EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201201736

Reactivity of 4-Vinyl-2*H*-1-benzopyran-2-ones in Diels–Alder Cycloaddition Reactions: Access to Coumarin-Based Polycycles with Cdc25 Phosphatase-Inhibiting Activity

Sergio Valente,[a,b,c][‡] Zhanjie Xu,[d,e][‡] Emilie Bana,[a] Clemens Zwergel,[a] Antonello Mai,[c] Claus Jacob,[d] Peter Meiser,[e] Denyse Bagrel,[a] Artur M. S. Silva,*[b] and Gilbert Kirsch*[a]

Keywords: Antitumor agents / Oxygen heterocycles / Polycycles / Diels–Alder / Cycloaddition

The reactivity of 4-(1-butoxyvinyl)-2*H*-chromen-2-one (**1**) and (*E*)-4-(2-butoxyvinyl)-2*H*-chromen-2-one (**2**) as diene in thermal Diels–Alder cycloaddition reactions with several electron-poor dienophiles is reported. Among several dienophiles used in this study 1,4-benzoquinone afforded cycloadducts 11-butoxy-1*H*-naphtho[1,2-*c*]chromene-1,4,5-trione (**3e**) and 1*H*-naphtho[1,2-*c*]chromene-1,4,5-trione (**4g**) that showed Cdc25 phosphatase-inhibition activity at low micromolar values, with both compounds more effective against Cdc25 A and Cdc25 C isoforms.

Introduction

A large number of natural products bear a 2*H*-1-benzopyran-2-one (coumarin) as part of their structure.[1] These compounds show a wide range of biological activities, such as antioxidants,[2] anticoagulants[3] and antifungal agents,[4] selective MAO-B inhibitors,[5] and inhibitors of hAChE and BACE,[6] NF*k*B,[7] Hsp90,[8] HIV-1 integrase[9] and Cdc25 phosphatases[10] (Figure 1). More recently, coumarin-based derivatives have been reported as inhibiting matrix metalloproteinase-7 expression,[11] and showing 17-β-HSD1[12] and cannabinoid receptor antagonist activity.[13]

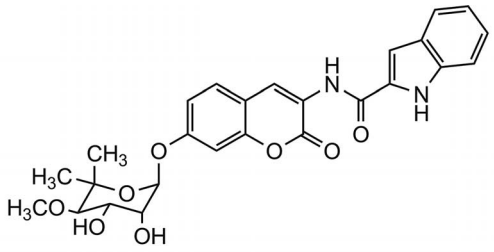
Among all natural compounds containing a coumarin scaffold, coumestrol (Figure 1) is a unique coumestan phytoestrogen, mimicking the biological activity of estrogens by inhibiting the activity of aromatase and hydroxysteroid dehydrogenase.[14] Despite decades of research, the total synthesis of the steroid nuclei by improved strategies continues to receive considerable attention. Numerous methods have been exploited for the total synthesis of steroids that appear widely in nature, and which possess practical medical importance. A certain group of steroid-derived compounds were reported as inhibitors of human Cdc25A protein phosphatase.[15] Furthermore, recently we described some coumarin derivatives endowed with good in-vitro inhibitory activity against Cdc25 A and C phosphatases.[10]

Steroids have become one of the preferred testing grounds for the development of more efficient methods of organic synthesis and the Diels–Alder reaction was shown to offer a versatile method for the stereoselective synthesis of steroids. The Diels–Alder cycloaddition reaction is well reviewed both for its application in total synthesis[16] and for synthetic routes of steroids and associated structures.[17]

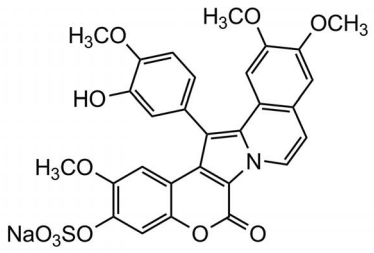
Recently our group optimized a method to provide a methyl-ketone substituent at the C4 position on the 2*H*-1-benzopyran-2-one scaffold through a very high α-regioselective Heck cross-coupling reaction by using tosylates as substrates (Scheme 1).[18] As an intermediate we obtained 4-(1-butoxyvinyl)-2*H*-chromen-2-one (**1**), a very useful diene. However, when we performed the cross-coupling reaction on 4-bromocoumarin, instead of 4-tosylate as starting reagent, with $Pd(OAc)_2$/DPPP or Pd_2dba_3/DPPF as the catalytic system, the reaction regioselectively afforded the β isomer (*E*)-4-(2-butoxyvinyl)-2*H*-chromen-2-one (**2**) in high yield (85%), and not a mixture of both isomers, as expected (Scheme 1).

In the present work we studied the reactivity of dienes **1** and **2** in (4π+2π) thermal Diels–Alder cycloaddition reactions with several dienophiles with the aim of building new

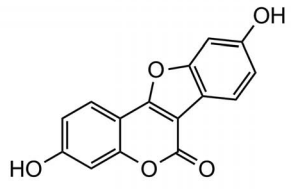
[a] Laboratoire d'Ingénierie Moléculaire et Biochimie Pharmacologique, SRSMC UMR 7565, Université de Lorraine, 1 Boulevard Arago, 57070 Metz, France
E-mail: kirsch@univ-metz.fr
gilbert.kirsch@univ-lorraine.fr
Homepage: www.limbp.univ-metz.fr
[b] Department of Chemistry & QOPNA, University of Aveiro, 3810-193 Aveiro, Portugal
E-mail: artur.silva@ua.pt
Homepage: https://sites.google.com/site/artursilvaua/
[c] Istituto Pasteur - Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, P.le A. Moro 5, 00185 Roma, Italy
[d] Division of Bioorganic Chemistry, School of Pharmacy, Saarland University, 66123 Saarbrücken, Germany
[e] Ursapharm Arzneimittel GmbH & Co KG, Industriestraße 35, 66129 Saarbrücken Germany
[‡] These authors contributed equally to this work.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201201736.



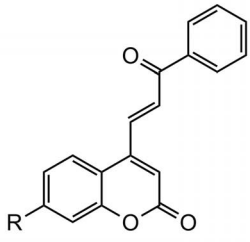
Novobiocin analogue as HSP90 inhibitor[8]



Lamellarin a 20-Sulfate
(HIV1 integrase inhibitor)[9]

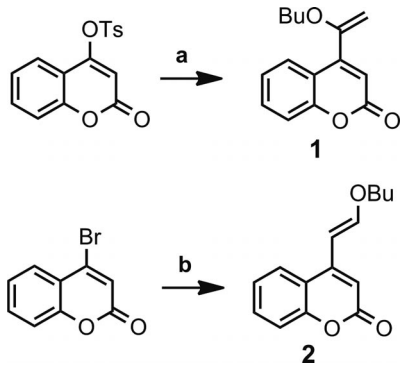


Coumestrol
Aromatase and hydroxysteroid dehydrogenase inhibitor[14]



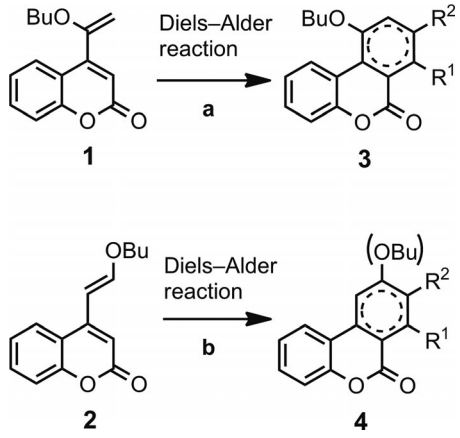
Cdc25 phosphatases inhibitor
R = -OCH_3, OH[10]

Figure 1. Some biologically active known coumarin-based derivatives.



