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Graphical abstract

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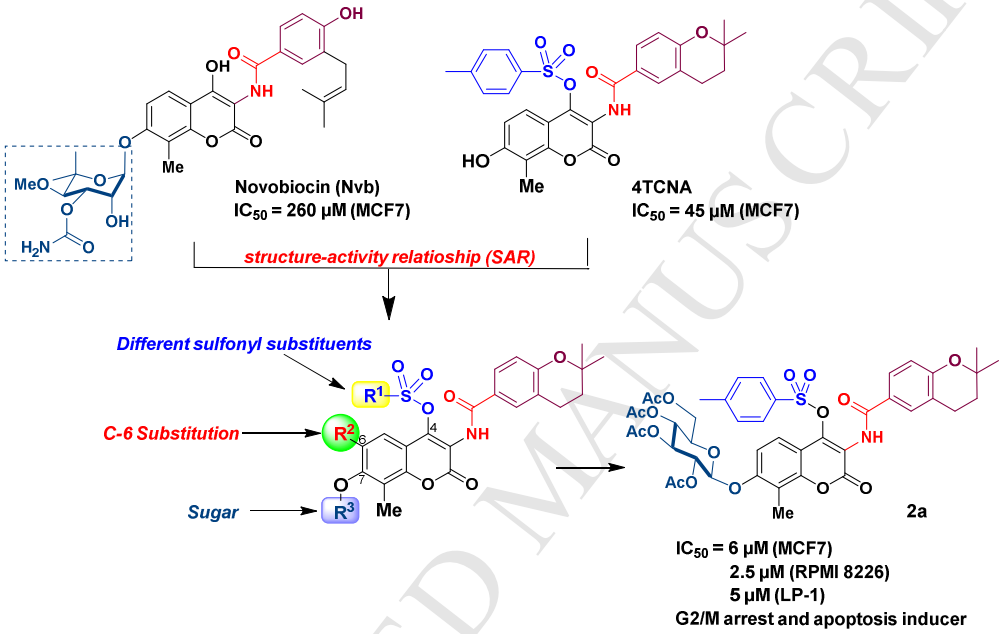

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Dedicated to the memory of Dr. Christine Radanyi

Abstract

A series of *substituted coumarins* **1-10** was designed and synthesized as a novel class of 4TCNA analogues. Compound **2a** showed excellent antiproliferative activity with mean GI_{50} values at a micromolar level in a diverse set of human cancer cells (GI_{50} = 2-30 µM) and induced a high apoptosis level in MCF-7 breast cancer cell line. The molecular signature of hsp90 inhibition was assessed by depletion of the Erα hsp90 client protein.

Keywords: Hsp90, 4TCNA, 6BrCaQ, cytotoxicity, apoptosis.

1. Introduction

Hsp90 is an emerging therapeutic target of interest for the treatment of cancer[1,2,3,4,5] and other diseases.[6, 7] As a chaperone protein, hsp90 is responsible for the conformational maturation of more than 250 hsp90-dependent client proteins which are directly associated with all hallmarks of cancer.[8, 9, 10, 11] The ATP binding site at the N-terminal domain of hsp90 has been extensively studied (especially following its cocrystallisation in the presence of geldanamycin) and the protein function may be inhibited by molecules competing with ATP binding, resulting in destabilization of the hsp90 client-protein heteroprotein complex, which leads to proteasome-mediated oncogenic client protein degradation.[12] Several structurally distinct hsp90 inhibitors are currently being evaluated for anticancer activity in numerous Phase II and several Phase III clinical trials, however, detriments such as heat shock induction and cytostatic activity associated with N-terminal inhibition has limited their potential use against cancer.[8]

The C-terminal domain of hsp90 has been implicated biochemically as the site of a possible second, cryptic ATP-binding site on the protein. Its contribution to the overall regulation of chaperone function is not clear, but some small molecules that bind to the C-terminal domain such as the antibiotic novobiocin[13] (Nvb, Figure 1), do not induce the pro-survival heat shock response, and in some cases, even cause hsp70 and hsp90 degradation.[14, 15] Novobiocin exhibit its antitumor activity against human breast SKBr3 cancer cell lines (~700 μM) through hsp90 inhibition. [16] It binds with poor affinity, and induces hsp90 client proteins degradation in a concentration-dependent manner. Although no cocrystal structure of hsp90 bound to C-terminal inhibitors has been reported, the structure and function of the hsp90 C-terminal binding site are under intense investigation. A better understanding of the role of this putative hsp90 C-domain site in regulating the function of the chaperone, as well as its potential as an anticancer drug target, requires further investigations. The identification of more potent site-specific inhibitors is needed and has led the development of specific C-terminal hsp90 inhibitors as potential anticancer drug modalities.

In this context, we reported a novel series of simplified 3-aminocoumarin analogues related to Nvb as a class of highly potent hsp90 inhibitors. We demonstrated that removal of the noviose moiety together with introduction of a tosyl substituent at C-4 coumarin nucleus lead to 4TCNA (Figure 1)[17, 18, 19, 20, 21] as a lead compound. In the pursuit of the development of more potent novobiocin analogues, we recently reported derivatives that contain a quinolein-2-one moiety in lieu of the coumarin unit. From these structure-relationship activity (SAR) studies, 6BrCaQ (Figure 1) [22, 23, 24] was found to be a potent derivative displaying the following IC_{50} values: 7 µM against MCF7 cells, 2 µM against MDA MB231 cells, 8 µM against Caco2 cells, 5 µM against IGROV-1 cells and 2 µM against ISHIKAWA cells. Further studies on its mode of action revealed that 6BrCaQ manifests downregulation of several hsp90 client proteins (HER2, Raf-1 and cdk-4), induces a high apoptosis level in MCF-7 breast cancer cell line (activation of caspases 7, 8 and 9) and promotes the subsequent cleavage of poly(ADP-ribose) polymerase (PARP) suggesting stimulation of apoptosis through both extrinsic and intrinsic pathways. In addition to these outstanding pro-apototic properties, 6BrCaQ was found to mediate cell death in a p23-dependant process and through a number of pathways in wich autophagy has a prominent place.[22]

Because of this novel and exciting activity, it was proposed that complementary 4TCNA analogues, built upon an optimized novoboicin scaffold could lead to more efficacious compounds. Herein, we planned to prepare a new series of analogues (Figure 1), in which various chemical modifications at the C-4, C-6 and C-7 positions of 4TCNA were performed in the aim to better understand the SAR in this novel series. In this article the synthesis and the biological

evaluation of novel 4TCNA analogues **1-10** are described. Preliminary *in vitro* efficacy of these compounds in terms of cytotoxicity, apoptosis and hsp90 client proteins degradation is reported.

