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Synthesis and biological evaluation of α-methylidene-δ-lactones with 3,4-dihydrocoumarin skeleton

Jakub Modranka [a], Anna Albrecht [a], Rafał Jakubowski [a], Henryk Krawczyk [a], Marek Różalski [b], Urszula Krajewska [b], Anna Janecka [c], Anna Wyrębska [c], Barbara Różalska [d], Tomasz Janecki [a,*]

[a] Institute of Organic Chemistry, Technical University of Łódź, Żeromskiego 116, 90-924 Łódź, Poland
[b] Department of Pharmaceutical Biochemistry, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland
[c] Department of Biomolecular Chemistry, Medical University of Łódź, Mazowiecka 6/8, 92-215 Łódź, Poland
[d] Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland

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ABSTRACT

A series of new 3-methylidenechroman-2-ones bearing various aromatic moieties and various substituents at position 4 were synthesized in a three step reaction sequence. Friedel–Crafts alkylation of phenols or naphthols using ethyl 3-methoxy-2-diethoxyphosphorylacrylate in the presence of trifluoromethanesulphonic acid gave 3-diethoxyphosphorylchromen-2-ones. These compounds were employed as Michael acceptors in the reaction with Grignard reagents to give adducts which were finally used as Horner–Wadsworth–Emmons reagents for the olefination of formaldehyde. All obtained 3-methylidenechroman-2-ones were tested against two human leukemia cell lines NALM-6 and HL-60 as well as MCF-7 breast cancer and HT-29 colon cancer adenocarcinomas. Several obtained methylidenechromanones displayed high cytotoxic activity with IC_{50} values below 1 μM, mainly against leukemia and MCF-7 cell lines. Investigation of structure–activity relationships revealed that the presence of additional, *ortho*-fused benzene ring and *n*-butyl or *i*-propyl group in position 4 enhances the activity. Selected methylidenechromanones were also tested on normal human umbilical vein endothelial cells (HUVEC) and chromanone **14o** was found to be eightfold more toxic against MCF-7 than normal cells. Furthermore, antimicrobial assays revealed that chromanone **14n** is highly active and bactericidal at concentration equal to MIC or 2MIC against nosocomial and community-associated staphylococci (MRSA) which are resistant to most or all available therapeutic classes of antimicrobial drugs.

1. Introduction

α-Methylidene-γ-lactones **1** are found, as a structural subunit, in many natural and synthetic compounds which show a wide spectrum of pharmacological activities ranging from anticancer and antibacterial to allergenic and antifungal.[1,2] These compounds have in common a very characteristic *exo*-methylidene moiety conjugated with an ester group, which is believed to be crucial for their biological activities. Indeed, α-methylidene-γ-lactones **1** have been shown to be strong Michael acceptors and to react with bionucleophiles, especially cysteine mercapto groups or free intracellular glutathione.[2–5]

Along with the increasing interest in α-methylidene-γ-lactones **1** also their homologues, α-methylidene-δ-lactones **2** (Fig. 1), have become the desirable target for both, the synthetic and biological studies.[6] However, when compared to α-methylidene-γ-lactones, α-methylidene-δ-lactones are less abundant in nature and their biological activity is hardly recognized. First α-alkylidene-δ-lactones, like vernolepin **3**, were isolated in the sixties of the last century from *Vernonia hymenolepis* and contained both the α-methylidene-δ-lactone and α-methylidene-γ-lactone moieties.[7] Later on, some natural compounds with α-methylene-δ-lactone moiety alone, such as teucriumlactone **4**,[8] crassin **5a**[9] and its acetate **5b**[10] or pentalenolactone E **6**[11] were also isolated. Crassin **5a** shows in vitro activity against KB cells and its acetate **5b** has antibiotic activity.[10] In turn, artemisitene **7** is the constituent of a Chinese medical herb *Artemisa annua* L. and has moderate antimalarial activity but has served as a template for the development of potent antimalarial agents.[12,13] Also synthesis and cytotoxicities of several steroidal α-methylene-δ-lactones **8** against HeLa S3 cells were reported.[14,15] Recently we performed a comparison of the cytotoxicities of synthetic β-aryl-δ-lactones with analogously substituted β-aryl-γ-lactones against several human and mouse cell lines showing that α-methylidene-δ-lactones can be as potent as corresponding α-methylidene-γ-lactones.[16]

Very interesting, although so far little recognized subgroup of α-methylidene-δ-lactones **2** are 3-methylidene-3,4-dihydrocouma-

* Corresponding author. Tel.: +48 426313220; fax: +48 426365530.
E-mail address: tjanecki@p.lodz.pl (T. Janecki).

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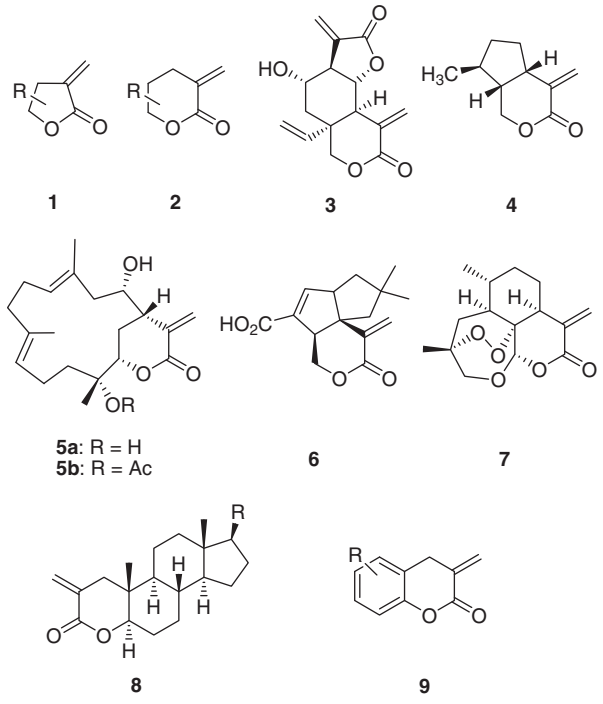


Figure 1. Structures of α-methylidene-γ- and δ-lactones.

rins (3-methylidenechroman-2-ones) **9**. It is well known that coumarin skeleton is frequently encountered in natural products which exhibit broad pharmacological activities[17–20] but, to the best of our knowledge, no natural products containing 3-methylidene-3,4-dihydrocoumarin skeleton are known. Surprisingly, even though several synthetic approaches to methylidenecoumarins **9** have been developed,[21–24] their biological activities have not been tested so far. Very recently we published a preliminary communication describing novel, simple and versatile methodology for the synthesis of variously substituted 3-methylidenechroman-2-ones via Horner–Wadsworth–Emmons approach which involves direct methylidation of the corresponding phosphorylated lactones.[25] Herein, we present the full account of this methodology along with pharmaceutical evaluation of the synthesized chromanones **14a–t**. All chromanones were tested for their cytotoxicity against two human leukemia cell lines NALM-6 and HL-60 as well as MCF-7 breast cancer and HT-29 colon cancer adenocarcinomas. Selected compounds were also tested for their toxicity against normal human umbilical vein endothelial cells (HUVEC), used as control. Furthermore, several 3-methylidenechroman-2-ones were evaluated for their antibacterial and antifungal activity.

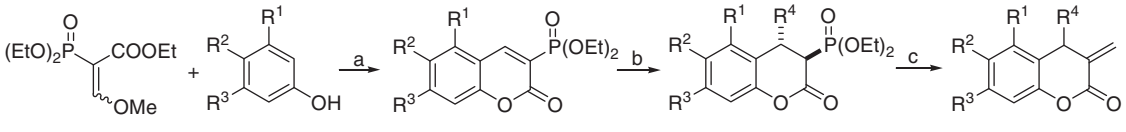
2. Results and discussion

2.1. Chemistry

Synthesis of 3-methylidenechroman-2-ones **14a–t** is shown in Scheme 1. Reaction of easily available ethyl 3-methoxy-2-diethoxyphosphorylacrylate **10**[26] with phenols **11a–c** or naphthols **11d,e** performed at room temperature, in the presence of trifluoromethanesulfonic acid or methanesulfonic acid as promoters gave phosphorylated chromenones **12a–e** in very good yields. Usually the reactions were completed in 6–18 days (see Section 4). Only the reaction with sesamol **11c** which was performed with the less acidic methanesulfonic acid instead of trifluoromethanesulphonic acid, due to the presence of acid sensitive acetal moiety, needed 60 days for completion. Reaction times are rather long and span from 6 to 60 days. However, we believe that simplicity of the operation, availability of the starting materials and high yields fully compensate this drawback. The reaction is obviously enhanced by stronger acidity of the catalyst (CF_3SO_3H vs CH_3SO_3H). Trifluoroacetic acid was also tested as promoter in this reaction but it was significantly less effective than trifluoromethanesulphonic acid. We believe that final chromenones **12a–e** are formed by Friedel–Crafts alkylation of phenols **11a–c** or naphthols **11d,e** with acrylate **10** followed by spontaneous lactonization and elimination of methanol. All reactions were fully regioselective, giving C-addition products exclusively. Structures of chromenones **12a–e** were fully confirmed by the analysis of their ^1H, ^{13}C and ^{31}P NMR spectra.

3-Diethoxyphosphorylchromen-2-ones **12a–e** were next used as Michael acceptors in the reaction with several Grignard reagents, such as methylmagnesium iodide, *n*-butylmagnesium iodide, *i*-propylmagnesium iodide and vinylmagnesium bromide. Additions were performed using 5 equiv of Grignard reagents in the presence of catalytic amount of CuI. Standard work up and purification of the crude products by column chromatography gave the expected 3-diethoxyphosphorylchroman-2-ones **13a–t** in good to excellent yields. Analysis of ^1H, ^{13}C and ^{31}P NMR spectra revealed that the reaction was fully diastereoselective and all chromanones **13a–t** were formed as single diastereomers of *trans* configuration. Diagnostic for *trans* diaxial arrangement of diethoxyphosphoryl group and R^4 substituent were coupling constants $^3J_{PCR4}$ = 14.7–18.6 Hz, $^3J_{PC5}$ = 0 Hz and $^3J_{H3H4}$ = 0.9–1.2 Hz (Fig. 2).[22,23]

Finally, olefination of the formaldehyde using 3-diethoxyphosphorylchroman-2-ones **13a–t** as the Horner–Wadsworth–Emmons reagents in the presence of *t*-BuOK or NaH gave, after standard work up and column chromatography, pure 3-methylidenechroman-2-ones **14a–t** in good yields (Table 1). Spectroscopic data (^1H, ^{13}C and ^{31}P NMR spectra) were in full agreement with their structures.