Scheme 1. Synthesis of starting dienes **1** and **2**. Reagents and conditions: (a) butyl vinyl ether, *N*,*N*-diisopropylethylamine (DIPEA), 1,3-bis(diphenylphosphanyl)propane (DPPP), $Pd(OAc)_2$, dioxane, 80 °C, 12 h; (b) butyl vinyl ether, DIPEA, 1,1′-bis(diphenylphosphanyl)ferrocene, Pd_2dba_3, dioxane, 80 °C.

coumarin-containing polycycles (steroid-like) or precursors of steroid structures and/or its manipulation, as shown in Scheme 2 and Table 1.



Scheme 2. Thermal Diels–Alder cycloaddition reaction of dienes **1** and **2** with several dienophiles. Reagents and conditions: (a) toluene, 100 °C or xylene, 130 °C or 1,2-dichlorobenzene, 180 °C, sealed tube; (b) toluene, 100 °C, sealed tube.

Results and Discussion

All Diels–Alder cycloaddition reactions were performed under thermal conditions in a sealed tube. In general the cycloaddition reactions with diene **1** were complete within 6 h, with the exception of the reaction with maleic anhydride leading to derivative **3d** (Table 1, Entry 4; 12 h) and within 4 h for diene **2**, with the exception of the reaction with methyl propiolate or 3-butyn-2-one leading to compounds **4b** and **4c** (Table 1, Entries 9 and 10; 12 h). The reactivity of both diene isomers **1** and **2** towards different dienophiles was similar only for dimethyl acetylene dicarboxylate (Table 1, Entries 1 and 8). Diene **2** showed higher reactivity for all other dienophiles relative to diene **1**, for which temperatures as high as 180 °C were necessary (Table 1, Entries 5 and 6) to reach sufficient energy for HOMO–LUMO overlapping, or longer reaction times were needed to complete the reaction. Every attempt to use mild conditions for these cycloaddition reactions, such as lower temperature and Lewis acid catalysts ($AlCl_3$, $ZnCl_2$, $BF_3{\cdot}Et_2O$), did not afford positive results for dienes **1** and **2**. When we reacted diene **1** with dimethyl acetylene dicarboxylate (Table 1, Entry 1), 1,4-benzoquinone (Table 1, Entry 5) or 1,4-naphthoquinone (Table 1, Entry 6), the cycloaddition was followed by spontaneous oxidation-aromatization of the new cycle onto the 2*H*-1-benzopyran-2-one nucleus giving unique aromatized compounds **3a**, **3e** and **3f**. However, reaction of diene **2** with the same dienophiles (Table 1, Entries 8, 14 and 15) afforded cycloadducts that spontaneously underwent rearrangement, butanol elimination and aromatization giving compounds **4a**, **4g**, and **4h** (Scheme 3 shows the reaction mechanism for **4a**). The same rearrangement occurred for other acetylene dienophiles (Table 1, Entries 9 and 10). In the other cases (Table 1, Entries 11–13 and 16) the first cycloadduct (expected after Diels–Alder reaction) was unstable and a [1,3]-proton shift occurs, as shown in Scheme 3 for **4d**. The general driving force is the reforming of the fully conjugated coumarin structure, whereas in the case of compounds **4a**–**c** the pres-

Table 1. Study of intermolecular thermal Diels–Alder cycloaddition reactions.[a]

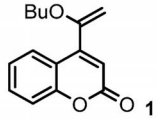
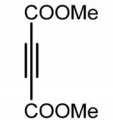
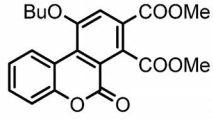
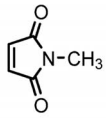
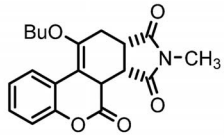
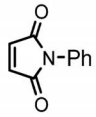
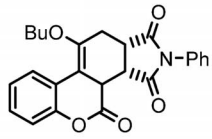
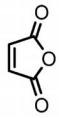
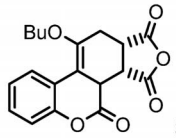
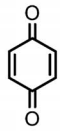
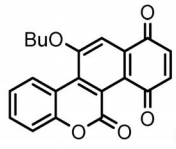
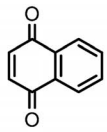
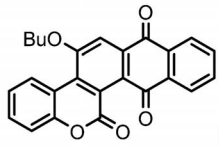
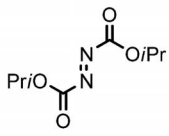
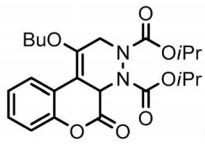
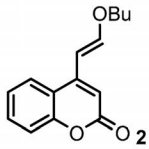
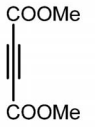
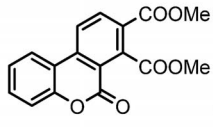
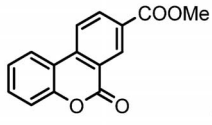
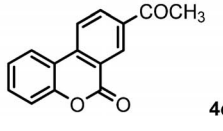
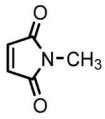
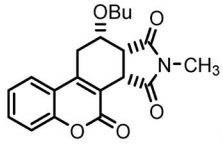
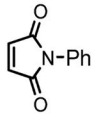
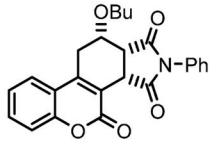
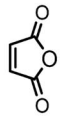
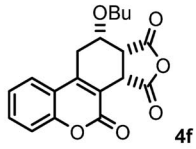
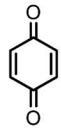
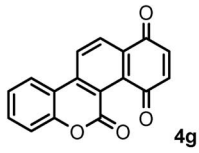
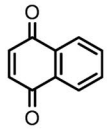
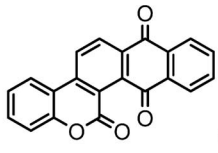
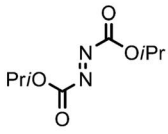
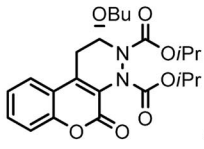
Entry	Diene	Dienophile	Cycloadduct	Solvent/time (h)/temp. (°C)	Yield (%)[b]
1	 **1**		 **3a**	toluene/3/100	55
2	**1**		 **3b**	toluene/6/100	76
3	**1**		 **3c**	toluene/6/100	73
4	**1**		 **3d**	xylene/12/130	56
5	**1**		 **3e**	ODCB/4/180	52
6	**1**		 **3f**	ODCB/4/180	48
7	**1**		 **3g**	toluene/6/100	57
8	 **2**		 **4a**	toluene/2/100	72
9	**2**		 **4b**	toluene/12/100	52
10	**2**		 **4c**	toluene/12/100	68

Table 1. (*Continued*)

Entry	Diene	Dienophile	Cycloadduct	Solvent/time (h)/temp. (°C)	Yield (%)[b]
11	**2**		 **4d**	toluene/3/100	80
12	**2**		 **4e**	toluene/3/100	78
13	**2**		 **4f**	toluene/4/100	67
14	**2**		 **4g**	toluene/4/100	54
15	**2**		 **4h**	toluene/4/100	58
16	**2**		 **4i**	toluene/4/100	72

[a] Reaction conditions: diene **1** or **2** (1.0 equiv.), dienophile (3 equiv.), in solvent. Reactions were performed in a sealed tube. [b] Greater than 95% purity as determined by MS.

ence of two double bonds and the elimination of butanol facilitates the formation of fully conjugated structures. When 1,4-benzoquinone or 1,4-naphthoquinone is used they play two roles, as dienophile and as oxidizing agent.