Figure 1. Structures of novobiocin, synthetic derivatives, 4TCNA, 6BrCaQ and targeted molecules.

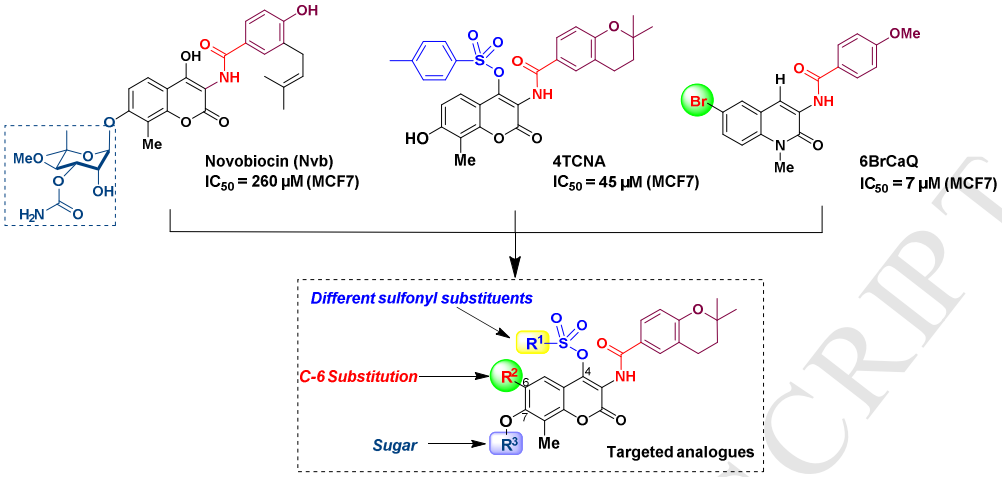


Figure 1.

2. Results and discussion

2.1. Chemistry.

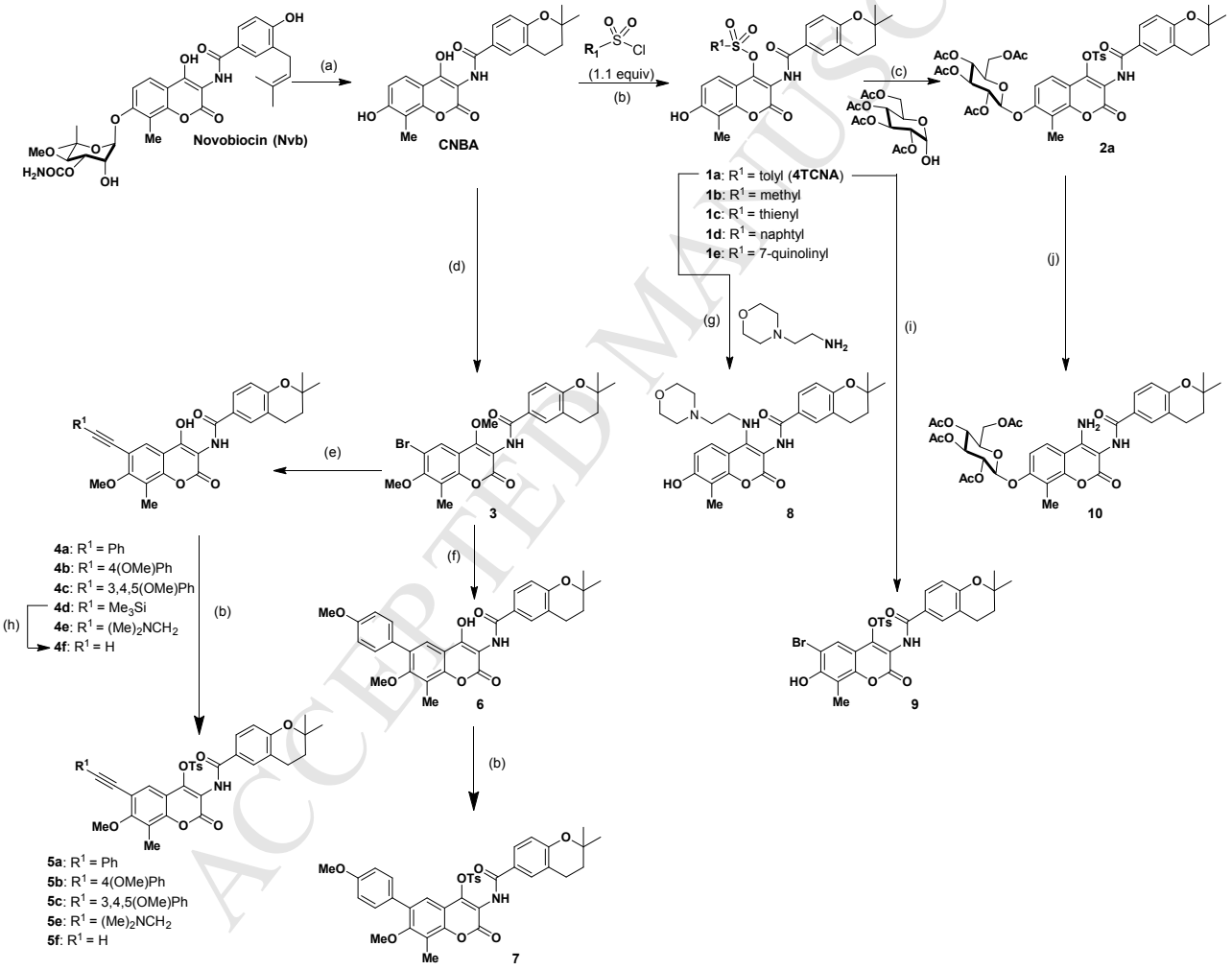
The synthesis of the target coumarin structures **1-10** is summarized in Scheme 1. Cyclonovobiocic acid (CNBA) was prepared as it was previously reported [17] by acidic hydrolysis of commercially available monosodium novobiocin salt. To extend upon our knowledge of 4TCNA and its SAR, several (hetero)aryl and alkyl sulfonyl moieties were appended to the 4-position of the coumarin ring of CNBA. Chemistry developed in our lab was used to attach selectively the desired sulfonyl substituents at C-4 postion rather than C-7 one.[17] Thus, treatment of CNBA with pyridine followed by substituted sulfonylchloride derivatives gave the best results for the C-4 sulfonylation process. Accordingly, the desired C-4-sulfonylated analogues **1a-e** were selectively obtained in acceptable yields (~50%).