11a R^1= H, R^2 = H, R^3 = OMe
11b R^1= OMe, R^2 = H, R^3 = OMe
11c R^1= H, R^2---R^3 = CH_2OCH_2
11d R^1---R^2 = CHCHCHCH, R^3 = H
11e R^1---R^2 = CHC(OH)CHCH, R^3 = H

Scheme 1. Reagents and conditions: (a) CF_3SO_3H (2 equiv) or $MeSO_3H$ (2 equiv), CH_2Cl_2, rt, 6–60 days, 73–95% yield. (b) R^4MgX (5 equiv), CuI (0.1 equiv), THF, rt, 48 h, 48–93% yield. (c) *t*-BuOK or NaH (1.2 equiv), $(CH_2O)_n$ (5 equiv), THF, rt, 1.5 h.

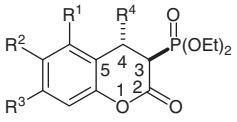


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Figure 2. *trans*-Arrangement of diethoxyphosphoryl group and R^4 substituent in **13**.

Table 1
Chromanones **14a–t** prepared

Compd	R^1	R^2	R^3	R^4	Yield %
14a	H	H	OMe	Me	69
14b	H	H	OMe	*n*-Bu	80
14c	H	H	OMe	*i*-Pr	62
14d	H	H	OMe	Vinyl	78
14e	OMe	H	OMe	Me	78
14f	OMe	H	OMe	*n*-Bu	77
14g	OMe	H	OMe	*i*-Pr	57
14h	OMe	H	OMe	Vinyl	71
14i	H	OCH_2O		Me	81
14j	H	OCH_2O		*n*-Bu	67
14k	H	OCH_2O		*i*-Pr	78
14l	H	OCH_2O		Vinyl	55
14m			H	Me	80
14n			H	*n*-Bu	70
14o			H	*i*-Pr	63
14p			H	Vinyl	45
14q			H	Me	75
14r			H	*n*-Bu	43
14s			H	*i*-Pr	55
14t			H	Vinyl	66

2.2. Bioactivity

2.2.1. Evaluation of cytotoxicity

Cytotoxic activity of all obtained 3-methylidenechroman-2-ones **14a–t** were evaluated in vitro against four human cancer cell lines: leukemia NALM-6 and HL-60 as well as MCF-7 breast cancer and HT-29 colon cancer adenocarcinomas. Carboplatin was used as a reference compound.[27] The IC_{50} values (Table 2) vary significantly and are dependent on the nature of substituent R^4 and, to the smaller extend, on the structure of the aromatic moiety. In general, the antiproliferative and cytotoxic activities of the tested compounds are high and, except chromanones **14a–d,i,p**, the IC_{50} values are below 10.0 μM for all four cell lines. It can also be noticed that cytotoxicity of chromanones **14a–t** is usually more pronounced for both leukemias and MCF-7 cells than for HT-9 cells. Aromatic moiety seems to have moderate influence on the activity, but in most cases the cytotoxicities of chromanones **14m–t** containing additional *ortho*-fused benzene ring are higher than those of the corresponding chromanones **14a–l** sharing the same R^4 substituent. We believe that increased lipophilicity of chromanones containing additional benzene ring may be crucial in enhancing the activity. However, it is worth to stress that also some chromanones without extra benzene ring are very active against specific cell lines, for example, **14c** and **14j** display IC_{50} values below 1 μM against both leukemia and MCF-7 cell lines and **14k** is very active against NALM-6 and MCF-7 cells, with IC_{50} values 0.7 μM and 0.87 μM, respectively. In most cases the presence of hydroxyl group in chromanones **14q–t** moderately enhances their cytotoxicity in comparison with the corresponding chromanones **14m–p,** sharing the same R^4 substituent. Noteworthy, vinyl substituted chromanone **14t** containing a hydroxy group is eightfold more active against NALM-6 cells than the corresponding chromanone **14p** lacking this group. In turn, a number and position of the electron releasing alkoxy groups, attached to the benzene ring in chromanones **14a–l,** have varying effects on cytotoxic activity, suggesting that steric, rather than electronic factors, account for the differing potencies of these compounds.

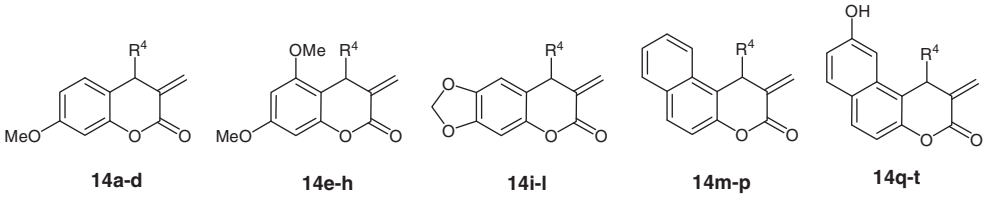
The influence of the R^4 substituent is more straightforward. When chromanones sharing the same aromatic moiety are compared, compounds containing *n*-butyl or *i*-propyl substituent are always more potent than compounds having methyl or vinyl substituent. In some cases this difference is really significant. For example chromanone **14c** (R^4 = *i*-Pr, IC_{50} = 0.56 μM) is 100-fold more active than **14a** (R^4 = Me, IC_{50} = 54.5 μM) or **14d** (R^4 = vinyl, IC_{50} = 51.7 μM) against HL-60. The same is true when cytotoxicities of **14j** and **14i** against NALM-6 are compared. For chromenones **14m–t** with *ortho*-fused benzene ring these differences are not that big but also meaningful. These results show that replacing methyl or vinyl with *n*-butyl or *i*-propyl substituent enhances the cytotoxic activity of chromanones **14a–t** against all tested cell lines.

Selected chromanones **14c–d** and **14m–p** were also tested on human umbilical vein endothelial cells (HUVEC), to evaluate their toxicity against normal cells (Table 1). In most cases toxicity of these compounds against normal and cancer cells were similar. However, a meaningful difference was observed for chromanones **14c** and **14o** which were 4- to 8-fold less toxic against HUVEC than against both leukemias and MCF-7 cancer cells. Both these chromanones share common *i*-Pr substituent in position 4. The best therapeutic index was found for **14o** against MCF-7 cells (IC_{50} HUVEC/IC_{50} MCF-7∼8).

2.2.2. Evaluation of antimicrobial activity

Chromanones **14b,c,n,o** were selected for the antimicrobial activity evaluation against a basic panel of reference Gram-positive (*Staphylococcus aureus* ATCC 29213), Gram-negative (*Escherichia coli* NCTC 8196, *Pseudomonas aeruginosa* NCTC 6749) bacteria and *Candida albicans* (ATCC 10231) fungi. The choice was based on the structure activity relationships revealed during the analysis of the cytotoxicity assays. Selected compounds contain *n*-butyl (**14b,n**) or *i*-propyl (**14c,o**) substituents which proved to be crucial for the high cytotoxic activity. They also have two different aromatic moieties—single benzene ring (**14b,c**) as well as two *ortho*-fused benzene rings (**14n,o**). The tested concentration of these chromanones ranged from 3.125 to 400.0 μg/mL (eight twofold serial dilutions). Tests showed that the growth of Gram-negative bacteria and yeasts *C. albicans*, was not significantly affected by these compounds (MIC exceeded 400 μg/mL). However, two of the tested compounds, chromanones **14b** and **14n,** showed satisfactory level of MIC (below 200 μg/mL) against *S. aureus* ATCC 29213 (Table 3). Therefore these chromanones were tested against a second panel of target strains—members of an important group of 'alert' human pathogens—*Staphylococcus* and *Enterococcus*.[28] These were *Enterococcus faecalis* ATCC 29212 and three clinical MRSA isolates derived from blood, cerebrospinal fluid and catheter's swab of hospitalized patients. It was shown that the growth of *E. faecalis* ATCC 29212

Table 2
In vitro cytotoxic activity of chromanones **14a–t** tested on four cancer cells and on normal HUVEC cells



14a-d **14e-h** **14i-l** **14m-p** **14q-t**

Compd	R^4	IC_{50}[a] (μM)				
		HL-60	NALM-6	MCF-7	HT-29	HUVEC
14a	Me	54.5 ± 5.2	53.6 ± 6.0	73 ± 5.8	70 ± 7.9	57.4 ± 2.4
14b	*n*-Bu	5.4 ± 1.0	6.2 ± 1.1	7.1 ± 0.8	10.2 ± 1.4	5.1 ± 0.5
14c	*i*-Pr	0.56 ± 0.02	0.85 ± 0.08	0.87 ± 0.11	1.7 ± 0.19	3.12 ± 0.36
14d	Vinyl	51.7 ± 5.43	56.6 ± 3.6	62 ± 7.2	42 ± 3.56	57.3 ± 2.86
14e	Me	5.2 ± 0.4	6.0 ± 0.4	4.1 ± 0.5	7.2 ± 0.8	—[b]
14f	*n*-Bu	3.4 ± 0.5	4.6 ± 0.4	3.3 ± 0.4	5.2 ± 0.3	—[b]
14g	*i*-Pr	1.3 ± 0.2	3.0 ± 0.4	2.1 ± 0.19	2.2 ± 0.3	—[b]
14h	Vinyl	8.4 ± 0.9	9.3 ± 0.4	6.2 ± 0.71	5.9 ± 0.42	—[b]
14i	Me	59.4 ± 5.3	60.7 ± 2.5	40.9 ± 5.6	38.4 ± 5.2	—[b]
14j	*n*-Bu	0.75 ± 0.06	0.60 ± 0.03	0.54 ± 0.05	1.8 ± 0.07	—[b]
14k	*i*-Pr	3.3 ± 0.4	0.7 ± 0.06	0.87 ± 0.07	1.5 ± 0.19	—[b]
14l	Vinyl	7.85 ± 0.45	7.82 ± 0.8	9.8 ± 1.2	6.2 ± 0.52	—[b]
14m	Me	4.8 ± 0.2	5.5 ± 0.3	5.17 ± 0.58	7.26 ± 0.77	6.69 ± 0.24
14n	*n*-Bu	0.72 ± 0.04	2.1 ± 0.3	2.75 ± 0.18	2.9 ± 0.24	3.83 ± 0.27
14o	*i*-Pr	0.70 ± 0.04	0.72 ± 0.43	0.48 ± 0.06	2.67 ± 0.28	4.03 ± 0.43
14p	Vinyl	5.7 ± 0.1	6.0 ± 0.2	4.7 ± 0.29	9.7 ± 1.65	6.1 ± 0.37
14q	Me	5.81 ± 0.4	5.77 ± 0.38	6.22 ± 0.5	4.3 ± 0.44	—[b]
14r	*n*-Bu	0.62 ± 0.06	0.56 ± 0.03	1.88 ± 0.11	1.73 ± 0.15	—[b]
14s	*i*-Pr	0.58 ± 0.05	0.59 ± 0.04	0.36 ± 0.04	0.49 ± 0.03	—[b]
14t	Vinyl	3.43 ± 0.9	0.79 ± 0.05	4.8 ± 0.83	6.5 ± 0.76	—[b]
Carboplatin	—	2.9 ± 0.1	0.7 ± 0.3	3.8 ± 0.45	4.2. ± 0.61	—[b]

[a] Compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SD from the concentration–response curves of at least three experiments.
[b] Not determined.