In order to elucidate the structure and to establish the stereochemistry of the obtained cycloadducts we carried out COSY, HSQC and NOESY NMR experiments of selected cycloadducts **3b** and **4d**. In the Diels–Alder reaction of diene **1** with electron poor dienophiles the *endo* cycloadduct was selectively obtained, as shown by the *syn* configuration of the coumarinic H-3 and the dienophile moiety (Figure 2; see NOESY experiment in Supporting Information). In the reaction of diene **2** the *endo* cycloadducts were also obtained, because the close proximity of the butoxy substituent with the dienophile moiety was observed (Scheme 3). The COSY and HSQC spectra of **4d** also proved the [1,3]-proton shift in the obtained cycloadduct, with two different methylene groups observed (one from the butoxy substituent and the other resulting from the 1,3-proton shift).

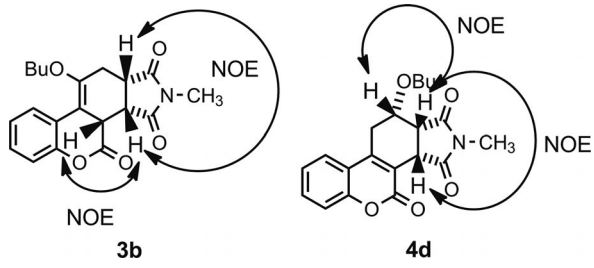
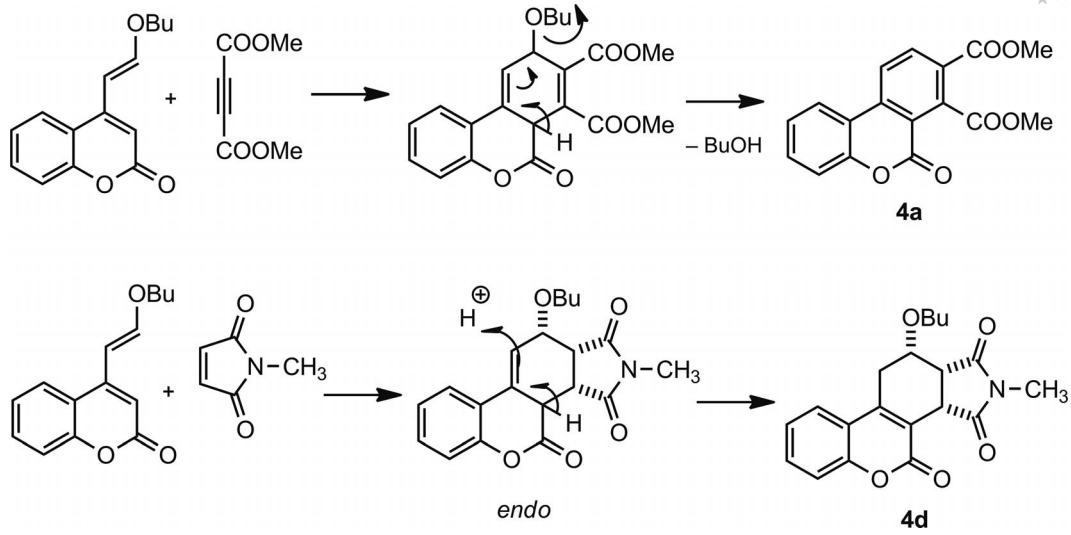


Figure 2. Main NOE correlations of compounds **3b** and **4d**.



Scheme 3. Proposed mechanisms for the Diels–Alder cycloadduct rearrangement.

Furthermore we explored the reactivity of both dienes **1** and **2** with the azo-dienophile, diisopropyl azodicarboxylate, to obtain polycyclic structures **3g** and **4i** (Table 1, Entries 7 and 16), which are useful starting materials to access to a pyridazino-coumarin scaffold. Both dienes **1** and **2** showed good reactivity with this dienophile, and within 4 h and 6 h (Table 1, Entries 16 and 7, respectively) the cycloaddition reactions were complete with good yields. We also studied whether the most reactive diene **2** behaved in a regioselective manner with non-symmetric dienophiles, such as methyl propiolate or 3-butyn-2-one (Table 1, Entries 9 or 10). As expected, the Diels–Alder reaction provided as sole product the regioisomer cycloadduct **4b** and **4c** that were obtained even under these conditions (dienophile with only one electronic withdrawing group) through butanol elimination and aromatization. However, longer reaction times (12 h) were needed by both methyl propiolate and 3-butyn-2-one (Table 1). The unequivocal assignment of the structure of compounds **4b** and **4c** is based on the multiplicity of the H-3 NMR signal. It appears as a broad singlet for **4b** and as a doublet with a small coupling constant (^4J = 2.0 Hz) for **4c**, which was not the case if the other isomer was obtained, for which three consecutive aromatic protons appear.

Following our recent work on coumarin-based derivatives as Cdc25 phosphatase inhibitors,[10] we decided to test some of the new compounds against the three Cdc25 isoforms, A, B and C. Cdc25 phosphatases are key enzymes regulating the cell cycle and are a valuable target for cancer treatment. Human glutathione-*S*-transferase (GST)-Cdc25 recombinant enzymes were used to evaluate the inhibitory potential of the compounds. Each isoenzyme was prepared as described previously.[19,20] Briefly, the GST-tagged Cdc25s were expressed in a bacterial expression system through isopropyl β-D-1-thiogalactopyranoside induction. After lysis of the bacteria, purification on a GSH-Agarose column gave the GST-Cdc25 recombinant proteins. Recom-