In another strategy, we wished to introduce a carbohydrate group at the 7-position of 4TCNA to further increase both solubility and polarity and to explore hydrogen bonding interactions. Numerous biologically active natural and non-natural products contain carbohydrates appended to their scaffolds that serve to increase solubility and provide interactions with their cognate receptor.[25, 26, 27, 28, 29] To this end, *O*-glycosylated 4TCNA **2a** derivative was prepared as described in scheme 1, by coupling the readily available α-glycopyranoside with the remaining phenol function of 4TCNA under Mitsunobu's conditions (DIAD, PPh_3 in THF).[30, 31, 32, 33, 34] The tosyl group of **2a** was then substituted by a free amine function by the use of ammonia in THF at RT affording the β-glycosylated derivative **10**.

For direct comparison to 6BrCaQ[22] in which the bromine atom at C-6 position play an important role for its activity, brominated 4TCNA derivative **9** was synthesized through a regioselective bromination by using bromine in AcOH at RT. The 6-bromo 4,7-dimethoxylated CNBA derivative **3** was also prepared in a good overall yield by selective bromination of the C-6 position using MPHT as the brominating agent[35, 36] (92% yield) followed by alkylation of both free C-4 and C-7 hydoxyl groups of CNBA by using an excess of dimethyl sulfate and K_2CO_3 (62% yield). The C-

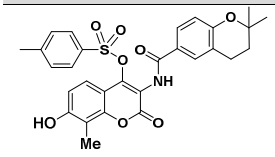
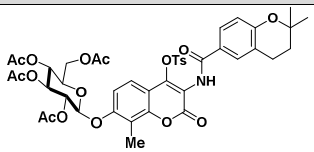
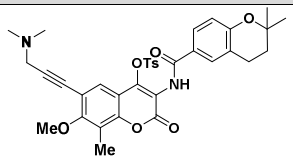
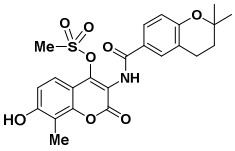
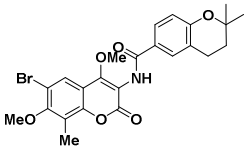
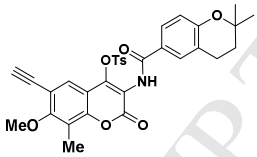
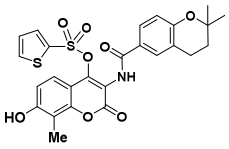
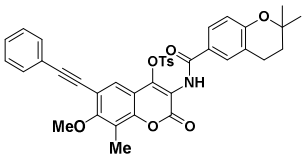
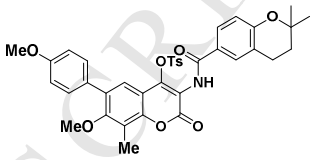
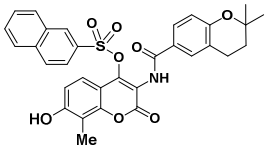
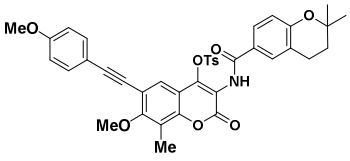
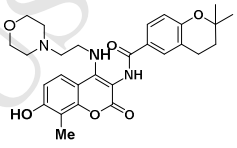
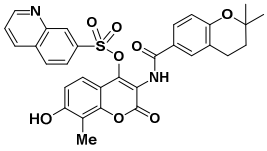
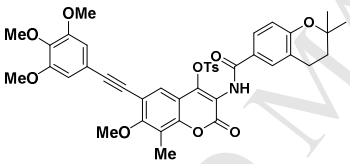
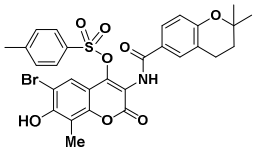
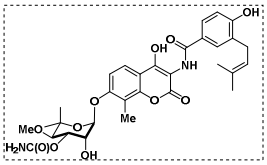
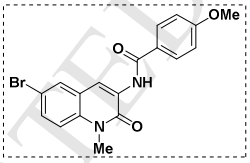
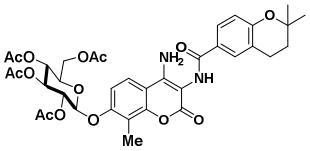
Br bond in **3** was then used for further functionalizations. In order to evaluate the importance of the C-6 position substitution in 4TCNA, we planned to introduce an alkynyl and aryl substituent by metal-catalyzed cross coupling reaction. For this purpose, treatment of compound **3** under Sonogashira conditions (CuI, $PdCl_2(PPh)_2$, PPh_3 in DMF/Et_2NH) allowed the introduction of various alkynes at the C-6 position of **3** together with a concomitant deprotection of the methoxy group at the C-4 position. The compound **6** bearing a 4-methoxyphenyl group at C-6 position was prepared in a 86% yield by using the same strategy using a Suzuki cross coupling reaction between **3** and 4-methoxyphenylboronic acid. Subsequent tosylation of the free hydroxyl group of **4a-e** and **6** under our previously reported protocol led to a series of derivatives **5a-f** and **7** in 55-84% yields.[17, 22] Finally, the aminoethylmorpholine group which is present in a plethora of biologically active compounds[37, 38, 39, 40, 41] was introduced in 4TCNA by nucleophilic displacement of the tosyl group by aminoethylmorpholine in dioxane providing compound **8** in a 71% yield.

Scheme 1. Synthesis of compounds **1-10**.