Table 3
Minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) of the **14b**,**n** against *S. aureus* strains

S. aureus strain[a] (reference, clinical)	MIC/MBC (μg/mL)		
	OX[a]	**14b**	**14n**
ATCC 29213 (MSSA)	0.25	50.0/100.0	6.25/12.5
A3 (MRSA)	>128.0	50.0/100.0	3.125/6.25
A7 (MRSA)	>128.0	50.0/100.0	6.25/6.25
D5 (MRSA)	>128.0	50.0/200.0	3.125/6.25

[a] Abbreviations: OX—oxacillin; MSSA—methicillin-susceptible *S. aureus*; MRSA—methicillin-resistant *S. aureus*.

was moderately inhibited by **14b** (MIC = 200.0 μg/mL) and by **14n** (MIC = 25.0 μg/mL). On the other hand, these compounds revealed potent antibacterial activities against multidrug resistant *S. aureus* strains A3, A7 and D5 (Table 3). MIC of the reference antibiotic—oxacillin is also given for the comparison. Furthermore the CFU counting test revealed ⩾99.9% reduction of the original inoculum by 2–4 MIC what proves bactericidal effect of **14b** and **14n** (Table 3).

Data shown in Table 3 clearly indicate that both chromanones tested are much more active against methicillin-resistant *S. aureus* than oxacillin. Furthermore, chromanone **14n**, with MIC values ranging from 3.125 μg/mL to 6.25 μg/mL, is much more potent than **14b**. Because both compounds share the same *n*-butyl substituent in position 4 the presence of *ortho*-fused benzene ring in **14n** must be crucial for the high activity of this compound. Moreover, chromanone **14n** is bactericidal at concentration equal to MIC or 2MIC, what proves its killing activity. It is important from the clinical point of view because bactericidal agents are preferred over the bacteriostatic ones, for the treatment of serious, life threatening infections.[28]

It is generally accepted that natural, semi-synthetic or synthetic compounds capable of inhibiting bacterial growth at a concentration lower than 10 μg/mL are considered highly active and worth further studies.[29] Chromanone **14n** meets this criteria and, what even more important, is active against nosocomial and community-associated staphylococci (MRSA) which are resistant to most or all available therapeutic classes of antimicrobial drugs.[28] Therefore we believe that chromanone **14n** can be considered a lead structure in developing a new class of antimicrobial agents of highly desirable properties.[30,31]

3. Conclusions

We developed novel and efficient synthesis of 3-methylidenechromanones **14a–t** which combine two biologically important structural units—α-methylidene-δ-lactone moiety incorporated into 3,4-dihydrocoumarin skeleton. Screening of the synthesized chromanones against several cancer cell lines showed that many of them have potent cytotoxic activity with IC_{50} values below 1 μM and revealed interesting structure-activity relationships. Both, the presence of an additional *ortho*-fused benzene ring and introduction of *n*-butyl or *i*-propyl substituent instead of methyl or vinyl group in position 4 significantly enhances activity. In turn tests on HUVEC showed that chromanones **14c,o** with *i*-propyl group in position 4 have improved therapeutic index against

MCF-6 cells. Very interesting results were obtained from antimicrobial tests. Tested chromanones **14b,c,n,o** were not active against Gram-negative bacteria and yeasts. However, chromanone **14n** showed high activity against nosocomial and community-associated staphylococci (MRSA) which are resistant to most or all available therapeutic classes of antimicrobial drugs. Presented here preliminary pharmacological results demonstrate that 3-methylidenechromanones **14** represent a very promising group of compounds which display a broad spectrum of high and desirable biological activities. We believe that further careful modifications of their structures, based on the structure–activity relationships found in this study, can provide good candidates for future drugs.

4. Experimental

4.1. Chemistry

NMR spectra were recorded on a Bruker DPX 250 instrument at 250.13 MHz for ^{1}H, 62.9 MHz for ^{13}C, and 101.3 MHz for ^{31}P NMR using tetramethylsilane as internal and 85% H_3PO_4 as external standard. ^{31}P NMR spectra were recorded using broadband proton decoupling. IR spectra were recorded on a Bruker Alpha ATR spectrophotometer. Melting points were determined in open capillaries and are uncorrected. Column chromatography was performed on Aldrich® silica gel 60 (230–400 mesh). Thin-layer chromatography was performed with precoated TLC sheets of silica gel 60 F_{254} (Aldrich®). The purity of tested compounds was determined by combustion elemental analyses (CH, elemental analyzer EuroVector 3018, Elementar Analysensysteme GmbH). Reagents and starting materials were purchased from commercial vendors and used without further purification. All organic solvents were dried over appropriate drying agents and distilled prior to use. Standard syringe techniques were used for transferring dry solvents.

4.1.1. General procedure for the synthesis of 3-diethoxyphosphorylchromen-2-ones 12a–e

To a solution of corresponding phenol **11a–c** (11 mmol) or naphthol **11d,e** (11 mmol) in CH_2Cl_2 (50 mL) trifluoromethanesulfonic acid (3.00 g, 20 mmol) or methanesulfonic acid (1.92 g, 20 mmol) and acrylate **10** (2.66 g, 10 mmol) were added and the resulting mixture was stirred at room temperature for the period of time given in Table 4. Next, saturated aqueous $NaHCO_3$ solution was added (100 mL). Extraction with CH_2Cl_2 (3 × 30 mL), drying ($MgSO_4$) and evaporation of the solvent gave a crude product, which was purified by crystallization from Et_2O.

4.1.1.1. Diethyl (7-methoxy-2-oxo-2*H*-chromen-3-yl)phosphonate (12a). 81%; mp 93–94 °C; IR 1725, 1601, 1228, 1134, 1016, 768 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.43 (d, J = 17.0 Hz, 1H), 7.46 (d, J = 8.7 Hz, 1H), 6.87 (dd, J = 8.7, 2.4 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 4.14–4.31 (m, 4H), 3.88 (s, 3H), 1.35 (t, J = 7.1 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.7, 158.4 (d, J = 15.0 Hz), 157.3, 153.2 (d, J = 7.0 Hz), 130.3, 113.2, 112.8 (d, J = 198.9 Hz), 111.5 (d, J = 14.3 Hz), 100.4, 62.9 (d, J = 5.8 Hz), 55.8, 16.2 (d, J = 6.4 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 12.47. Anal. Calcd for $C_{14}H_{17}O_6P$: C, 53.85; H, 5.49. Found: C, 53.63; 5.52.

Table 4
Conditions for the preparation of 3-diethoxyphosphorylchromen-2-ones **12a–e**

Product	Catalyst	Time (d)
12a	CF_3SO_3H	10
12b	CF_3SO_3H	6
12c	$MeSO_3H$	60
12d	CF_3SO_3H	10
12e	CF_3SO_3H	18

4.1.1.2. Diethyl (5,7-dimethoxy-2-oxo-2*H*-chromen-3-yl)phosphonate (12b). 88%; mp 97–98 °C; IR 1732, 1600, 1256, 1136, 1032, 784 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.77 (d, J = 17.4 Hz, 1H), 6.20–6.50 (m, 2H), 4.00–4.45 (m, 4H), 3.91 (s, 3H), 3.88 (s, 3H), 1.36 (t, J = 7.1 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 165.9, 158.6 (d, J = 15.7 Hz), 158.1, 158.0, 148.6 (d, J = 8.2 Hz), 109.9 (d, J = 201.1 Hz), 103.3 (d, J = 14.2 Hz), 94.7, 92.5, 62.7 (d, J = 7.1 Hz), 55.9, 55.8, 16.1 (d, J = 6.4 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 13.39. Anal. Calcd for $C_{15}H_{19}O_7P$: C, 52.64; H, 5.60. Found: C, 52.32; 5.74.

4.1.1.3. Diethyl (6-oxo-6*H*-[1,3]dioxolo[4,5-*g*]chromen-7-yl)phosphonate (12c). 73%; mp 108–109 °C; IR 1726, 1610, 1224, 1140, 1021, 771 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.30 (d, J = 17.1 Hz, 1H), 6.83 (s, 1H), 6.74 (s, 1H), 6.06 (s, 2H), 4.09–4.26 (m, 4H), 1.30 (t, J = 7.0 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 158.4 (d, J = 14.8 Hz), 153.4, 153.2, 153.0 (d, J = 7.3 Hz), 145.2, 113.0 (d, J = 198.9 Hz), 111.7 (d, J = 14.6 Hz), 105.7, 102.7, 97.9, 63.1 (d, J = 5.9 Hz), 16.2 (d, J = 6.4 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 12.31. Anal. Calcd for $C_{14}H_{15}O_7P$: C, 51.54; H, 4.63. Found: C, 51.50; 4.75.

4.1.1.4. Diethyl (3-oxo-3*H*-benzo[*f*]chromen-2-yl)phosphonate (12d). 88%; mp 149–150 °C; IR 1732, 1604, 1256, 1136, 1024, cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.30 (d, J = 17.6 Hz, 1H), 8.37 (d, J = 9.0 Hz, 1H), 8.10 (d, J = 7.2 Hz, 1H), 7.93 (d, J = 7.2 Hz, 1H), 7.75 (t, J = 9.0 Hz, 1H), 7.61 (t, J = 9.0 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 4.15–4.39 (m, 4H), 1.25 (t, J = 7.0 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 158.3, 158.1, 155.8, 149.0 (d, J = 7.4 Hz), 135.8, 130.1, 129.1, 129.0, 126.4, 121.5, 116.5, 115.7 (d, J = 197.0 Hz), 112.2, 63.9 (d, J = 5.9 Hz), 16.2 (d, J = 6.3 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 12.38. Anal. Calcd for $C_{17}H_{17}O_5P$: C, 61.45; H, 5.16. Found: C, 61.40; 5.20.