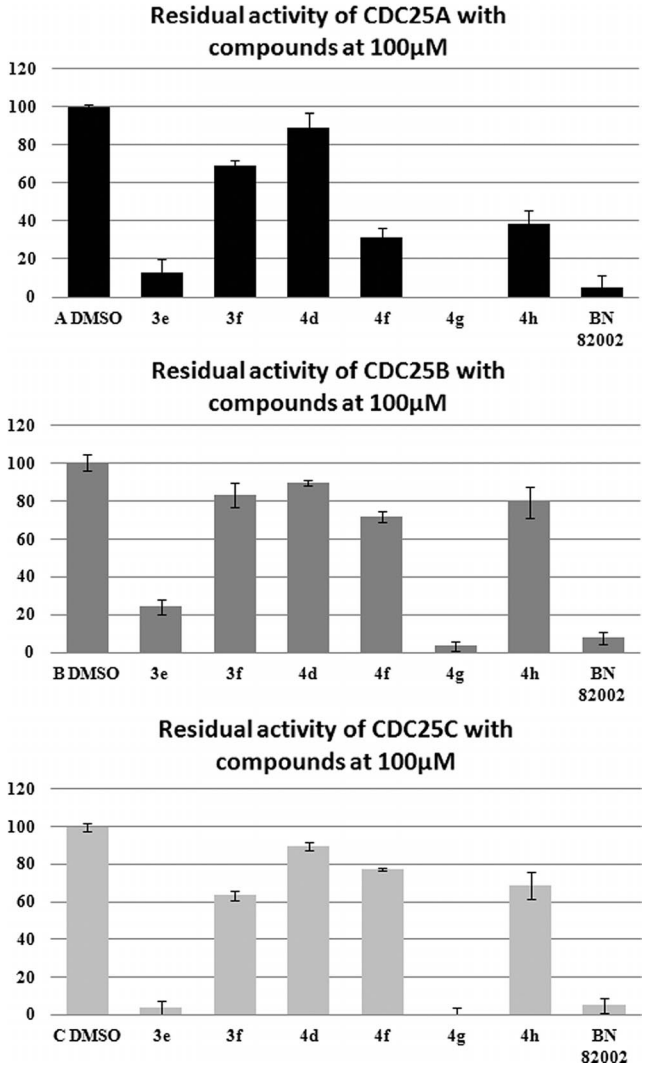
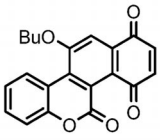
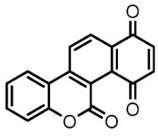


Figure 3. Inhibitory activity (expressed as residual percentage of inhibition) of compounds **3e**, **3f**, **4d**, **4f**–**h**. Tested at 100 µM against Cdc25A, Cdc25B and Cdc25C phosphatases. Values are means of three independent experiments.

binant Cdc25 A and Cdc25 C are full-length enzymes whereas Cdc25 B is truncated (active site only).

The enzymatic activity was measured by a dephosphorylation assay with 3-*O*-methyl fluorescein phosphate as described previously.[21] To test the inhibitory potential of the new compounds, **3e**, **3f**, **4d**, and **4f**–**h** were assayed at 100 μM final concentration relative to BN82002 (Sigma Aldrich), which was used as a reference inhibitor drug at 10 μM. Inhibitory activity with dimethyl sulfoxide (DMSO) was used to establish residual activity of the enzyme (as a percentage relative to DMSO reference; Figure 3). Analysis of the inhibition assay revealed compounds **3e** and **4g** as the most potent inhibitors among these coumarin derivatives. Compound **3e** showed about 80% inhibition for Cdc25 A and B, and more than 95% for Cdc25 C, and **4g** completely inhibited enzymatic activity of Cdc25 A and C at that concentration and inhibited more than 95% activity of Cdc25 B. Next we determined IC_{50} values for the most potent compounds, **3e** and **4g** (Table 2). Statistical calculations were performed with a generalized log linear regression model (Poisson regression), as described by Maul.[22]

Table 2. Inhibitory activity (IC_{50} values) against Cdc25A, B and C phosphatases of compounds **3e** and **4g**.[a]

Compd.	Structure	Phosphatases (IC_{50}, μM)		
		Cdc25A	Cdc25B	Cdc25C
3e		13.2	46.1	9.0
4g		5.8	14.4	2.3

[a] The IC_{50} values were determined by testing seven different concentrations of compounds (from 0 to 150 μM). For each compound, each concentration was separately tested in 3 independent microplates, at the rate of 3 wells per microplate. The statistical evaluation of IC_{50} was made with software specially designed for calculating the median inhibitory concentration for toxicity tests.[20]

The new quinone-based tetracycle **4g** showed an interesting inhibition in the low micromolar range against all three Cdc25 phosphatases, being the most active for Cdc25 C (2.3 μM). It was also more potent (up to almost 4 fold for Cdc25C) than analogue **3e** bearing a butoxy group. Therefore this compound could be considered as a new lead for further structural optimization and deeper in-cell studies in order to validate its anticancer properties.

Conclusions

In summary, we have described the reactivity of 4-(1- and 2-butoxyvinyl)-2*H*-1-benzopyran-2-one in [4+2] thermal Diels–Alder cycloaddition reactions with several electron-poor dienophiles, to access coumarin-based polycyclic compounds. Among these heterocycles we identified **3e** and **4g** as new Cdc25 phosphatases inhibitors.

Experimental Section

General: The solvents used were purchased from Carlo–Erba and the reagents from Acros Organics or Alfa Aesar. Melting points were determined with a Stuart SMP3 apparatus. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC 250 MHz spectrometer in $CDCl_3$ or [D_6]DMSO. Mass spectra were recorded with a Micro-Tof-Q 98. 2D COSY ($^1H/^1H$), 2D HSQC ($^1H/^{13}C$; delay for one bond *J* C/H couplings were optimized for 145 Hz) and NOESY (mixing time of 800 ms) experiments were performed in a Bruker Avance 300 MHz spectrometer. Chemical shifts are reported relative to the internal reference tetramethylsilane. All reactions were routinely checked by thin-layer chromatography performed with aluminum-backed silica gel plates (Merck DC, Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light. Column chromatography was performed with silica gel LC 60A (70–200 micron).

4-(1-Butoxyvinyl)-2*H*-chromen-2-one (1):[18] A mixture of 2-oxo-2*H*-chromen-4-yl tosylate (0.1 g, 0.37 mmol), butyl vinyl ether (1.5 mmol, 0.19 mL), DIPEA (1.13 mmol, 0.19 mL) $Pd(OAc)_2$ (0.002 g, 0.009 mmol), DPPP (0.004 g, 0.010 mmol), in dry dioxane (3.0 mL) was stirred under N_2 in a sealed tube at 80 °C for 12 h. After this time the solvent was removed under vacuum and the residue purified by column chromatography (ethyl acetate/cyclohexane, 1:7) to provide pure **1** (85.87 mg, 95%) as a colorless oil. R_f = 0.3 (silica gel, cyclohexane/EtOAc, 7:1). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.97 (t, *J* = 7.4 Hz, 3 H, C*H*$_3$CH$_2$CH$_2$CH$_2$O–), 1.44–1.51 (m, 2 H, CH$_3$C*H*$_2$CH$_2$CH$_2$O–), 1.74–1.79 (m, 2 H, CH$_3$CH$_2$C*H*$_2$CH$_2$O–), 3.91 (t, *J* = 6.4 Hz, 2 H, CH$_3$CH$_2$CH$_2$C*H*$_2$O–), 4.53 and 4.55 (2d, *J* = 3.0 and 2.9 Hz, 2 H, =C*H*$_2$), 6.48 (s, 1 H, 3-H), 7.24–7.29 (m, 1 H, Ar–H), 7.31–7.35 (m, 1 H, Ar–H), 7.49–7.55 (m, 1 H, Ar–H), 7.78 (dd, *J* = 7.0 and 1.2 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.7, 19.3, 30.8, 68.1, 89.1, 114.8, 117.1, 117.7, 124.1, 126.8, 131.7, 150.9, 154.0, 156.8, 161.0 ppm. HRMS: calcd. for $C_{15}H_{16}O_3Na$ [M + Na]$^+$ 267.1099; found 267.1103.