[a]Reagents and conditions: (a) HCl (12 M), EtOH, reflux, 1 h (b) Pyridine, 0° C to RT, 2.5 h. (c), DIAD (2 equiv), PPh_3 (2 equiv), THF, -78 °C to RT, 24 h (d) (*i*) MPHT (2 equiv), MeCN, 80 °C, 16 h (*ii*) K_2CO_3 (6 equiv), Me_2SO_4 (6 equiv), DMF, RT, 12 h, (e) $PdCl_2(PPh_3)_2$ (6 mol%), CuI (6 mol%), alkyne (1 equiv), PPh_3 (20 mol%), DMF/Et_2NH (1:3), 120 °C, 30 min; (f) (*i*) $Pd(OAc)_2$ (5 mol%), Xphos (20 mol%), KF (3 equiv), THF, reflux, 24 h (*ii*) DMF/Et_2NH (1:3), 120 °C, 30 min. (g) Dioxane, sealed tube, 100 °C. (h) K_2CO_3 (2 equiv), MeOH, RT, 2 h. (i) Br_2, AcOH, 24 h, RT. (j) NH_3 (gas), THF, RT, 24 h.

Table 1. Cytotoxic effect of **1**, **3**, **5** and **7-10** derivatives against MCF-7 cell line

Compounds	Viab. [%][a]	Compounds	Viab. [%][a]	Compounds	Viab. [%][a]
 **1a (4TCNA)**	**36**	 **2a**	**10**	 **5e**	**96**
 **1b**	**61**	 **3**	**90**	 **5f**	**82**
 **1c**	**62**	 **5a**	**90**	 **7**	**89**
 **1d**	**33**	 **5b**	**89**	 **8**	**87**
 **1e**	**22**	 **5c**	**92**	 **9**	**65**
 **Novobiocin**	**ND**	 **6BrCaQ**	**10**	 **10**	**71**

[a] Value of the anti-proliferative effect (% of viable cells compared to untreated cells 100%) of analogues **1**, **3**, **5** and **7-10** in MCF-7 cell line at a concentration of 10^{-4} M (MTT method). ND: Not determined

2.2. Biological results.

Upon completion of their syntheses, the *in vitro* activity of coumarin derivatives **1, 3, 5** and **7-10** was evaluated by their growth-inhibitory potency in MCF-7 cell line at concentration of 10^{-4} M. The quantification of cell survival in this cell line was established by using MTT assays after 72 h exposure (Table 1), and GI_{50} values were determined at the concentration required to produce 50% inhibition (Table 1).

As shown in Table 1, compounds **1b** and **1c** bearing a mesityl and thienylsulfonyl groups, respectively, at C-4 position of the coumarin moiety displayed slight effect in the growth of MCF-7 cells (61% and 62% survival, respectively), while compounds **1d** and **1e** with a naphtylsulfonyl and 7-quinoleinesulfonyl, respectively, were able to decrease the cell viability in MCF-7 cells until 22-33% compared to the reference compound 4TCNA (36% survival) indicating a possible π-staking interactions of the bulky aromatic substituents at the C-4 position with the binding

pocket. Another important observation is that β–glycosylated-4TCNA (**2a**) induced a significant decrease of the cell viability in MCF-7 cells (10% survival) while analogue **10** affects slightly the growth of MCF-7 cells (71% survival). These results clearly suggest that the presence of the β–(OAc)-glucose at the C-7 position of the coumarin nucleus and a tosyl group at the C-4 position are very important to affect cell viability (Table 1). A comparison of 6BrCaQ with 6-bromocoumarin derivative **9** (6Br-4TCNA) revealed that introduction of bromine atom at C-6 position of the coumarin nucleus induced a significant increase of the cell viability in MCF-7 cells (65% survival, respectively) vs 33% survival for 4TCNA and 10% survival for 6BrCaQ. In addition, substitution of the C-6 position by alkynyl- or aryl substituents did not produce compounds that inhibit cell growth more effectively (see compounds **5a-f** and **7** , survival > 80%) than the reference compound 4TCNA (**1a**, 33%) suggesting that functionalization of the position 6 of 4TCNA with and aryl or alkynyl appendage significantly alters the mode of binding.

Then, the growth inhibitory activities against MCF-7 breast cancer cell line were measured for the selected coumarins derivatives (**1a**, **1d-e** and **2a**). All the compounds shown in Table 2 behave as stronger cell growth inhibitors in regard to Nvb. Compounds **1d** and **1e** having a naphtylsulfonyl and quinoleinsulfonyl groups at the C-4 position exhibited approximatively a same growth inhibition activity than that of 4TCNA, which itself is ~4x more active than Nvb. Interestingly, **2a** having a *O*-glycoside group at the C-7 position of the coumarin nucleus showed a significant ability to inhibit cell growth and is ~40-fold more potent than the parent compound Nvb, 4-fold more active than 4TCNA (**1a**) and displays the same activity than that of our best lead compound 6BrCaQ (GI_{50} = 7 µM). This result suggests that activity increased as hydrogen bond donor/acceptor, polarity and solubility properties of the inhibitor increased.

Table 2. GI_{50} (µM) values for anti-proliferative effects of selected compounds **1a**, **1d-e** and **2a**[a] against breast cancer cell lines (MCF-7)[b]

Compound	MCF-7 (µM)
1a (**4TCNA**)	50
1d	67
1e	64
2a	6
6BrCaQ	7
Novobiocin (Nvb)	260

[a] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). Values are means of three experiments, carried out in sextuplate.[b] MCF-7, human breast cancer cells.

To further characterize the cytotoxicity profiles of these compounds, we next investigated the effect of the most active compound **2a** on the proliferation of six tumor cell lines hormone-independent breast cancer (MDA-MB-231), human breast cancer cells resistant to Tamoxifen (MCF7 Tamoxifen^R), human breast cancer cells resistant to Fulvestrant (MCF-7 Faslodex^R), human myeloma (plasmatocytoma) cells (RPMI 8226 and LP-1) and prostate cancer cells (PC-3). The results of this study, summarized in Table 3, revealed that compound **2a**, as 6BrCaQ, strongly inhibited the growth of all examined tumor cell lines with GI_{50} ranging from 2 to 30 µM, and this effect did not depend on the cell type. The RPMI 8226 and LP-1 cancer cell lines were significantly more sensitive (GI_{50} ~ 2-5 µM), while MCF-7 Tamoxifen^R, MCF7 Faslodex^R, and PC-3 cancer cell line were less responsive (GI_{50} ~ 25-30 µM) to growth inhibition by **2a**. Consequently, *O*-glycosylation at 7-position of the coumarin core appears to be a suitable moiety in this series for antiproliferative activities.