4.1.1.5. Diethyl (9-hydroxy-3-oxo-3*H*-benzo[*f*]chromen-2-yl) phosphonate (12e). 95%; mp 178–180 °C; IR 1730, 1604, 1252, 1138, 1028, cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.58 (d, J = 18.6 Hz, 1H), 8.67 (d, J = 2.1 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.28 (dd, J = 8.9, 2.1 Hz, 1H), 7.19 (d, J = 9.0 Hz, 1H), 5.30 (s, 1H), 4.08–4.38 (m, 4H), 1.10 (t, J = 7.0 Hz, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 158.6, 158.4, 156.6, 150.7 (d, J = 8.1 Hz), 136.3, 131.7, 130.6, 124.7, 118.5, 112.8, 112.8 (d, J = 187.4 Hz), 112.8, 111.3, 105.2, 63.9 (d, J = 6.0 Hz), 16.2 (d, J = 6.3 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 14.10. Anal. Calcd for $C_{17}H_{17}O_6P$: C, 58.62; H, 4.92. Found: C, 58.55; 4.99.

4.1.2. General procedure for the synthesis of 3-diethoxyphosphorylchroman-2-ones 13a–l and 3-diethoxyphosphoryldihydrobenzochromen-2-ones 13m–t

To a solution of the corresponding chromenones **12a–c** (1 mmol) or benzochromenones **12d,e** (1 mmol) and a catalytic amount of CuI (19 mg, 0.1 mmol) in THF (10 mL) a solution of Grignard reagent (5 mmol) was added dropwise, under an argon atmosphere at rt (Me, *n*-Bu and *i*-Pr magnesium iodides were prepared from the corresponding alkyl halides and magnesium in Et_2O; vinylmagnesium bromide was purchased from ALDRICH®). The solution was stirred for 48 h. After this time the reaction mixture was quenched with H_2O (2 mL), acidified to pH ca. 1.5 with 10% aq HCl solution and extracted with $CHCl_3$ (4 × 10 mL). The organic extracts were washed with brine (10 mL) and dried over $MgSO_4$. Evaporation of the solvent gave the crude product which was purified by column chromatography (eluent: $CHCl_3$–acetone, 99:1).

4.1.2.1. Diethyl (7-dimethoxy- 4-methyl-2-oxochroman-3-yl)phosphonate (13a). 75%; yellow oil; R_f = 0.43 ($CHCl_3$–acetone, 98:2); IR 1762, 1625, 1257, 1229, 1147, 1015 cm^{-1}; ^{1}H NMR

(250 MHz, $CDCl_3$) δ 7.13 (d, J = 8.4 Hz, 1H), 6.68 (dd, J = 8.4, 2.5 Hz, 1H), 6.60 (d, J = 2.5 Hz, 1H), 4.06–4.18 (m, 2H), 3.72–3.85 (m, 1H), 3.78 (s, 3H), 3.45–3.59 (m, 2H), 3.29 (dd, J = 25.0, 1.2 Hz, 1H), 1.35 (t, J = 7.1 Hz, 3H), 1.30 (dd, J = 7.2, 1.8 Hz, 3H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.5 (d, J = 6.0 Hz), 159.7, 151.4, 128.1, 117.3, 110.6, 102.1, 62.9 (d, J = 6.8 Hz), 62.7 (d, J = 6.9 Hz), 55.3, 47.2 (d, J = 127.7 Hz), 31.6 (d, J = 4.3 Hz), 23.6 (d, J = 18.6 Hz), 15.9 (d, J = 6.3 Hz), 15.7 (d, J = 6.1 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.86. Anal. Calcd for $C_{15}H_{21}O_6P$: C, 54.88; H, 6.45. Found: C, 54.71; 6.55.

4.1.2.2. Diethyl (4-butyl-7-methoxy-2-oxochroman-3-yl)phosphonate (13b). 81%; yellow oil; IR R_f = 0.47 ($CHCl_3$–acetone, 98:2); 1761, 1625, 1257, 1190, 1146, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.08 (d, J = 8.4 Hz, 1H), 6.66 (dd, J = 8.4, 2.5 Hz, 1H), 6.58 (d, J = 2.5 Hz, 1H), 4.04–4.15 (m, 2H), 3.70–3.85 (m, 1H), 3.77 (s, 3H), 3.27–3.51 (m, 2H), 3.35 (dd, J = 25.5, 1.0 Hz, 1H), 1.47–1.56 (m, 2H), 1.31 (t, J = 7.0 Hz, 3H), 1.19–1.30 (m, 4H), 0.93 (t, J = 7.0 Hz, 3H), 0.83 (t, J = 6.7 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.7 (d, J = 5.9 Hz), 159.6, 151.5, 129.0, 115.9, 110.2, 102.0, 62.8 (d, J = 6.8 Hz), 62.7 (d, J = 7.0 Hz), 55.3, 45.7 (d, J = 127.8 Hz), 36.7 (d, J = 17.0 Hz), 36.4 (d, J = 4.3 Hz), 28.2, 22.1, 15.9 (d, J = 6.2 Hz), 15.7 (d, J = 6.0 Hz), 13.6; ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.18. Anal. Calcd for $C_{18}H_{27}O_6P$: C, 58.37; H, 7.35. Found: C, 58.30; 7.49.

4.1.2.3. Diethyl (4-isopropyl-7-methoxy-2-oxochroman-3-yl)phosphonate (13c). 86%; yellow oil; IR R_f = 0.45 ($CHCl_3$–acetone, 98:2); 1758, 1625, 1588, 1255, 1230, 1148, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.09 (d, J = 8.4 Hz, 1H), 6.67 (dd, J = 8.4, 2.6 Hz, 1H), 6.59 (d, J = 2.6 Hz, 1H), 4.05–4.17 (m, 2H), 3.72–3.85 (m, 1H), 3.79 (s, 3H), 3.43–3.53 (m, 1H), 3.43 (dd, J = 26.4, 1.0 Hz, 1H), 3.21 (ddd, J = 13.1, 6.1, 1.0 Hz, 1H), 1.75–1.88 (m, 1H), 1.32 (t, J = 7.0 Hz, 3H), 0.94 (t, J = 7.1 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.1 (d, J = 6.2 Hz), 159.6, 151.9, 129.9, 113.9, 110.2, 101.6, 62.8 (d, J = 8.0 Hz), 62.7 (d, J = 7.5 Hz), 55.2, 43.2 (d, J = 127.4 Hz), 42.3 (d, J = 4.4 Hz), 34.2 (d, J = 15.9 Hz), 18.9, 18.8, 15.9 (d, J = 6.2 Hz), 15.6 (d, J = 6.0 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.92. Anal. Calcd for $C_{17}H_{25}O_6P$: C, 57.30; H, 7.07. Found: C, 57.17; 7.22.

4.1.2.4. Diethyl (7-methoxy-2-oxo-4-vinylchroman-3-yl)phosphonate (13d). 75%; yellow oil; IR R_f = 0.42 ($CHCl_3$–acetone, 98:2); 1760, 1624, 1256, 1226, 1143, 1013 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.12 (d, J = 8.4 Hz, 1H), 6.70 (dd, J = 8.4, 2.5 Hz, 1H), 6.60 (d, J = 2.5 Hz, 1H), 5.85 (ddd, J = 17.9, 10.2, 5.8, 1H), 5.09 (ddd, J = 10.2, 1.4, 0.7, 1H), 4.86 (ddd, J = 17.9, 1.6, 0.7, 1H), 4.04–4.26 (m, 3H), 3.79–3.90 (m, 1H), 3.78 (s, 3H), 3.46–3.59 (m, 1H), 3.45 (dd, J = 24.8, 1.2 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.1 (d, J = 5.8 Hz), 160.1, 151.9, 137.6 (d, J = 18.4 Hz), 129.1, 116.1, 113.4, 110.8, 102.1, 63.1 (d, J = 6.7 Hz), 62.9 (d, J = 6.9 Hz), 55.4, 45.3 (d, J = 126.9 Hz), 39.6 (d, J = 3.3 Hz), 16.0 (d, J = 6.2 Hz), 15.7 (d, J = 6.0 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.82. Anal. Calcd for $C_{16}H_{21}O_6P$: C, 56.47; H, 6.22. Found: C, 56.39; 6.36.

4.1.2.5. Diethyl (5,7-dimethoxy- 4-methyl-2-oxochroman-3-yl)phosphonate (13e). 83%; yellow oil; R_f = 0.47 ($CHCl_3$–acetone, 98:2); IR 1764, 1624, 1260, 1216, 1140, 1024 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.22–6.26 (m, 2H), 4.05–4.16 (m, 2H), 3.86–3.92 (m, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.60–3.78 (m, 1H), 3.26 (dd, J = 25.1, 0.9 Hz, 1H), 1.33 (t, J = 7.0 Hz, 3H), 1.23 (dd, J = 7.1, 1.6 Hz, 3H), 1.01 (t, J = 7.0 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.1 (d, J = 5.7 Hz), 158.5, 155.4, 150.3, 104,7, 93.1, 91.8, 61.3 (d, J = 7.3 Hz), 61.1 (d, J = 7.8 Hz), 55.7, 54.0, 45.5 (d, J = 127.6 Hz), 24.3 (d, J = 4.4 Hz), 19.9 (d, J = 18.3 Hz), 14.3 (d, J = 6.2 Hz), 14.2 (d, J = 6.0 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.13. Anal. Calcd for $C_{16}H_{23}O_7P$: C, 53.63; H, 6.47. Found: C, 53.85; 6.78.

4.1.2.6. Diethyl (4-butyl-5,7-dimethoxy-2-oxochroman-3-yl)phosphonate (13f). 64%; yellow oil; R_f = 0.45 ($CHCl_3$–acetone, 98:2); IR 1760, 1628, 1252, 1216, 1140, 1020 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.22–6.27 (m, 2H), 4.04–4.16 (m, 2H), 3.81 (s, 3H), 3.78 (s, 3H), 3.67–3.88 (m, 2H), 3.55–3.64 (m, 1H), 3.37 (dd, J = 25.6, 1.0 Hz, 1H), 1.48–1.54 (m, 2H), 1.31 (t, J = 7.0 Hz, 3H), 1.21–1.30 (m, 4H), 1.01 (t, J = 7.0 Hz, 3H), 0.85 (t, J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.2 (d, J = 5.5 Hz), 159.4, 156.6, 151.5, 104.7, 94.0, 92.7, 62.0 (d, J = 6.9 Hz), 61.9 (d, J = 7.1 Hz), 54.8, 54.5, 44.2 (d, J = 128.0 Hz), 34.5 (d, J = 16.6 Hz), 29.6 (d, J = 4.4 Hz), 27.2, 21.4, 15.2 (d, J = 6.4 Hz), 15.0 (d, J = 6.1 Hz), 12.9; ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.53. Anal. Calcd for $C_{19}H_{29}O_7P$: C, 56.99; H, 7.30. Found: C, 56.78; 7.33.

