/s-0037-1610793.
38. Douka, M. D.; Litinas, K. E. *Arkivoc* **2021**, *viii*, 107-118. https://doi.org/10.24820/ark.5550190.p011.432.
39. Kallitsakis, M. G.; Carotti, A.; Catto, M.; Peperidou, A.; Hadjipavlou-Litina, D. J.; Litina, K. E. *Open Med. Chem. J.* **2017,** *11***,** 196-211. https://doi.org/10.2174/1874104501711010196.
40. Vronteli, A.; Hadjipavlou-Litina, D. J.; Konstantinidou, M.; Litinas, K. E. *Arkivoc* **2015**, *iii*, 111-123. https://doi.org/10.3998/ark.5550190.p009.004
41. Kallitsakis, M. G.; Yapez, M.; Soriano, E.; Marco-Contelles, J.; Hadjipavlou-Litina, D. J.; Litinas. K. E. *Future Med. Chem.* **2015**, *7*, 103–110. https://doi.org/10.4155/fmc.14.158
42. Nkosana,N. K.; Czyzyk, D.; Siegel, Z. S.; Cote, J. M.; Taylor, E. A. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 594–600. https://doi.org/10.1016/j.bmcl.2018.01.040
43. Konrádová, D.; Kozubíková, H.; Doležal, K.; Pospíšil, J. *Eur. J. Org. Chem*. **2017**, 5204–5213. https://doi.org/10.1002/ejoc.201701021
44. Bjerre, J.; Nielsen, E. H.; Bols, M. *Eur. J. Org. Chem*. **2008**, 745–752. https://doi.org/10.1002/ejoc.200700954
45. Vlachou, E.-E.N.; Pontiki, E.; Hadjipavlou-Litina, D. J.; Litinas, K. E. *Organics* **2023**, *4*, 364-385. https://doi.org.10.3390/org4030027
46. Mehta, R. H.; Sethna. S. *J. Ind. Chem. Soc*., **1964**, 41, 122-124. Chem Abstrs. **1964**, *60*, 15820h.
47. Nicolaides, D. N.; Bezergannidou-Balouctsi, C.; Litinas, K. E.; Malamisou-Xenikaki, E.; Mentzafos, D.; Terzis, A. *Tetrahedron* **1993**, *49*, 9127-9136.

https://doi.org/10.1016/S0040-4020(01)91229-3
48. Dey, B. B.; Kutti, V. A. *Proceed. Nat. Inst. Sci. India* **1940**, *6*, 641-669. Chem Abstrs. 1942, 36, 83[4].
49. Sigala, I.; Tsamis, K.I.; Gousia, A.; Alexiou, G.; Voulgaris, S.; Giannakouros, T.; Kyritsis, A.P.; Nikolakaki, E. *Tumour Biol.* **2016**, *37*, 8699–8707. https://doi.org.10.1007/s13277-015-4738-7