Table 3. Cytotoxicity (GI_{50} µM, 72 h)[a]

Compounds	MCF-7	MDA-MB-231[c]	MCF-7 Tamoxifen^R	MCF-7 Faslodex^R	RPMI 8226	LP-1	PC-3
2a	**6**	**10**	**30**	**25**	**2.5**	**5**	**25**
6BrCaQ	**7**	**2**	**ND**	**ND**	**5**	**6.2**	**10**

[a] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). Values are means of three experiments, carried out in triplicate or sextuplate. [b] MCF-7: human breast cancer cells. MCF7 Tamoxifen^R human breast cancer cells resistant to Tamoxifen. MCF7 Faslodex^R: human breast cancer cells resistant to Foslodex. MDA-MB231: hormone-independent breast cancer. RPMI 8226 and LP-1 : human myeloma cells. PC-3: human prostate cancer cells. ND. not determined

To gain further insight in the mechanism of action, the most cytotoxic compound **2a** was assayed for its effect on cell cycle distribution. The results presented in Table 4, demonstrate that highly increased accumulation of MCF-7 cells in the G_2/M phase of the cellular cycle was observed after treatment with **2a** (55% as compared to 7.0% in the control). Prolonged incubation with compound **2a** (72 h) increases G_2/M phase up to 60%. SubG1 phase, which represents apoptotic or necrotic cells, was found to be also increased until 27% upon **2a** exposure for 72 h treatment than in the control (9.5%). All these data reveal **2a** to be a more potent inducer of G2/M arrest than other 4TCNA derivatives in MCF-7 cells while 6BrCaQ remains the strongest inducer of apoptosis.

Table 4. Cell cycle and apoptosis analysis of MCF-7 cells treated with analogue **4TCNA**, **6BrCaQ** and **2a** (100 µM) for 48 h and 72 h.[a]

compound	Sub G1		G0/G1 (%)		S (%)		G2/M (%)	
	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
DMSO	5	9.5	87	60.5	6	20.5	7	19.0
4TCNA	6	36.0	44.0	37.0	30.5	35.5	25.5	27.5
6BrCaQ	29.5	47.0	34.81	40.43	17.14	18.09	48.0	41.5
2a	13	27	40	30.0	5.5	9.5	55	60.0

[a] Data represent percentage of cells in Sub G1 and G2/M phases of the cell cycle. The results are the mean of two independent experiments in which no more than 2.5 variations were measured.

In order to confirm that the introduction of a glucose moiety at 7-postion of 4TCNA did not alter inhibitory activity against hsp90, MCF-7 cells were exposed to various compounds (24 h at 100 µM) in the presence or not of the proteasome inhibitor MG132, then western blot analyses of cell lysates. Given that inhibition of hsp90 leads to a proteasome–mediated degradation of client proteins, we wondered at first, if the selected compounds could affect the stability of the transcription factor ER which is an hsp90-client protein. As shown in Figure 2A, **2a** is able to induce a proteasome-mediated loss of ERα protein in the same manner than 4TCNA (Figure 2B), suggesting inhibition of hsp90 and disruption of heteroprotein complexes. The activity of these two compounds was inhibited by MG-132. One can note that analogues **1d** and **1e** which displayed GI_{50} values of 67 to 64 µM, respectively, (Table 2) induced also a proteasome-mediated ERα degradation (Figure 2C).

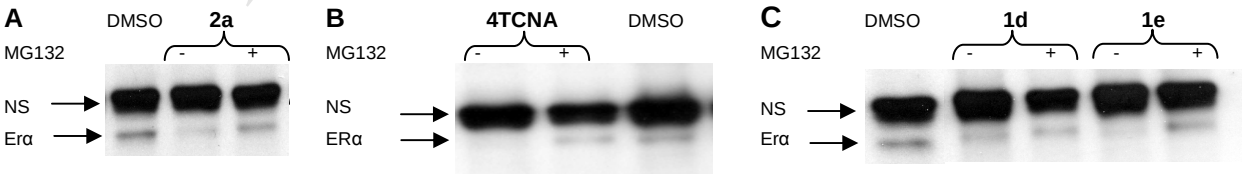


Figure 2 Effects of quinolone analogues **1d**, **1e**, **2a** and **4TCNA** on ERα stability. MCF-7 cells were grown and exposed to hsp90 inhibitors (**1d**, **1e**, **2a** and **4TCNA**, 100 µM) as described in Experimental Section in the presence (+) or absence (−) of the proteasome inhibitor MG132 (5 µM) for 24 h and cell lysates were analyzed by Western blotting with regard to the levels of ERα. DMSO was used as a control, NS = nonspecific protein band detected in these conditions and serving as a control of constant protein loading.

Finally, to provide evidence that the growth inhibitory activity manifested by **2a** resulted from hsp90 inhibition, **2a** was evaluated by its ability to induce degradation of other hsp90-dependent client proteins such as Her2, Raf and cdk4. Unfortunately, **2a** proved to be inactive in regard to proteasome-mediated degradation of these selected hsp90-dependent client proteins. These data suggest that this analogue may selectively affect different other hsp90/client protein clusters/complexes than 4TCNA and 6BrCaQ as suggested for Celastrol, which preferentially affect hsp90-cdc37 interaction.[42, 43, 44]

3. Conclusions

We designed and synthesized a series of substituted 4TCNA derivatives **1-10**. From these SAR studies, 7-*O*-glycosylated 4TCNA (**2a**) was found to display the most stronger antiproliferative activity against a panel of cancer cell lines and induces a high apoptosis level in MCF-7 breast cancer cell line. Morever, by contrast to other hsp90 client proteins (Her2, Raf and cdk4), **2a** manifests downregulation of the hsp90 client protein ERα (the key target responsible for hormone-dependent breast cancer tumor growth).[45] Taken in concert, these data suggest that compounds from this family of hsp90 inhibitors may target different cluster of Hsp90 client proteins, potentially being targeted not only in case of hormone-independent and/or –resistant breast cancers but also in other cancers such as multiple myeloma.