4.1.2.7. Diethyl (4- isopropyl-5,7-dimethoxy-2-oxochroman-3-yl)phosphonate (13g). 85%; yellow oil; R_f = 0.44 ($CHCl_3$–acetone, 98:2); IR 1764, 1612, 1592, 1260, 1216, 1128, 1024 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.22–6.27 (m, 2H), 4.04–4.16 (m, 2H), 3.84–3.95 (m, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 3.52–3.67 (m, 2H), 3.43 (dd, J = 26.6, 0.9 Hz, 1H), 1.85–1.99 (m, 1H), 1.31 (t, J = 6.4 Hz, 3H), 1.01 (t, J = 7.1 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.9 (d, J = 5.8 Hz), 159.7, 157.2, 152.0, 103.7, 94.2, 92.8, 62.4 (d, J = 5.4 Hz), 62.3 (d, J = 6.2 Hz), 55.0, 54.8, 41.2 (d, J = 128.1 Hz), 36.2 (d, J = 4.5 Hz), 32.2 (d, J = 15.4 Hz), 18.9, 17.5, 15.4 (d, J = 6.6 Hz), 15.3 (d, J = 6.4 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 20.61. Anal. Calcd for $C_{18}H_{27}O_7P$: C, 55.95; H, 7.04. Found: C, 56.21; 7.19.

4.1.2.8. Diethyl (5,7-dimethoxy-2-oxo-4-vinylchroman-3-yl)phosphonate (13h). 89%; yellow oil; R_f = 0.40 ($CHCl_3$–acetone, 98:2); IR 1764, 1604, 1252, 1216, 1128, 1028 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.24–6.29 (m, 2H), 5.79 (ddd, J = 17.0, 10.1, 5.6, 1H), 5.06 (ddd, J = 10.1, 1.4, 0.8, 1H), 4.91 (ddd, J = 17.0, 1.6, 0.8, 1H), 4.06–4.18 (m, 2H), 3.81–3.90 (m, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.58–3.69 (m, 1H), 3.45 (dd, J = 25.0, 1.1 Hz, 1H), 1.30 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.3 (d, J = 5.4 Hz), 160.6, 157.4, 152.5, 136.5 (d, J = 18.3 Hz), 115.3, 102.8, 94.9, 93.5, 63.0 (d, J = 7.2 Hz), 62.9 (d, J = 7.2 Hz), 55.8, 55.4, 44.9 (d, J = 127.1 Hz), 33.8 (d, J = 3.1 Hz), 16.0 (d, J = 6.4 Hz), 15.8 (d, J = 6.1 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.73. Anal. Calcd for $C_{17}H_{23}O_7P$: C, 55.13; H, 6.26. Found: C, 55.31; 6.43.

4.1.2.9. Diethyl (8-methyl-6-oxo-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-7-yl)phosphonate (13i). 68%; yellow oil; R_f = 0.50 ($CHCl_3$–acetone, 98:2); IR 1758, 1625, 1255, 1216, 1154, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.66 (s, 1H), 6.57 (s, 1H), 5.95 (s, 2H), 4.06–4.18 (m, 2H), 3.60–3.91 (m, 2H), 3.39–3.53 (m, 1H), 3.25 (dd, J = 25.1, 1.1 Hz, 1H), 1.32 (t, J = 7.1 Hz, 3H), 1.28 (dd, J = 7.2, 1.7 Hz, 3H), 1.03 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.4 (d, J = 5.8 Hz), 147.2, 145.0, 144.3, 117.4, 106.6, 101.5, 96.6, 63.1 (d, J = 7.2 Hz), 62.9 (d, J = 7.4 Hz), 46.8 (d, J = 128.0 Hz), 32.2 (d, J = 4.3 Hz), 23.3 (d, J = 18.7 Hz), 16.0 (d, J = 6.3 Hz), 15.8 (d, J = 6.2 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.05. Anal. Calcd for $C_{15}H_{19}O_7P$: C, 52.64; H, 5.60. Found: C, 52.52; 5.69.

4.1.2.10. Diethyl (8-butyl-6-oxo-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-7-yl)phosphonate (13j). 74%; yellow oil; R_f = 0.52 ($CHCl_3$–acetone, 98:2); IR 1758, 1638, 1503, 1255, 1153, 1017 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.61 (s, 1H), 6.56 (s, 1H), 5.94 (s, 2H), 4.07–4.19 (m, 2H), 3.77–3.92 (m, 1H), 3.54–3.70 (m, 1H), 3.32 (dd, J = 25.7, 1.0 Hz, 1H), 3.19–3.30 (m, 1H), 1.47–1.58 (m, 2H), 1.15–1.39 (m, 7H), 1.01 (t, J = 7.1 Hz, 3H), 0.85 (t,

J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.7 (d, J = 5.8 Hz), 147.2, 145.3, 144.0, 116.2, 107.4, 101.4, 96.6, 62.9 (d, J = 7.3 Hz), 62.7 (d, J = 7.3 Hz), 45.3 (d, J = 128.0 Hz), 37.0 (d, J = 4.3 Hz), 36.4 (d, J = 17.0 Hz), 28.3, 22.1, 15.9 (d, J = 6.3 Hz), 15.7 (d, J = 6.2 Hz), 13.6; ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.99. Anal. Calcd for $C_{18}H_{25}O_7P$: C, 56.25; H, 6.56. Found: C, 56.03; 6.74.

4.1.2.11. Diethyl (4-isopropyl-6-oxo-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-7-yl)phosphonate (13k). 82%; yellow oil; R_f = 0.52 ($CHCl_3$–acetone, 98:2); IR 1758, 1625, 1588, 1255, 1230, 1148, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.59 (s, 1H), 6.53 (s, 1H), 5.93 (s, 2H), 4.03–4.15 (m, 2H), 3.78–3.88 (m, 1H), 3.56–3.67 (m, 1H), 3.37 (dd, J = 26.5, 0.8 Hz, 1H), 3.09 (dd, J = 15.6, 6.1 Hz, 1H), 1.72–1.86 (m, 1H), 1.29 (t, J = 7.0 Hz, 3H), 0.99 (t, J = 7.0 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.4 (d, J = 6.1 Hz), 147.4, 146.0, 144.1, 114.5, 108.5, 101.6, 96.6, 63.1 (d, J = 6.9 Hz), 62.9 (d, J = 7.3 Hz), 43.3 (d, J = 127.5 Hz), 43.0 (d, J = 4.6 Hz), 34.3 (d, J = 15.9 Hz), 19.2, 19.1, 16.2 (d, J = 6.3 Hz), 16.0 (d, J = 6.2 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 20.14. Anal. Calcd for $C_{17}H_{23}O_7P$: C, 55.13; H, 6.26. Found: C, 55.01; 6.40.

4.1.2.12. Diethyl (6-oxo-8-vinyl-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-7-yl)phosphonate (13l). 48%; yellow oil; R_f = 0.47 ($CHCl_3$–acetone, 98:2); IR 1759, 1638, 1255, 1216, 1153, 1013 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.66 (s, 1H), 6.59 (s, 1H), 5.97 (s, 2H), 5.83 (ddd, J = 16.8, 10.2, 6.0 Hz, 1H) , 1H), 5.11 (dd, J = 10.2, 0.6 Hz, 1H), 4.92 (dd, J = 16.8, 0.6 Hz, 1H), 4.07–4.20 (m, 2H), 3.83–3.93 (m, 1H), 3.61–3.71 (m, 1H), 3.41 (dd, J = 24.9, 1.3 Hz, 1H), 1.33 (t, J = 7.1 Hz, 3H), 1.04 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.2 (d, J = 5.7 Hz), 147.7, 145.8, 144.5, 137.2 (d, J = 18.5 Hz), 116.4, 113.7, 107.5, 101.7, 96.7, 63.2 (d, J = 6.9 Hz), 63.0 (d, J = 7.1 Hz), 45.1 (d, J = 126.9 Hz), 40.3 (d, J = 3.6 Hz), 16.1 (d, J = 6.5 Hz), 15.9 (d, J = 6.2 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.34. Anal. Calcd for $C_{16}H_{19}O_7P$: C, 54.24; H, 5.41. Found: C, 54.07; 5.64.

4.1.2.13. Diethyl (1-methyl-3-oxo-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13m). 95%; yellow oil; R_f = 0.52 ($CHCl_3$–acetone, 98:2); IR 1764, 1440, 1392, 1260, 1232, 1224, 1160, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.96–8.00 (m, 1H), 7.76–7.87 (m, 2H), 7.56–7.63 (m, 1H), 7.44–7.50 (m, 1H), 7.21–7.26 (m, 1H), 4.24–4.39 (m, 1H), 4.04–4.16 (m, 2H), 3.59–3.69 (m, 1H), 3.47 (dd, J = 25.3, 1.2 Hz, 1H), 3.28–3.38 (m, 1H), 1.44 (dd, J = 7.2, 1.8 Hz, 3H), 1.27 (t, J = 7.1 Hz, 3H), 0.73 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.7 (d, J = 5.8 Hz), 148.3, 131.0, 130.3, 129.1, 128.7, 127.5, 125.2, 122.4, 118.5, 117.0, 63.2 (d, J = 6.7 Hz), 62.8 (d, J = 6.9 Hz), 47.0 (d, J = 127.6 Hz), 28.5 (d, J = 4.2 Hz), 21.7 (d, J = 18.4 Hz), 16.1 (d, J = 6.2 Hz), 15.6 (d, J = 6.1 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.72. Anal. Calcd for $C_{18}H_{21}O_5P$: C, 62.07; H, 6.08. Found: C, 62.37; 6.23.