(*E*)-4-(2-Butoxyvinyl)-2*H*-chromen-2-one (2): A mixture of 4-bromo-2*H*-chromen-2-one (0.450 g, 2.0 mmol), butyl vinyl ether (8.0 mmol, 1.04 mL), DIPEA (6.0 mmol, 1.05 mL), $Pd_2(dba)_3$ (0.027 g, 0.03 mmol), DPPF (0.033 g, 0.06 mmol), in dry dioxane (3.0 mL) was stirred under N_2 in a sealed tube at 80 °C for 12 h. After this time the solvent was removed under vacuum and the residue purified by column chromatography (ethyl acetate/*n*-hexane, 1:5) to provide pure **2** (0.415 g, 85%) as a colorless solid, m.p. 100–102 °C. R_f = 0.3 (silica gel, *n*-hexane/EtOAc, 3:1). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.3 Hz, 3 H, C*H*$_3$CH$_2$CH$_2$CH$_2$O–), 1.40–1.52 (m, 2 H, CH$_3$C*H*$_2$CH$_2$CH$_2$O–), 1.69–1.81 (m, 2 H, CH$_3$CH$_2$C*H*$_2$CH$_2$O–), 4.04 (t, *J* = 6.5 Hz, 2 H, CH$_3$CH$_2$CH$_2$C*H*$_2$O–), 6.23–6.28 (d, *J* = 12.4 Hz, 1 H, –C*H*=CHO–), 6.32 (s, 1 H, 3-H), 7.22–7.36 (m, 3 H, Ar–H and –C*H*COO–), 7.50–7.56 (m, 1 H, Ar–H), 7.66–7.70 (d, *J* = 12.5 Hz, 1 H, –CH=C*H*O–), 7.97–8.00 (d, *J* = 8.0 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.7, 19.0, 31.2, 71.2, 98.2, 106.7, 117.3, 118.8, 123.9, 124.3, 131.6, 149.9, 153.7, 154.8, 161.3 ppm. HRMS: calcd. for $C_{11}H_8O_3Na$ [M + Na]$^+$ 267.1099; found 267.1103.

General Procedure for Thermal Diels–Alder Cycloaddition Reaction: A mixture of diene **1** or **2** (1 equiv.) and the appropriate dienophile

(3 equiv.) in the respective dry solvent (10 mL; see Table 1) was stirred in a sealed tube (for the reaction time and temperature, see Table 1). When the starting material was consumed the reaction was cooled to room temperature, the solvent was removed under vacuum and organic residue purified by column chromatography (ethyl acetate/cyclohexane, ratio as noted) to provide desired cycloadducts **3a**–**g** and **4a**–**i**.

Dimethyl 10-Butoxy-6-oxo-6*H*-benzo[*c*]chromene-7,8-dicarboxylate (3a): Colorless solid, m.p. 141–143 °C. R_f = 0.3 (silica gel, cyclohexane/EtOAc, 3:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.06 (t, J = 7.9 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.59–1.64 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 2.00–2.04 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 3.96 (s, 3 H, –COOCH_3), 4.05 (s, 3 H, –COOCH_3), 4.31 (t, J = 5.4 Hz, 2 H, CH$_3$CH$_2$CH$_2$CH_2O–), 7.30–7.39 (m, 2 H, Ar–H), 7.50–7.56 (m, 1 H, Ar–H), 7.95 (s, 1 H, Ar–H), 9.07–9.10 (m, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.8, 19.4, 30.9, 53.0, 53.1, 69.8, 116.6, 117.1, 117.7, 120.7, 124.5, 128.1, 128.4, 129.0, 130.8, 131.1, 151.0, 156.8, 158.8, 164.5, 168.5 ppm. HRMS: calcd. for $C_{21}H_{20}O_7Na$ [M + Na]$^+$ 407.1209; found 407.1215.

10-Butoxy-2-methyl-11,11a-dihydrochromeno[3,4-*e*]isoindole-1,3,4-(2*H*,3a*H*,3b*H*)-trione (3b): Colorless solid, m.p. 112–114 °C. R_f = 0.25 (silica gel, light petroleum/EtOAc, 3:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.95 (t, J = 6.1 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.37–1.50 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.65–1.75 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 2.31–2.39 (m, 1 H, –C*H*HCHCON–), 2.89 (s, 3 H, –NCH_3), 3.18–3.23 (d, J = 14.2 Hz, 1 H, –CH*H*CHCON–), 3.33–3.39 (t, 1 H, CH$_2$C*H*CON–), 3.48–3.50 (m, 1 H, –C*H*COO–), 3.75–3.82 (m, 1 H, CH$_3$CH$_2$CH$_2$C*H*HO–), 3.90–3.97 (m, 1 H, CH$_3$CH$_2$CH$_2$CH*H*O–), 3.99–4.03 (m, 1 H, CHC*H*CON–), 7.05–7.10 (m, 2 H, Ar–H), 7.16–7.21 (m, 1 H, Ar–H), 8.35 (d, J = 6.6 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.7, 19.0, 25.1, 26.2, 31.7, 38.4, 39.2, 44.4, 68.9, 105.2, 117.3, 117.5, 124.6, 127.4, 128.2, 148.2, 151.0, 166.4, 176.6, 178.2 ppm. HRMS: calcd. for $C_{20}H_{21}NO_5Na$ [M + Na]$^+$ 355.1420; found 355.1424.

10-Butoxy-2-phenyl-11,11a-dihydrochromeno[3,4-*e*]isoindole-1,3,4-(2*H*,3a*H*,3b*H*)-trione (3c): Colorless solid, m.p. 144–146 °C. R_f = 0.25 (silica gel, light petroleum/EtOAc, 3:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.81–0.87 (t, J = 7.5 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.33–1.42 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.61–1.69 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 2.40 (dd, J = 6.8, 11.6 Hz, 1 H, –C*H*HCH$_2$CON–), 3.24 (d, J = 11.6 Hz, 1 H, –CH*H*CH$_2$CON–), 3.43–3.49 (m, 2 H, CH$_2$C*H*CON– and –C*H*COO–), 3.73–3.82 (m, 1 H, CH$_3$CH$_2$CH$_2$C*H*HO–), 3.87–3.96 (m, 1 H, CH$_3$CH$_2$CH$_2$CH*H*O–), 4.12 (dd, J = 4.0, 6.9 Hz, 1 H, CHC*H*CON–), 6.99–7.31 (m, 8 H, Ar–H), 8.30–8.33 (d, J = 7.5 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.7, 19.1, 26.7, 31.8, 38.7, 39.5, 44.7, 69.1, 77.2, 105.5, 117.4 (2 C), 124.7, 126.0 (2 C), 127.2, 128.2, 128.8, 129.1 (2 C), 131.2, 151.0, 166.4, 175.7, 177.3 ppm. HRMS: calcd. for $C_{25}H_{23}NO_5Na$ [M + Na]$^+$ 440.1474; found 440.1477.