4. Experimental

4.1. Chemistry.

4.1.a. General considerations

The compounds were all identified by usual physical methods, i.e. ^{1}H-NMR, ^{13}C-NMR, IR, MS. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ with a Bruker Avance 300 or Bruker Avance 400. ^{1}H chemical shifts are reported in ppm from an internal standard TMS or of residual chloroform (7.27 ppm). The following abbreviations are used: m (multiplet), s (singlet), d (doublet), br s (broad singlet), t (triplet), dd (doublet of doublet), td (triplet of doublet). ^{13}C chemical shifts are reported in ppm from the central peak of $CDCl_3$ (77.14). IR spectra were measured on a Bruker Vector 22 spectrophotometer (neat, cm^{-1}). Elemental analyses were performed with a Perkin-Elmer 240 analyser. Mass spectra were obtained with a LCT Micromass spectrometer. Analytical TLC was performed on Merck precoated silica gel 60F plates. Merck silica gel 60 (230-400 mesh) was used for column chromatography. Visualization was achieved with UV light and phosphomolybdic acid reagent unless otherwise stated. Monosodium novobiocin salt was purchased from Sigma-Aldrich; all other reagents were of high grade and were used without further purification. Analogues CNBA and 4TCNA were prepared according to our previously reported procedure.[17]

4.1.b. General procedure for the selective C-4 sulfonylation of CNBA.

To a 50 mL ice-cooled round bottom flask charged with CNBA (200 mg, 0.51 mmol, 1 eq.) and pyridine (10 mL), was added slowly a solution of the corresponding sulfonyl chloride (2.78 mmol, 1.1 eq) in pyridine (5 mL). After 2.5 hrs at 0 °C, the mixture was diluted in AcOEt (25 mL) and the organic phase washed with a 1N HCl solution (6 x 10mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Further purification on silica gel chromatography (*c*-hexane/acétate d'éthyle : 6/4) delivered the corresponding C4-sulfonylated product.

*4.1.b.1. 3-(2,2-dimethylchroman-6-carboxamido)-7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl methanesulfonate (**1b**)*

After purification, **1b** was obtained in 41% yield (98 mg, 0.21 mmol). R_f: 0.14 (*c*-hexane/AcOEt: 6/4). m.p.: 189-191 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 10.82 (s, 1H), 9.76 (s, 1H), 7.79 (s, 1H), 7.73 (d, 1H, *J* = 8.4 Hz), 7.51 (d, 1H, *J* = 8.8 Hz), 7.01 (d, 1H, *J* = 8.8 Hz), 6.80 (d, 1H, *J* = 8.4 Hz), 3.53 (s, 3H), 2.80 (t, 2H, *J* = 6.4 Hz), 2.21 (s, 3H), 1.81 (t, 2H, *J* = 6.4 Hz), 1.31 (s, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6, δ): 165.3, 159.8, 159.7, 156.9, 152.5, 150.7, 129.9, 127.2, 124.1, 122.1, 120.6, 116.5, 112.8, 112.7, 111.0, 108.1, 75.1, 39.9, 31.7, 26.5 (2C), 21.6, 7.9. MS (ESI$^+$) *m/z*: 473.9 $[M+H]^+$, 495.9 $[M+Na]^+$. IR (cm^{-1}): 3286, 2931, 1698, 1667, 1636, 1607, 1588, 1489, 1360, 1319, 1255, 1177, 1157, 1138, 1118, 1093, 1022, 947, 822, 665.

*4.1.b.2. 3-(2,2-dimethylchroman-6-carboxamido)-7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl thiophene-2-sulfonat (**1c**)*

After purification, **1c** was obtained in 48% yield (132 mg, 0.24 mmol). R_f: 0.23 (CH_2Cl_2/AcOEt: 9/1). m.p.: 198-200 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 10.81 (bs, 1H), 9.62 (s, 1H), 8.09-8.07 (m, 1H), 7.89 (dd, 1H, J_1 = 3.8 Hz, J_2 = 1.4 Hz), 7.61 (s, 1H), 7.57 (d, 1H, *J* = 8.4 Hz), 7.18 (d, 1H, *J* = 8.7 Hz), 7.13-7.10 (m, 1H), 6.89 (d, 1H, *J* = 8.7 Hz), 6.76 (d, 1H, *J* = 8.4 Hz), 2.80 (t, 2H, *J* = 6.4 Hz), 2.19 (s, 3H), 1.82 (t, 2H, *J* = 6.4 Hz), 1.32 (s, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6, δ): 164.7, 159.7, 159.5, 156.7, 151.8, 150.5, 137.5, 136.6, 133.6, 129.9, 128.3, 127.2, 124.1, 121.4, 120.3, 116.2, 113.4, 112.6, 111.1, 107.7, 75.0, 31.8, 26.5 (2C), 21.6, 7.9. MS (ESI$^+$) *m/z*: 564.1 $[M+Na]^+$. IR (cm^{-1}): 1696, 1582, 1490, 1357, 1256, 1193, 1016, 731, 703, 672, 604, 579.

*4.1.b.3. 3-(2,2-dimethylchroman-6-carboxamido)-7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl naphthalene-2-sulfonate (**1d**)*

After purification, **1d** was obtained in 49% yield (146 mg, 0.25 mmol). R_f: 0.30 (Et_2O). m.p.: 192-194 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 10.82 (s, 1H), 9.41 (s, 1H), 8.57 (s, 1H), 8.01 (d, 1H, *J* = 7.9 Hz), 7.93-7.84 (m, 3H), 7.71-7.58 (m, 2H), 7.45 (d, 1H, *J* = 8.7 Hz), 7.20 (d, 1H, *J* = 8.5 Hz), 7.15 (s, 1H), 6.97 (d, 1H, *J* = 8.8 Hz), 6.41 (d, 1H, *J* = 8.5 Hz), 2.53-2.50 (m, 2H), 2.21 (s, 3H), 1.72 (t, 2H, *J* = 6.4 Hz), 1.28 (s, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6, δ): 164.2, 159.7, 159.5, 156.3, 152.4, 150.6, 134.9, 132.5, 131.1, 129.8, 129.6, 129.5 (2C), 129.2, 127.6 (2C), 126.7, 123.2, 121.8, 121.6, 119.7, 115.7, 113.0, 112.7, 111.1, 108.3, 74.8, 31.7, 26.5 (2C), 21.4, 7.9. MS (ESI$^+$) *m/z*: 586.1 $[M+H]^+$, 608.1 $[M+Na]^+$, 624.1 $[M+K]^+$. IR (cm^{-1}): 3292, 2971, 1693, 1648, 1589, 1484, 1361, 1322, 1253, 1177, 1154, 1120, 1092, 1074, 1016, 948, 859, 809, 761, 653, 617.