4.1.2.14. Diethyl (1-butyl-3-oxo-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13n). 89%; yellow oil; R_f = 0.54 ($CHCl_3$–acetone, 98:2); IR 1764, 1464, 1392, 1260, 1236, 1224, 1156, 1020 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.95–7.99 (m, 1H), 7.76–7.83 (m, 2H), 7.55–7.62 (m, 1H), 7.44–7.50 (m, 1H), 7.21–7.24 (m, 1H), 4.02–4.14 (m, 2H), 3.53–3.67 (m, 3H), 3.25–3.35 (m, 1H), 1.65–1.78 (m, 2H), 1.27–1.47 (m, 4H), 1.27 (t, J = 6.5 Hz, 3H), 0.86 (t, J = 7.2 Hz, 3H), 0.73 (t, J = 7.6 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.4 (d, J = 5.8 Hz), 148.1, 130.5, 130.1, 128.6, 128.1, 126.8, 124.5, 122.1, 117.4, 116.3, 62.5 (d, J = 6.7 Hz), 62.2 (d, J = 7.1 Hz), 44.0 (d, J = 128.1 Hz), 34.8 (d, J = 16.6 Hz), 32.6 (d, J = 4.4 Hz), 27.8, 21.8, 15.5 (d, J = 6.2 Hz), 15.1 (d, J = 6.0 Hz), 13.2; ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.51. Anal. Calcd for $C_{21}H_{27}O_5P$: C, 64.61; H, 6.97. Found: C, 64.42; 7.11.

4.1.2.15. Diethyl (1-isopropyl-3-oxo-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13o). 93%; yellow oil; R_f = 0.52 ($CHCl_3$–acetone, 98:2); IR 1764, 1625, 1464, 1440, 1392, 1256, 1216, 1160, 1024 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.94–7.98 (m, 1H), 7.77–7.86 (m, 2H), 7.55–7.62 (m, 1H), 7.42–7.49 (m, 1H), 7.21–7.24 (m, 1H), 4.03–4.14 (m, 2H), 3.53–3.68 (m, 3H), 3.31–3.37 (m, 1H), 2.17–2.20 (m, 1H), 1.27 (t, J = 7.6 Hz, 3H), 1.06 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.73 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.7 (d, J = 6.0 Hz), 147.3, 130.9, 129.4, 127.6, 127.0, 125.5, 123.3, 121.4, 115.1, 115.0, 61.4 (d, J = 6.6 Hz), 61.1 (d, J = 6.9 Hz), 39.4 (d, J = 128.0 Hz), 37.5 (d, J = 4.6 Hz), 31.3 (d, J = 15.9 Hz), 18.6, 16.0, 14.3 (d, J = 6.2 Hz), 13.9 (d, J = 6.1 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 20.32. Anal. Calcd for $C_{20}H_{25}O_5P$: C, 63.82; H, 6.69. Found: C, 64.01; 6.93.

4.1.2.16. Diethyl (6-oxo-8-vinyl-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13p). 59%; yellow oil; R_f = 0.49 ($CHCl_3$–acetone, 98:2); IR 1768, 1512, 1464, 1392, 1256, 1156, 1024 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.91–7.94 (m, 1H), 7.77–7.87 (m, 2H), 7.55–7.61 (m, 1H), 7.44–7.51 (m, 1H), 7.23–7.26 (m, 1H), 5.98 (ddd, J = 17.0, 10.2, 5.8 Hz, 1H), 5.15 (dd, J = 10.2, 1.6 Hz, 1H), 4.92 (dd, J = 17.0, 1.6 Hz, 1H), 4.81 (ddd, J = 14.0, 5.8, 1.1 Hz, 1H), 4.05–4.26 (m, 2H), 3.63–3.70 (m, 1H), 3.63 (dd, J = 25.1, 1.1 Hz, 1H), 3.29–3.42 (m, 1H), 1.29 (t, J = 7.0 Hz, 3H), 0.76 (t, J = 7.0 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 161.5 (d, J = 5.6 Hz), 147.4, 134.4, 134.1, 129.1 (d, J = 14.8 Hz), 128.0, 126.9, 125.9, 123.6, 121.0, 115.4, 115.1, 113.1, 61.5 (d, J = 6.7 Hz), 61.2 (d, J = 6.9 Hz), 43.5 (d, J = 126.6 Hz), 34.9 (d, J = 3.6 Hz), 14.3 (d, J = 6.1 Hz), 13.9 (d, J = 6.0 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 18.68. Anal. Calcd for $C_{19}H_{21}O_5P$: C, 63.33; H, 5.87. Found: C, 63.49; 5.98.

4.1.2.17. Diethyl (9-hydroxy-1-methyl-3-oxo-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13q). 49%; yellow oil; R_f = 0.47 ($CHCl_3$–acetone, 98:2); IR 1756, 1448, 1232, 1216, 1160, 1012 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.57 (bs, 1H), 7.64–7.68 (m, 2H), 7.34–7.36 (m, 1H), 6.99–7.07 (m, 2H), 4.25–4.43 (m, 1H), 4.13–4.22 (m, 2H), 3.64–3.74 (m, 1H), 3.50 (dd, J = 25.3, 1.0 Hz, 1H), 3.21–3.34 (m, 1H), 1.40 (dd, J = 7.1, 1.9 Hz, 3H), 1.36 (t, J = 6.4 Hz, 3H), 0.73 (t, J = 7.0 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.1 (d, J = 5.9 Hz), 157.1, 149.2, 132.5, 130.7, 129.5, 126.4, 118.2, 116.9, 114.1, 105.2, 64.2 (d, J = 6.5 Hz), 64.1 (d, J = 6.9 Hz), 47.5 (d, J = 128.7 Hz), 29.0 (d, J = 3.9 Hz), 21.9 (d, J = 18.6 Hz), 16.6 (d, J = 6.2 Hz), 16.1 (d, J = 6.2 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.60. Anal. Calcd for $C_{18}H_{21}O_6P$: C, 59.34; H, 5.81. Found: C, 59.45; 5.96.

4.1.2.18. Diethyl (1-butyl-9-hydroxy-3-oxo-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13r). 57%; orange oil; R_f = 0.48 ($CHCl_3$–acetone, 98:2); IR 1756, 1448, 1232, 1216, 1160, 1012 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.99 (bs, 1H), 7.66–7.71 (m, 2H), 7.32–7.33 (m, 1H), 7.00–7.06 (m, 2H), 4.14–4.26 (m, 2H), 4.00–4.13 (m, 1H), 3.55–3.71 (m, 2H), 3.18–3.28 (m, 1H), 1.58–1.81 (m, 2H), 1.37 (t, J = 7.0 Hz, 3H), 1.24–1.33 (m, 4H), 0.81 (t, J = 7.3 Hz, 3H), 0.73 (t, J = 7.0 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.0 (d, J = 5.8 Hz), 156.6, 149.1, 132.4, 130.2, 129.1, 126.0, 117.7, 116.0, 113.6, 105.1, 63.7 (d, J = 6.6 Hz), 63.6 (d, J = 7.2 Hz), 44.6 (d, J = 129.2 Hz), 35.2 (d, J = 16.9 Hz), 33.3 (d, J = 4.1 Hz), 28.5, 22.5, 16.2 (d, J = 6.2 Hz), 15.7 (d, J = 6.1 Hz), 13.8; ^{31}P NMR (101 MHz, $CDCl_3$) δ 20.54. Anal. Calcd for $C_{21}H_{27}O_6P$: C, 62.06; H, 6.70. Found: C, 61.83; 6.99.

4.1.2.19. Diethyl (9-hydroxy-1-isopropyl-3-oxo-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13s). 51%; yellow oil; R_f = 0.50 ($CHCl_3$–acetone, 98:2); IR 1756, 1440, 1224, 1160, 1024 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.95 (bs, 1H),7.66–7.69 (m, 2H), 7.30–7.33 (m, 1H), 7.00–7.06 (m, 2H), 4.06–4.23 (m, 2H),

3.91–3.99 (m, 1H), 3.59–3.61 (m, 2H), 3.26–3.33 (m, 1H), 2.19–2.27 (m, 1H), 1.37 (t, J = 7.0 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H), 0.70 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.7 (d, J = 6.6 Hz), 154.4, 147.8, 131.1, 128.6, 127.5, 124.5, 115.7, 113.1, 112.0, 103.9, 61.9 (d, J = 7.2 Hz), 61.8 (d, J = 7.7 Hz), 39.2 (d, J = 129.0 Hz), 37.6, 31.0 (d, J = 15.8 Hz), 18.6, 15.9, 14.4 (d, J = 6.0 Hz), 13.9 (d, J = 6.2 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 21.39. Anal. Calcd for $C_{20}H_{25}O_6P$: C, 61.22; H, 6.42. Found: C, 61.52; 6.12.

4.1.2.20. Diethyl (9-hydroxy-3-oxo-1-vinyl-2,3-dihydro-1*H*-benzo[*f*]chromen-2-yl)phosphonate (13t). 63%; yellow oil; R_f = 0.47 ($CHCl_3$–acetone, 98:2); IR 1764, 1457, 1395, 1240, 1156, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.65–7.72 (m, 2H), 7.30–7.32 (m, 1H), 7.01–7.06 (m, 2H), 5.93 (ddd, J = 16.9, 10.2, 5.9 Hz, 1H), 5.12 (dd, J = 10.2, 1.3 Hz, 1H), 4.96 (dd, J = 16.9, 1.3 Hz, 1H), 4.72 (ddd, J = 14.5, 5.9, 1.1 Hz, 1H), 4.15–4.26 (m, 2H), 3.66 (dd, J = 25.2, 1.1 Hz, 1H), 3.57–3.70 (m, 1H), 3.21–3.32 (m, 1H), 1.37 (t, J = 6.9 Hz, 3H), 0.75 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.2 (d, J = 5.8 Hz), 156.7, 149.5, 135.6 (d, J = 18.5 Hz), 132.4, 130.2, 129.7, 125.9, 117.7, 117.2, 113.6, 112.8, 105.1, 63.8 (d, J = 7.1 Hz), 63.7 (d, J = 7.4 Hz), 45.5 (d, J = 128.1 Hz), 36.7 (d, J = 3.3 Hz), 16.1 (d, J = 6.2 Hz), 15.7 (d, J = 6.1 Hz); ^{31}P NMR (101 MHz, $CDCl_3$) δ 19.36. Anal. Calcd for $C_{19}H_{21}O_6P$: C, 60.64; H, 5.62. Found: C, 60.51; 5.72.

4.1.3. General procedure for the synthesis of 3-methylidenechroman-2-ones 14a–t

To a solution of the corresponding chromanones **13a**–**t** (0.5 mmol) in THF (5 mL), *t*-BuOK (for **13b**–**h**,**j**–**t**; 67 mg, 0.6 mmol) or NaH (for **13a**,**i**; 14 mg, 0.6 mmol) was added and the resulting mixture was stirred at rt for 30 min. Then paraformaldehyde (75 mg, 2.5 mmol) was added in one portion. After 1.5 h, the reaction mixture was quenched with brine (5 mL), THF was removed under reduced pressure and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography (eluent: CH_2Cl_2).