10-Butoxy-11,11a-dihydro-1*H*-isobenzofuro[4,5-*c*]chromene-1,3,4-(3a*H*,3b*H*)-trione (3d): Colorless solid, m.p. 190–192 °C. R_f = 0.30 (silica gel, cyclohexane/EtOAc, 2:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.83 (t, J = 6.5 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.16–1.28 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.34–1.40 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 1.77–1.87 (m, 1 H, –C*H*HCHCOO–), 2.97 (dd, J = 3.8, 9.0 Hz, 1 H, –CH*H*CHCOO–), 3.19–3.27 (m, 1 H, CH$_3$CH$_2$CH$_2$CH*H*O–), 3.42–3.51 (m, 1 H, CH$_3$CH$_2$CH$_2$C*H*HO– and –CH$_2$C*H*COO–), 4.85 (d, J = 10.5 Hz, 1 H, –C*H*CHCOO–), 5.04–5.05 (m, 1 H, –CHC*H*COO–), 7.36–7.46 (m, 2 H, Ar–H), 7.63 (t, J = 8.8 Hz, 1 H, Ar–H), 7.75 (d, J = 8.0 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.7, 18.8, 30.1, 31.2, 35.4, 38.2, 67.0, 70.1, 117.6, 117.7, 117.8, 123.2, 124.9, 132.7, 149.7, 153.1, 160.3, 170.0, 173.7 ppm. HRMS: calcd. for $C_{19}H_{18}O_6Na$ [M + Na]$^+$ 365.1001; found 365.1007.

11-Butoxy-1*H*-naphtho[1,2-*c*]chromene-1,4,5-trione (3e): Yellow solid, m.p. 136–138 °C. R_f = 0.35 (silica gel, light petroleum/EtOAc, 5:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.05–1.10 (m, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.60–1.67 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 2.03–2.08 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 4.39 (t, J = 5.4 Hz, 2 H, CH$_3$CH$_2$CH$_2$CH_2O–), 6.89 (d, J = 8.5 Hz, 1 H, –C*H*CO–), 7.10 (d, J = 8.6 Hz, 1 H, –C*H*CO–), 7.29–7.41 (m, 2 H, Ar–H), 7.52–7.56 (m, 1 H, Ar–H), 7.82 (s, 1 H, Ar–H), 9.00–9.03 (m, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.8, 19.4, 30.8, 70.2, 111.2, 116.5, 117.2, 124.3, 124.8, 126.7, 128.8, 130.7, 131.6, 134.2, 135.4, 141.2, 151.7, 157.8, 159.4, 183.4 (2 C) ppm. HRMS: calcd. for $C_{21}H_{16}O_5Na$ [M + Na]$^+$ 371.0895; found 371.0901.

5-Butoxy-1*H*-anthra[1,2-*c*]chromene-7,12,13-trione (3f): Yellow solid, m.p. 167–169 °C. R_f = 0.35 (silica gel, light petroleum/EtOAc, 5:1). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.08 (t, J = 7.5 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.61–1.69 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 2.05–2.10 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 4.42 (t, J = 6.5 Hz, 2 H, CH$_3$CH$_2$CH$_2$CH_2O–), 7.30–7.34 (m, 1 H, Ar–H), 7.39–7.42 (m, 1 H, Ar–H), 7.53–7.57 (m, 1 H, Ar–H), 7.75–7.82 (m, 2 H, Ar–H), 8.01 (s, 1 H, Ar–H), 8.23 (d, J = 7.3 Hz, 2 H, Ar–H), 9.04 (d, J = 8.5 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.8, 19.4, 30.9, 70.2, 111.5, 116.6, 117.2, 123.0, 124.2, 126.7, 127.3, 128.8, 131.1, 131.4, 131.5, 132.2, 132.2, 134.7, 135.7, 136.0, 151.92, 158.0, 159.5, 181.8, 182.7 ppm. HRMS: calcd. for $C_{25}H_{18}O_5Na$ [M + Na]$^+$ 421.1052; found 421.1058.

Diisopropyl 1-Butoxy-5-oxo-4a,5-dihydro-2*H*-chromeno[3,4-*c*]pyridazine-3,4-dicarboxylate (3g): Colorless oil. R_f = 0.25 (silica gel, cyclohexane/EtOAc, 7:1). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 1.03 (t, J = 6.8 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.18–1.33 [m, 12 H, –NCOOCH(CH_3)$_2$], 1.36–1.42 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.55–1.66 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 3.77–3.81 (m, 1 H, –C*H*HCOBu), 3.84–3.86 (m, 1 H, –CH*H*COBu), 3.89–3.93 (m, 1 H, CH$_3$CH$_2$CH$_2$C*H*HO–), 4.78 (m, 1 H, CH$_3$CH$_2$CH$_2$C*H*HO–), 4.84–4.99 [m, 2 H, –NCOOC*H*(CH$_3$)$_2$], 5.41 (s, 1 H, –NC*H*COO–), 6.99 (m, 1 H, Ar–H), 7.02–7.21 (m, 2 H, Ar–H), 7.78–7.81 (m, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 13.6, 19.0, 22.2, 22.6, 26.8, 29.6, 31.7, 41.8, 43.4, 68.5, 71.3, 116.7, 120.8, 124.2, 127.7, 128.4, 148.4, 148.6, 163.3 ppm. HRMS: calcd. for $C_{23}H_{30}N_2O_7Na$ [M + Na]$^+$ 469.1945; found 469.1933.

Dimethyl 6-Oxo-6*H*-benzo[*c*]chromene-7,8-dicarboxylate (4a): Colorless solid, m.p. 230–232 °C. R_f = 0.3 (silica gel, cyclohexane/EtOAc, 4:1). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 3.87 (s, 6 H, –COOCH_3), 7.47–7.50 (m, 2 H, Ar–H), 7.63–7.66 (m, 1 H, Ar–H), 8.41–8.45 (m, 2 H, Ar–H), 8.66–8.69 (d, J = 8.8 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 52.5, 52.9, 116.5, 117.2, 117.8, 124.1, 124.6, 125.0, 127.3, 132.4, 135.5, 137.6, 138.8, 151.1, 158.0, 164.1, 167.1 ppm. HRMS: calcd. for $C_{17}H_{12}O_6Na$ [M + Na]$^+$ 335.0526; found 335.0531.

Methyl 6-Oxo-6*H*-benzo[*c*]chromene-8-carboxylate (4b): Colorless solid, m.p. 230–232 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 3.92 (s, 3 H, –COOCH_3), 7.42–7.48 (m, 2 H, Ar–H), 7.61–7.64 (m, 1 H, Ar–H), 8.41 (t, J = 7.8 Hz, 2 H, Ar–H), 8.60 (d, J = 8.5 Hz, 1 H, Ar–H), 8.74 (s, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]-DMSO): δ = 52.5, 117.0, 117.4, 121.0, 123.4, 124.3, 125.0, 129.8, 130.6, 131.9, 134.7, 138.0, 151.2, 159.6, 164.9 ppm. HRMS: calcd. for $C_{15}H_{11}O_4$ [M + H]$^+$ 255.0652; found 255.0661.