*4.1.b.4. 3-(2,2-dimethylchroman-6-carboxamido)-7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl quinoline-7-sulfonate (**1e**)*

After purification, **1e** was obtained in 52% yield (155 mg, 0.27 mmol). R_f: 0.28 (CH_2Cl_2/AcOEt: 9/1). m.p.: 293-295 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 10.81 (s, 1H), 9.07 (s, 1H), 9.02 (dd, 1H, J_1 = 4.0 Hz, J_2 = 1.4 Hz), 8.29-8.23 (m, 2H), 8.08 (d, 1H, *J* = 8.2 Hz), 7.59-7.47 (m, 3H), 7.03 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.8 Hz), 6.98-6.95 (m, 2H), 6.52 (d, 1H, *J* = 8.5 Hz), 2.63 (t, 2H, *J* = 6.3 Hz), 2.20 (s, 3H), 1.82 (t, 2H, *J* = 6.3 Hz), 1.34 (s, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6, δ): 164.0, 159.7, 159.5, 156.4, 153.3, 151.8, 150.7, 142.5, 136.3, 135.6, 132.9, 132.4, 129.2, 128.5, 126.7, 125.1, 123.0, 122.4, 122.0, 119.7, 115.9, 112.9, 112.6, 111.0, 108.6, 74.9, 31.7, 26.5 (2C), 21.6, 7.9. MS (ESI$^+$) *m/z*: 587.2 $[M+H]^+$, 609.2 $[M+Na]^+$. IR (cm^{-1}): 1606, 1474, 1352, 1259, 1178, 1118, 1091, 995, 842, 735, 691, 604, 584, 552.

*4.1.c. 6-bromo-3-(2,2-dimethylchroman-6-carboxamido)-7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate (**9**)*

To a round bottom flask charged with 4TCNA (300 mg, 0.55 mmol, 1 eq.) and acetic acid (5 mL), was added dropwise bromine (31 µL, 0.61 mmol, 1.1 eq) at room temperature. The mixture was stirred at room temperature for 24 hrs, then hydrolised with a saturated solution of $Na_2S_2O_3$ and extracted with AcOEt (3 x 20 mL). The combines organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Further purification on silica gel chromatography delivered compound **9** in 37 % yield (128 mg, 0.20 mmol).

Rf: 0.39 (c-hexane/AcOEt: 6/4). m.p.: 169-171 °C. ^{1}H-NMR (300 MHz, CDCl3, δ): 7.76 (d, 2H, *J* = 8.2 Hz), 7.60-7.55 (m, 2H), 7.47 (dd, 1H, *J* = 8.6 Hz, *J* = 1.9 Hz), 7.22 (s, 1H), 7.20 (d, 2H, *J* = 8.4 Hz), 6.79 (d, 1H, *J* = 8.5 Hz), 2.82 (t, 2H, *J* = 6.6 Hz), 2.34 (s, 3H), 2.32 (s, 3H), 1.85 (t, 2H, *J* = 6.6 Hz), 1.37 (s, 6H), the -OH proton was not observed. ^{13}C-NMR (75 MHz, CDCl3, δ): 164.9, 160.1, 158.1, 153.9, 149.4, 148.1, 146.4, 132.9, 130.1 (3C), 128.1 (2C), 127.2, 123.7, 123.6, 121.0, 117.3, 114.4, 113.9, 110.8, 107.2, 75.5, 32.5, 26.9 (2C), 22.3, 21.7, 9.3. MS (APCI$^+$) m/z: 628.0 ([M+H]$^+$, ^{79}Br), 630.0 ([M+H]$^+$, ^{81}Br). IR (cm^{-1}): 1733, 1602, 1482, 1365, 1256, 1152, 1119, 1090, 684, 618, 577, 553.

*4.4. N-(6-bromo-4,7-dimethoxy-8-methyl-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**3**)*

MPHT [35, 36] (i.e. *N*-methylpyrrolidin-2-one hydrotribromide; 4.4 g, 10.12 mmol, 2 eq) was added portionwise to an heterogeneous solution of CNBA (2 g, 5.06 mmol, 1 eq) in acetonitrile (80 mL). The reaction mixture was stirred at 80 °C for 14 hrs. The white precipitate formed is then filtered and washed with *c*-hexane and acetonitrile, dried under high vacuum to deliver the 6-Br CNBA intermediate in 92% yield (2.2 g, 4.64 mmol).

R_f: 0.19 (*c*-hexane/AcOEt: 8/2). m.p.: 244-246 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 14.21 (s, 1H), 8.69 (s, 1H), 8.01 (s, 1H), 7.73-7.65 (m, 2H), 6.87 (d, 1H, *J* = 8.4 Hz), 5.98 (s, 1H), 2.86 (t, 2H, *J* = 6.7 Hz), 2.41 (s, 3H), 1.86 (t, 2H, *J* = 6.7 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.1, 161.3, 158.8, 152.8, 152.2, 149.2, 129.7, 127.1, 124.0, 122.2, 121.5, 117.9, 113.1, 111.6, 107.0, 103.4, 75.8, 32.3, 26.9 (2C), 22.3, 9.3. MS (ESI$^+$) *m/z*: 473.2 ([M+H]$^+$, ^{79}Br), 475.2 ([M+H]$^+$, ^{81}Br). IR (cm^{-1}): 3516, 3373, 2924, 1682, 1642, 1608, 1637, 1486, 1341, 1266, 1142, 1114, 1094, 945, 875, 830, 775, 750, 672, 622, 554, 540.