4.1.3.1. 7-Methoxy-4-methyl-3-methylenechroman-2-one (14a). 69%; yellow oil; R_f = 0.55 (CH_2Cl_2); IR 1755, 1608, 1442, 1258, 1192, 1014 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.08 (d, J = 8.4 Hz, 1H), 6.68 (dd, J = 8.4, 2.6 Hz, 1H), 6.61 (d, J = 2.6 Hz, 1H), 6.33 (dd, J = 1.0, 0.9 Hz, 1H), 5.73 (dd, J = 1.3, 0.9 Hz, 1H), 3.78 (s, 3H), 3.72–3.75 (m, 1H), 1.39 (d, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.5, 159.6, 150.8, 138.2, 127.3, 127.0, 118.9, 110.9, 102.5, 55.5, 36.6, 22.8. Anal. Calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.30; 5.81.

4.1.3.2. 4-Butyl-7-methoxy-3-methylenechroman-2-one (14b). 80%; yellow oil; R_f = 0.53 (CH_2Cl_2); IR 1747, 1613, 1460, 1285, 1138, 1026 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.03 (d, J = 8.4 Hz, 1H), 6.67 (dd, J = 8.4, 2.5 Hz, 1H), 6.60 (d, J = 2.5 Hz, 1H), 6.33 (d, J = 0.8 Hz, 1H), 5.66 (d, J = 0.8 Hz, 1H), 3.78 (s, 3H), 3.53 (t, J = 7.2 Hz, 1H), 1.51–1.70 (m, 2H), 1.20–1.32 (m, 4H), 0.85 (t, J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.7, 159.6, 150.9, 136.9, 128.3, 128.0, 118.2, 110.8, 102.5, 55.5, 43.1, 37.9, 28.2, 22.3, 13.9. Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 73.01; 7.52.

4.1.3.3. 4-Isopropyl-7-methoxy-3-methylenechroman-2-one (14c). 62%; yellow oil; R_f = 0.56 (CH_2Cl_2); IR 1739, 1587, 1459, 1273, 1126, 1028 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.02 (d, J = 8.4 Hz, 1H) , 1H), 6.68 (dd, J = 8.4, 2.6 Hz, 1H), 6.61 (d, J = 2.6 Hz, 1H), 6.39 (d, J = 1.1 Hz, 1H), 5.65 (d, J = 1.1 Hz, 1H), 3.79 (s, 3H), 3.31 (t, J = 6.1 Hz, 1H), 1.75–1.89 (m, 1H), 0.90 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.1, 159.6, 151.4, 135.4, 129.3, 129.1, 116.7, 110.6, 102.2, 55.4, 49.6, 35.1, 19.4, 18.6. Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: C, 72.14; 7.08.

4.1.3.4. 7-Methoxy-3-methylene-4-vinylchroman-2-one (14d). 78%; yellow oil; R_f = 0.49 (CH_2Cl_2); IR 1749, 1558, 1463, 1282, 1152, 1027 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.07 (d, J = 8.5 Hz, 1H), 6.68 (dd, J = 8.5, 2.6 Hz, 1H), 6.61 (d, J = 2.6 Hz, 1H), 6.47 (d, J = 1.1 Hz, 1H), 5.79 (d, J = 1.1 Hz, 1H), 5.78 (ddd, J = 17.0, 10.0, 6.6 Hz, 1H), 5.24 (ddd, J = 10.0, 1.8, 1.0 Hz, 1H), 5.07 (ddd, J = 17.0, 1.8, 1.0 Hz, 1H), 4.27 (dd, J = 6.6, 1.0 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 161.1, 158.3, 149.3, 135.5, 133.2, 127.5, 126.8, 115.9, 113.4, 109.2, 100.7, 55.8, 53.7, 43.7. Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.12; 5.74.

4.1.3.5. 5,7-Dimethoxy-4-methyl-3-methylenechroman-2-one (14e). 78%; yellow oil; R_f = 0.55 (CH_2Cl_2); IR 1756, 1440, 1224, 1160, 1024 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.32 (d, J = 1.1 Hz, 1H), 6.23–6.26 (m, 2H), 5.72 (d, J = 1.1 Hz, 1H), 3.99 (q, J = 7.1 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 1.27 (d, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 161.7, 158.3, 155.2, 149.4, 136.5, 125.9, 106.2, 93.2, 92.1, 53.9, 53.7, 30.6, 21.5. Anal. Calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.98; 5.81.

4.1.3.6. 4-Butyl-5,7-dimethoxy-3-methylenechroman-2-one (14f). 77%; yellow oil; R_f = 0.57 (CH_2Cl_2); IR 1752, 1592, 1220, 1144, 1096 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.32 (d, J = 1.2 Hz, 1H), 6.23–6.26 (m, 2H), 5.65 (d, J = 1.2 Hz, 1H), 3.83 (t, J = 6.8 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 1.46–1.63 (m, 2H), 1.23–1.29 (m, 4H), 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 161.1, 160.3, 157.2, 151.7, 136.8, 128.2, 107.7, 95.2, 94.1, 55.9, 55.7, 37.5, 35.9, 28.2, 22.6, 14.1. Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found: C, 69.76; 7.41.

4.1.3.7. 4-Isopropyl-5,7-dimethoxy-3-methylenechroman-2-one (14g). 57%; yellow oil; R_f = 0.55 (CH_2Cl_2); IR 1748, 1592, 1464, 1304, 1104, 1048 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.37 (d, J = 1.3 Hz, 1H), 6.23–6.26 (m, 2H), 5.62 (d, J = 1.3 Hz, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 3.66 (d, J = 1.3 Hz, 1H), 1.86–1.95 (m, 1H), 0.92 (d, J = 6.9 Hz, 3H), 0.78 (d, J = 6.9 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.4, 160.1, 157.3, 151.9, 134.5, 129.1, 106.6, 95.0, 93.8, 55.6, 55.4, 43.6, 33.0, 19.8, 17.8. Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.95; 7.11.

4.1.3.8. 5,7-Dimethoxy-3-methylene-4-vinylchroman-2-one (14h). 71%; yellow oil; R_f = 0.50 (CH_2Cl_2); IR 1748, 1596, 1464, 1304, 1100, 1052 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.42 (d, J = 0.9 Hz, 1H), 6.25–6.29 (m, 2H), 5.84 (ddd, J = 17.1, 10.1, 4.9 Hz, 1H), 5.77 (dd, J = 1.0, 0.9 Hz, 1H), 5.04 (ddd, J = 10.1, 1.8, 0.9 Hz, 1H), 4.90 (ddd, J = 17.1, 1.8, 0.9 Hz, 1H), 4.55 (dd, J = 4.9, 1.0 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.1, 160.5, 157.1, 151.6, 137.4, 134.8, 128.9, 114.7, 104.6, 95.0, 94.0, 55.8, 55.5, 40.4. Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.45; 5.58.

4.1.3.9. 8-Methyl-7-methylene-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-6-one (14i). 81%; yellow oil; R_f = 0.58 (CH_2Cl_2); IR 1741, 1639, 1480, 1245, 1154, 1032 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.60 (s, 1H), 6.55 (s, 1H), 6.30 (d, J = 1.0 Hz, 1H), 5.93 (s, 2H), 5.70 (d, J = 1.0 Hz, 1H), 3.66 (q, J = 7.1 Hz, 1H), 1.35 (d, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.4, 147.0, 144.4, 144.3, 137.7, 127.1, 119.1, 105.8, 101.5, 99.1, 37.2, 22.9. Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.92; 4.76.

4.1.3.10. 8-Butyl-7-methylene-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-6-one (14j). 67%; yellow oil; R_f = 0.55 (CH_2Cl_2); IR 1745, 1631, 1481, 1257, 1151, 1033 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 6.58 (s, 1H), 6.56 (s, 1H), 6.33 (d, J = 0.9 Hz, 1H), 5.95 (s, 2H), 5.66 (d, J = 0.9 Hz, 1H), 3.45 (t, J = 7.2 Hz, 1H), 1.48–1.67 (m, 2H), 1.18–1.33 (m, 4H), 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.6, 147.2, 144.6, 144.3, 136.6, 128.0, 118.5, 106.7, 101.6, 99.3, 43.8, 37.6, 29.7, 28.2, 22.3, 13.9. Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.05; 6.39.

4.1.3.11. 8-Isopropyl-7-methylene-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-6-one (14k). 78%; yellow oil; R_f = 0.58 (CH_2Cl_2); IR 1731, 1621, 1478, 1303, 1241, 1142, 1025 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 6.56 (s, 1H), 6.53 (s, 1H), 6.36 (s, 1H), 5.94 (s, 2H), 5.62 (s, 1H), 3.23 (t, J = 6.1 Hz, 1H), 1.81 (sp, J = 6.7 Hz Hz, 1H), 0.90 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.3, 145.5, 143.3, 142.4, 133.2, 127.4, 115.3, 105.8, 99.8, 97.2, 48.5, 33.2, 17.8, 16.8. Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.12; 5.91.

4.1.3.12. 7-Methylene-8-vinyl-7,8-dihydro-6*H*-[1,3]dioxolo[4,5-*g*]chromen-6-one (14l). 55%; yellow oil; R_f = 0.60 (CH_2Cl_2); IR 1732, 1642, 1479, 1263, 1151, 1033 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 6.60 (s, 1H), 6.58 (s, 1H), 6.46 (dd, J = 1.2, 1.0 Hz, 1H), 5.95 (s, 2H), 5.79 (dd, J = 1.5, 1.0 Hz, 1H), 5.77 (ddd, J = 17.0, 10.0, 6.7 Hz, 1H), 5.25 (ddd, J = 10.0, 1.1, 0.9 Hz, 1H), 5.09 (ddd, J = 17.0, 1.1, 1.0 Hz, 1H), 4.19–4.30 (m, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.8, 147.5, 144.7, 144.5, 137.0, 134.5, 129.2, 117.8, 115.3, 106.7, 101.7, 99.2, 46.1. Anal. Calcd for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 67.75; 4.50.