8-Acetyl-6*H*-benzo[*c*]chromen-6-one (4c): Colorless solid, m.p. 176–178 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.71 (s, 3 H,

–COCH_3), 7.37–7.43 (m, 2 H, Ar–H), 7.54–7.58 (m, 1 H, Ar–H), 8.13 (d, J = 8.0 Hz, 1 H, Ar–H), 8.23 (d, J = 8.5 Hz, 1 H, Ar–H), 8.44 (dd, J = 1.8, 7.3 Hz, 1 H, Ar–H), 8.94 (d, J = 2.0 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 26.6, 117.2, 118.0, 121.2, 122.3, 123.5, 124.9, 131.3, 131.8, 133.6, 136.9, 138.5, 151.8, 160.6, 196.3 ppm. HRMS: calcd. for $C_{15}H_{11}O_3$ [M + H]$^+$ 239.0703, found 239.0715.

11-Butoxy-2-methyl-11,11a-dihydrochromeno[3,4-*e*]isoindole-1,3,4-(2*H*,3a*H*,3b*H*)-trione (4d): Colorless solid, m.p. 181–183 °C. R_f = 0.3 (silica gel, cyclohexane/EtOAc, 2:1). ^{1}H NMR (250 MHz, [D$_6$]-DMSO): δ = 0.69 (t, J = 7.3 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 0.99–1.08 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.14–1.22 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 2.75–2.83 (m, 1 H, –CHHCHOBu), 2.78 (s, 3 H, –NCH_3), 3.13–3.22 (m, 1 H, –CHCON–), 3.30–3.33 (m, 1 H, CH$_3$CH$_2$CH$_2$CHHO–), 3.37–3.45 (m, 1 H, –CHHCHOBu), 3.51–3.53 (m, 1 H, CH$_3$CH$_2$CH$_2$CHHO–), 4.16 (s, 1 H, –CHCON–), 4.24 (d, J = 7.3 Hz, 1 H, –CHOBu), 7.34–7.43 (m, 2 H, Ar–H), 7.63 (t, J = 8.3 Hz, 1 H, Ar–H), 7.86 (d, J = 7.2 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 13.3, 18.2, 24.0, 27.3, 31.1, 38.0, 43.8, 68.0, 70.6, 116.3, 116.5, 118.8, 124.5, 124.7, 131.8, 146.4, 152.0, 159.0, 174.4, 176.2 ppm. HRMS: calcd. for $C_{20}H_{21}NO_5Na$ [M + Na]$^+$ 378.1312; found 378.1311.

11-Butoxy-2-phenyl-11,11a-dihydrochromeno[3,4-*e*]isoindole-1,3,4-(2*H*,3a*H*,10*H*)-trione (4e): Colorless solid, m.p. 162–164 °C. R_f = 0.35 (silica gel, cyclohexane/EtOAc, 2:1). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 0.65 (t, J = 7.3 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$-O–), 0.97–1.12 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.22–1.33 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 2.85 (d, J = 17.5 Hz, 1 H, –CHHCHOBu), 3.25–3.35 (m, 1 H, –CHCON–), 3.42–3.49 (m, 2 H, CH$_3$CH$_2$CH$_2$CH_2O–), 3.60 (d, J = 17.5 Hz, 1 H, –CHHCHOBu), 4.30–4.32 (m, 1 H, –CHOBu), 4.51 (d, J = 15.5 Hz, 1 H, –CHCON–), 7.23 (d, J = 7.0 Hz, 2 H, Ar–H), 7.37–7.53 (m, 5 H, Ar–H), 7.58–7.64 (m, 1 H, Ar–H), 7.91 (d, J = 8.0 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 13.3, 18.3, 26.9, 31.2, 38.3, 43.9, 67.8, 70.7, 116.4, 118.7, 124.5, 124.7, 126.6 (2 C), 128.0, 128.1, 128.8 (2 C), 131.8, 132.5, 146.5, 152.0, 159.0, 173.4, 175.3 ppm. HRMS: calcd. for $C_{25}H_{23}NO_5Na$ [M + Na]$^+$ 440.1468; found 440.1454.

11-Butoxy-11,11a-dihydro-1*H*-isobenzofuro[4,5-*c*]chromene-1,3,4(3a*H*,3b*H*)-trione (4f): Colorless solid, m.p. 191–193 °C. R_f = 0.3 (silica gel, cyclohexane/EtOAc, 3:1). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 0.69 (t, J = 7.4 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$-O–), 1.05–1.11 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.20–1.28 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 2.83 (d, J = 18.7 Hz, 1 H, CH, –CHHCHOBu), 3.45 (s, 1 H, –CHCOO–), 3.47–3.50 (m, 1 H, CH$_3$CH$_2$CH$_2$CHHO–), 3.54–3.59 (m, 1 H, –CHHCHOBu), 3.61–3.63 (m, 1 H, CH$_3$CH$_2$CH$_2$CHHO–), 4.29 (s, 1 H, –CHCOO–), 4.71 (d, J = 8.7 Hz, 1 H, –CHOBu), 7.38–7.47 (m, 2 H, Ar–H), 7.66 (t, J = 7.2 Hz, 1 H, Ar–H), 7.89 (d, J = 8.1 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 13.3, 18.2, 26.9, 30.9, 38.0, 44.8, 68.0, 69.8, 114.5, 116.5, 118.5, 124.6, 124.9, 132.1, 146.9, 152.0, 158.7, 168.9, 171.3 ppm. HRMS: calcd. for $C_{19}H_{18}O_6Na$ [M + Na]$^+$ 365.0996; found 365.1014.

1*H*-Naphtho[1,2-*c*]chromene-1,4,5-trione (4g): Yellow solid, m.p. 131–133 °C. R_f = 0.2 (silica gel, cyclohexane/EtOAc, 7:3). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 7.05 (d, J = 10.4 Hz, 1 H, –CHCO–), 7.28 (d, J = 10.4 Hz, 1 H, –CHCO–), 7.45–7.47 (m, 2 H, Ar–H), 7.63 (m, 1 H, Ar–H), 8.34 (d, J = 8.5 Hz, 1 H, Ar–H), 8.42 (d, J = 8.5 Hz, 1 H, Ar–H), 8.79 (d, J = 8.7 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 115.6, 116.5, 117.0, 118.4, 124.6, 124.8, 126.7, 130.5, 132.5, 133.2, 136.4, 136.5, 140.3, 151.6, 156.5, 182.8, 184.3 ppm. HRMS: calcd. for $C_{17}H_8O_4Na$ [M + Na]$^+$ 299.0315; found 299.0308.

7*H*-Anthra[1,2-*c*]chromene-7,12,13-trione (4h): Yellow solid, m.p. 167–169 °C. R_f = 0.2 (silica gel, cyclohexane/EtOAc, 7:3). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 7.43–7.51 (m, 2 H, Ar–H), 7.65–7.71 (m, 1 H, Ar–H), 7.91–7.96 (m, 2 H, Ar–H), 8.06–8.09 (m, 1 H, Ar–H), 8.14–8.18 (m, 1 H, Ar–H), 8.45 (d, J = 8.3 Hz, 1 H, Ar–H), 8.53 (d, J = 8.5 Hz, 1 H, Ar–H), 8.84 (d, J = 8.8 Hz, 1 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 116.6, 117.0, 119.0, 124.6, 124.8, 126.3, 126.4, 127.0, 131.2, 132.0, 132.6, 134.0, 134.7, 134.8, 135.3, 138.7, 140.6, 151.7, 156.7, 180.8, 183.1 ppm. HRMS: calcd. for $C_{21}H_{10}O_4Na$ [M + Na]$^+$ 349.0471; found 349.0483.

Diisopropyl 2-Butoxy-5-oxo-4a,5-dihydro-2*H*-chromeno[3,4-*c*]pyridazine-3,4-dicarboxylate (4i): Colorless oil. R_f = 0.3 (silica gel, cyclohexane/EtOAc, 4:1). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 0.92 (t, J = 7.3 Hz, 3 H, CH_3CH$_2$CH$_2$CH$_2$O–), 1.21–1.26 (m, 2 H, CH$_3$CH_2CH$_2$CH$_2$O–), 1.30–1.34 [m, 12 H, –NCOOCH(CH_3)$_2$], 1.48–1.52 (m, 2 H, CH$_3$CH$_2$CH_2CH$_2$O–), 2.94 (d, J = 17.5 Hz, 1 H, –CHHCHOBu), 3.32 (dd, J = 6.5, 17.5 Hz, 1 H, –CHHCHOBu), 3.52–3.61 (m, 1 H, CH$_3$CH$_2$CH$_2$CHHO–), 3.82–3.91 (m, 1 H, CH$_3$CH$_2$CH$_2$CHHO–), 4.91–5.06 [m, 2 H, –NCOOCH(CH$_3$)$_2$], 5.85 (d, J = 6.5 Hz, 1 H, –CHOBu), 7.29–7.35 (m, 2 H, Ar–H), 7.44–7.50 (m, 2 H, Ar–H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 14.1, 18.9, 22.6 (4 C), 31.1, 31.9, 71.1 (2 C), 72.4, 81.4, 116.8, 117.0, 118.4, 123.1, 124.4, 125.1, 125.6, 130.9, 132.7, 151.6, 153.7 ppm. HRMS: calcd. for $C_{23}H_{30}N_2O_7Na$ [M + Na]$^+$ 469.1945; found 469.1932.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR and ^{13}C NMR spectra copies for all compounds and NOESY, COSY and HSQC experiments for compounds **3b** and **4d** are provided.

Acknowledgments

The research leading to these results has received funding from the European Community (Seventh Framework Programme FP7/2007-2013) under grant agreement number 215009. Thanks are also due to the University of Aveiro, Fundação para a Ciência e a Tecnologia (FCT) and the Fundo Europeu de Desenvolvimento Regional (FEDER) for funding the Organic Chemistry Research Unit (project PEst-C/QUI/UI0062/2011) and Portuguese National NMR Network (RNRMN). FEDER is also acknowledged for financing the purchase of a Flash 2000 Thermoscientific CHNS-O microanalyzer.

[1] I. Kostova, *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 29–46.
[2] a) I. Kostova, *Mini-Rev. Med. Chem.* **2006**, *6*, 365–74; b) A. Beillerot, J. C. Domínguez, G. Kirsch, D. Bagrel, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1102–1105.
[3] L. Wu, X. Wang, W. Xu, F. Farzaneh, R. Xu, *Curr. Med. Chem.* **2009**, *16*, 4236–60.
[4] P. Curir, F. Galeotti, M. Dolci, E. Barile, V. Lanzotti, *J. Nat. Prod.* **2007**, *70*, 1668–71.
[5] a) L. Pisani, G. Muncipinto, T. F. Miscioscia, O. Nicolotti, F. Leonetti, M. Catto, C. Caccia, P. Salvati, R. Soto-Otero, E. Mendez-Alvarez, C. Passeleu, A. Carotti, *J. Med. Chem.* **2009**, *52*, 6685–706; b) M. J. Matos, D. Viña, C. Picciau, F. Orallo, L. Santana, E. Uriarte, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5053–5055.
[6] L. Piazzi, A. Cavalli, F. Colizzi, F. Belluti, M. Bartolini, F. Mancini, M. Recanatini, V. Andrisano, A. Rampa, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 423–426.

[7] G. Appendino, L. Maxia, M. Bascope, P. J. Houghton, G. Sanchez-Duffhues, E. Munoz, O. Sterner, *J. Nat. Prod.* **2006**, *69*, 1101–1104.
[8] A. Donnelly, B. S. Blagg, *Curr. Med. Chem.* **2008**, *15*, 2702–17.
[9] T. Yamaguchi, T. Fukuda, F. Ishibashi, M. Iwao, *Tetrahedron Lett.* **2006**, *47*, 3755–57, and references cited therein.
[10] S. Valente, E. Bana, E. Viry, D. Bagrel, G. Kirsch, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5827–30.
[11] T. Yamazaki, T. Tokiwa, *Biol. Pharm. Bull.* **2010**, *33*, 1716–22.
[12] S. Starcević, P. Brozic, S. Turk, J. Cesar, T. L. Rizner, S. Gobec, *J. Med. Chem.* **2011**, *54*, 248–261.
[13] A. Behrenswerth, N. Volz, J. Toräng, S. Hinz, S. Bräse, C. E. Müller, *Bioorg. Med. Chem.* **2009**, *17*, 2842–51.
[14] C. H. Blomquist, P. H. Lima, J. R. Hotchkiss, *Steroids* **2005**, *70*, 507–14.
[15] H. Peng, W. Xie, D. M. Otterness, J. P. Cogswell, R. T. McConnell, H. L. Carter, G. Powis, R. T. Abraham, L. H. Zalkow, *J. Med. Chem.* **2001**, *44*, 834–34.
[16] K. C. Nicolaou, S. A. Snyder, T. Montagnon, G. Vassilikogiannakis, *Angew. Chem.* **2002**, *114*, 1742–1773; *Angew. Chem. Int. Ed.* **2002**, *41*, 1668–98.
[17] O. Malika Ibrahim, *Steroids* **2009**, *74*, 133–62.
[18] S. Valente, G. Kirsch, *Tetrahedron Lett.* **2011**, *52*, 3429–3432.
[19] L. Brault, D. Bagrel, *Life Sci.* **2008**, *82*, 315–23.
[20] L. Brault, M. Denance, E. Banaszak, S. El Maadidi, E. Battaglia, D. Bagrel, M. Samadi, *Eur. J. Med. Chem.* **2007**, *42*, 243–247.
[21] The enzymatic activity of the GST-Cdc25 recombinant enzyme was determined in 96-well plates in Tris-HCl (50 mM), NaCl (50 mM), ethylenediaminetetraacetic acid (1 mM) and sodium acetate buffer (SAB) (0.1%), pH 8.1 buffer containing 3-*O*-methylfluorescein phosphate (500 μM) as substrate. The Gst-Cdc25 proteins, diluted in assay buffer, were used at a final concentration of 1 μg/well. After 2 h at 30 °C, 3-*O*-methylfluorescein fluorescent emission was measured with a CytoFluor system PerSeptive Applied Biosystems; excitation filter: 475 nm and emission filter: 510 nm.
[22] A. Maul, *Environ. Monit. Assess.* **1992**, *23*, 153.

Received: December 23, 2012
Published Online: March 27, 2013