4.1.3.13. 1-Methyl-2-methylene-1*H*-benzo[*f*]chromen-3(2*H*)-one (14m). 80%; yellow oil; R_f = 0.61 (CH_2Cl_2); IR 1756, 1440, 1224, 1160, 1024 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.76–7.97 (m, 3H), 7.58–7.65 (m, 1H), 7.46–7.52 (m, 1H), 7.25–7.28 (m, 1H), 6.44 (s, 1H), 5.88 (s, 1H), 4.47 (q, J = 7.2 Hz, 1H), 1.51 (d, J = 7.2 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 161.5, 145.6, 136.2, 129.4, 128.4, 127.3, 127.2, 126.4, 125.5, 123.4, 120.4, 118.2, 115.8, 33.1, 21.8. Anal. Calcd for $C_{15}H_{12}O_2$: C, 80.34; H, 5.39. Found: C, 80.01; 5.23.

4.1.3.14. 1-Butyl-2-methylene-1*H*-benzo[*f*]chromen-3(2*H*)-one (14n). 70%; yellow oil; R_f = 0.63 (CH_2Cl_2); IR 1752, 1312, 1220, 1132, 1072 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.76–7.94 (m, 3H), 7.57–7.64 (m, 1H), 7.46–7.52 (m, 1H), 7.24–7.28 (m, 1H), 6.46 (s, 1H), 5.80 (s, 1H), 4.23 (dt, J = 9.5, 4.3 Hz, 1H), 1.56–1.81 (m, 2H), 1.26–1.47 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.6, 146.8, 135.1, 130.2, 129.2, 128.0, 127.7, 126.2, 124.1, 121.3, 118.6, 116.5, 39.6, 34.6, 27.2, 21.4, 12.9. Anal. Calcd for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.01; 6.98.

4.1.3.15. 1-Isopropyl-2-methylene-1*H*-benzo[*f*]chromen-3(2*H*)-one (14o). 63%; yellow oil; R_f = 0.63 (CH_2Cl_2); IR 1756, 1318, 1224, 1132, 1068 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.84–7.90 (m, 2H), 7.78 (d, J = 8.9 Hz, 1H), 7.54–7.61 (m, 1H), 7.43–7.50 (m, 1H), 7.25 (d, J = 8.9 Hz, 1H), 6.52 (d, J = 1.0 Hz, 1H), 5.76 (t, J = 1.0 Hz, 1H), 4.14 (dd, J = 4.1, 1.0 Hz, 3H), 2.14–2.27 (m, 1H), 1.07 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.9, 148.2, 133.5, 131.1, 130.6, 130.0, 129.1, 128.9, 127.0, 124.9, 122.7, 118.5, 117.3, 46.0, 33.4, 20.3, 17.1. Anal. Calcd for $C_{17}H_{16}O_2$: C, 80.93; H, 6.39. Found: C, 80.61; 6.54.

4.1.3.16. 2-Methylene-1-vinyl-1*H*-benzo[*f*]chromen-3(2*H*)-one (14p). 45%; yellow oil; R_f = 0.65 (CH_2Cl_2); IR 1752, 1318, 1224, 1136, 1064 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.76–7.94 (m, 3H), 7.57–7.64 (m, 1H), 7.46–7.52 (m, 1H), 7.24–7.28 (m, 1H), 6.52 (s, 1H), 5.96 (ddd, J = 17.0, 10.1, 4.8 Hz, 1H), 5.91 (s, 1H), 5.13 (dd, J = 10.1, 1.7 Hz, 1H), 4.94–5.00 (m, 1H), 4.95 (dd, J = 17.0, 1.7 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 162.9, 148.2, 136.7, 134.8, 131.1, 130.5, 129.7, 129.5, 128.9, 127.4, 125.2, 122.6, 117.6, 116.5, 116.4, 43.2. Anal. Calcd for $C_{16}H_{12}O_2$: C, 81.34; H, 5.12. Found: C, 81.63; 5.32.

4.1.3.17. 9-Hydroxy-1-methyl-2-methylene-1*H*-benzo[*f*]chromen-3(2*H*)-one (14q). 75%; yellow oil; R_f = 0.54 (CH_2Cl_2); IR 1756, 1440, 1224, 1160, 1024 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.65–7.76 (m, 2H), 7.27–7.28 (m, 1H), 7.05–7.13 (m, 2H), 6.40 (s, 1H), 5.82 (s, 1H), 4.28 (q, J = 7.2 Hz, 1H), 2.06 (s, 1H), 1.44 (d, J = 7.2 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.7, 155.2, 148.0, 138.0, 131.7, 130.9, 128.9, 128.1, 126.4, 118.5, 117.0, 115.0, 104.9, 35.0, 23.1. Anal. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 74.71; 4.87.

4.1.3.18. 1-Butyl-9-hydroxy-2-methylene-1*H*-benzo[*f*]chromen-3(2*H*)-one (14r). 43%; yellow oil; R_f = 0.57 (CH_2Cl_2); IR 1756, 1320, 1220, 1132, 1064 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.65–7.77 (m, 2H), 7.05–7.25 (m, 3H), 6.43 (s, 1H), 5.75 (s, 1H), 4.09 (dt, J = 9.5, 4.2 Hz, 1H), 2.20 (s, 1H), 1.54–1.90 (m, 2H), 1.19–1.41 (m, 4H), 0.86 (t, J = 7.1 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.0, 155.0, 148.5, 136.1, 131.8, 130.9, 128.8, 126.5, 118.2, 116.9, 115.0, 105.0, 40.2, 35.2, 28.2, 22.4, 13.9. Anal. Calcd for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43. Found: C, 76.82; 6.62.

4.1.3.19. 9-Hydroxy-1-Isopropyl-2-methylene-1*H*-benzo[*f*]chromen-3(2*H*)-one (14s). 55%; yellow oil; R_f = 0.52 (CH_2Cl_2); IR 1752, 1318, 1220, 1132, 1068 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.67–7.70 (m, 2H), 7.18–7.19 (m, 1H), 7.04–7.10 (m, 2H), 6.51 (d, J = 1.0 Hz, 1H), 5.73 (t, J = 1.0 Hz, 1H), 3.98 (dd, J = 4.3, 1.0 Hz, 3H), 2.11–2.26 (m, 1H), 2.18 (s, 1H), 1.06 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.9 Hz, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 164.2, 154.7, 149.0, 133.6, 132.3, 130.9, 130.1, 129.0, 126.6, 117.2, 116.7, 115.1, 105.5, 46.3, 33.1, 20.4, 17.2. Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 76.31; 6.29.

4.1.3.20. 9-Hydroxy-2-methylene-1-vinyl-1*H*-benzo[*f*]chromen-3(2*H*)-one (14t). 66%; yellow oil; R_f = 0.65 (CH_2Cl_2); IR 1750, 1324, 1220, 1141, 1044 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.74 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.26 (d, J = 2.1 Hz, 1H), 7.14 (dd, J = 8.8, 2.1 Hz, 1H), 7.06 (d, J = 8.9 Hz, 1H), 6.49 (s, 1H), 5.88 (ddd, J = 17.0, 10.1, 5.1 Hz, 1H), 5.83 (s, 1H), 5.06 (dd, J = 10.1, 1.7 Hz, 1H), 4.91 (dd, J = 17.0, 1.7 Hz, 1H), 4.79 (d, J = 5.1 Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 163.7, 155.3, 148.6, 136.4, 134.6, 132.1, 130.7, 129.7, 129.4, 126.3, 117.2, 116.5, 114.9, 114.7, 105.3, 43.1. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.10; 4.93.

4.2. Biological evaluation

4.2.1. Cytotoxicity assay by MTT

Cytotoxicity of the compounds was assessed by the mitochondrial reduction assay on two leukemia cell lines, promyelocytic HL-60 and lymphoblastic NALM-6 and on two solid tumor-derived cell lines, breast cancer MCF-7 and colon cancer HT-29 adenocarcinomas. Cells were purchased from the European Collection of Cell Cultures (ECACC). Leukemia cells were cultured in RPMI 1640 medium, while MCF-7 and HT-29 cells in DMEM (Dulbecco's Modified Eagle Medium), both supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Paisley, UK) and antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin). Normal human umbilical vein endothelial cells (HUVECs) and all reagents for cell culture were purchased from Cascade Biologics (Portland, Oregon,

USA). The HUVECs were cultured according to the manufacturer's instructions and the cells underwent 3–8 passages. Cells were grown in 37 °C in a humidified atmosphere of 5% CO_2 in air. Exponentially growing cells were seeded at 8×10^3/well on 96-well plates (Nunc, Roskilde, Denmark). After 24 h, the tested compounds (freshly prepared in DMSO and diluted with complete culture medium to obtain the concentration range from 10^{-7} to 10^{-3} M) were added and the plates were incubated for 48 h. Afterwards, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL in PBS) was added and incubation was continued for 2 h. The metabolically active cells reduced MTT to blue formazan crystals. Then, the MTT-containing medium was carefully aspirated and 100 μL DMSO was added to dissolve the crystals. After shaking 10 min in the dark absorbance was read at 560 nm on an ELISA–plate reader (ELX 800, Bio-Tek, USA) and compared with control (untreated cells). The IC_{50} values were calculated from concentration–response curves.

4.2.2. Evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The reference *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* NCTC 8196, *Pseudomonas aeruginosa* NCTC 6749, *Candida albicans* ATCC 10231 and three *S. aureus* clinical strains (MRSA—methcillin resistant, from our own collection), specified as A3, A7, and D5 were used. Sterile stock solutions of each tested compound were prepared in DMSO at the concentration of 20.0 mg/mL. The concentration range used in the antimicrobial tests was 3.125–400.0 μg/mL, prepared in Mueller-Hinton broth (Difco, USA) or RPMI-1640 (Sigma, Germany). Prior to the assay, the strains were evaluated for antibiotic susceptibility, using the standard broth microdilution MIC tests, according to CLSI recommendations.[32] Minimum inhibitory concentration (MIC) values were specified as the concentration of compound at which microbial growth was inhibited during 24 h culture (the turbidity was thus equal to A600 of the culture medium used as a blank control (0.06). The solubilizer—DMSO at a concentration of 2.0% (the same as the highest final concentration used for the compound dilutions)—had no antimicrobial activity itself. The lowest concentration of compounds bactericidal to $\geqslant$99.9% of the original inoculum was determined by subculturing 10 μL from the wells of suspected MIC, 2 MIC, 4 MIC, to the broth media without antimicrobial agent. No visible growth after subsequent 24 h incubation, means minimum bactericidal concentration (MBC). The compound was regarded bactericidal if MBC was not greater than four times the MIC. All test methods met acceptable standards based on recommended quality control ranges for all comparator antibiotics and the appropriate ATCC quality control strains.

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