To a solution of 6-Br CNA intermediate (390 mg, 0.82 mmol, 1 eq) and K_2CO_3 (0.7 g, 4.93 mmol, 6 eq) in dry DMF (5 mL), stirred at room temperature under a nitrogen atmosphere, was added dimethyl sulfate (466 µL, 4.93 mmol, 6 eq). After 12 hrs the mixture is diluted with AcOEt (20 mL) and the organic phase washed with a saturated solution of NH_4Cl (3 x 15 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Further purification on silica gel chromatography delivered compound **3** in 62 % yield (255 mg, 0.51 mmol).

R_f: 0.59 (CH_2Cl_2/AcOEt : 95/5). m.p.: 232-234 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 8.04 (s, 1H), 7.94 (s, 1H), 7.68 (d, 1H, *J* = 2.1 Hz), 7.63 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.3 Hz), 6.74 (d, 1H, *J* = 8.5 Hz), 4.12 (s, 3H), 3.85 (s, 3H), 2.76 (t, 2H, *J* = 6.7 Hz), 2.39 (s, 3H), 1.80 (t, 2H, *J* = 6.7 Hz), 1.34 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 166.9, 162.8, 158.6, 157.9, 157.8, 149.2, 129.6, 127.1, 125.1, 123.8, 121.2, 121.0, 117.4, 115.2, 113.1, 104.7, 75.3, 60.9, 59.4, 32.4, 26.9 (2C), 22.3, 9.7. MS (APCI$^+$) *m/z*: 502.1 ([M+H]$^+$, ^{79}Br), 504.2 ([M+H]$^+$, ^{81}Br). IR (cm^{-1}): 1714, 1642, 1619, 1490, 1348, 1261, 1179, 1121, 1047, 685.

4.1.d. General procedure for the one-pot Sonogashira coupling/4-MeO deprotection.

A flame-dried resealable Schlenk tube was charged with compound **3** (100 mg, 0.20 mmol, 1 eq), CuI (6 mol %), $PdCl_2(PPh_3)_2$ (6 mol %), PPh_3 (20 mol %). The Schlenk tube was capped with a rubber septum, evacuated and backfilled with argon (three times). Triethylamine (0.5 mL) and dry DMF (1.5 mL) were added, followed by a dropwise

addition of the terminal alkyne (0.40 mmol, 1.2 eq). The septum was replaced with a Teflon screwcap, the Schlenk tube sealed and the mixture was stirred at 120 °C for 30 minutes. The resulting suspension was cooled to room temperature and filtred through Celite eluting with ethyl acetate and dichloromethane. The filtrate was then concentrated under reduced pressure. Purification of the residue by silica gel column chromatography gave the desired product.

*4.1.d.1 N-(4-hydroxy-7-methoxy-8-methyl-2-oxo-6-(phenylethynyl)-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**4a**)*

After purification, **4a** was obtained in 63% yield (64 mg, 0.13 mmol). R_f: 0.42 (*c*-hexane/Et_2O: 5/5). m.p.: 194-196 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 14.18 (s, 1H), 8.70 (s, 1H), 8.01 (s, 1H), 7.70-7.66 (m, 2H), 7.57-7.52 (m, 2H), 7.38-7.33 (m, 3H), 6.86 (d, 1H, *J* = 8.3 Hz), 4.07 (s, 3H), 2.85 (t, 2H, *J* = 6.7 Hz), 2.38 (s, 3H), 1.85 (t, 2H, *J* = 6.7 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.1, 161.6, 161.3, 158.7, 152.4, 149.4, 131.5 (2C), 129.8, 128.5, 128.4 (2C), 127.1, 126.4, 123.1, 122.3, 121.5, 119.3, 117.9, 113.9, 113.2, 104.0, 93.8, 84.9, 75.8, 61.2, 32.3, 26.9 (2C), 22.3, 9.0. MS ($APCI^+$) *m/z*: 510 $[M+H]^+$. IR (cm^{-1}): 1687, 1632, 1490, 1327, 1268, 1111, 727, 614, 584, 575.

*4.1.d.2. N-(4-hydroxy-7-methoxy-6-((4-methoxyphenyl)ethynyl)-8-methyl-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**4b**)*

After purification, **4b** was obtained in 55 % yield (59 mg, 0.11 mmol). R_f: 0.30 (*c*-hexane/Et_2O: 5/5). m.p.: 167-169 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 14.09 (s, 1H), 8.62 (s, 1H), 7.92 (s, 1H), 7.66-7.56 (m, 2H), 7.41 (d, 2H, *J* = 8.5Hz), 6.81 (d, 2H, *J* = 8.5Hz), 6.80-6.75 (m, 1H), 3.99 (s, 3H), 3.76 (s, 3H), 2.78 (t, 2H, *J* = 6.6Hz), 2.31 (s, 3H), 1.78 (t, 2H, *J* = 6.7Hz), 1.30 (6, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.0, 161.4, 161.3, 159.8, 158.7, 152.4, 149.1, 132.9 (2C), 129.7, 127.1, 126.1, 122.3, 121.5, 119.2, 117.9, 115.2, 114.2, 114.0 (2C), 113.2, 104.0, 93.9, 83.6, 75.7, 61.1, 55.3, 32.3, 26.9 (2C), 22.3, 9.0. MS ($APCI^+$) *m/z*: 540.0 $[M+H]^+$. IR (cm^{-1}): 1687, 1632, 1569, 1535, 1512, 1487, 1371, 1317, 1252, 1152, 1114, 1033, 832, 752, 625, 575, 543.

*4.1.d.3 N-(4-hydroxy-7-methoxy-8-methyl-2-oxo-6-((3,4,5-trimethoxyphenyl)ethynyl)-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**4c**)*

After purification, **4c** was obtained in 33 % yield (79 mg, 0.13 mmol). R_f: 0.42 (Et_2O/*c*-hexane : 5/5). m.p.: 205-207 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 14.20 (s, 1H), 8.71 (s, 1H), 8.04 (s, 1H), 7.71-7.67 (m, 2H), 6.87 (d, 1H, *J* = 8.3 Hz), 6.80 (s, 2H), 4.06 (s, 3H), 3.90 (s, 6H), 3.88 (s, 3H), 2.86 (t, 2H, *J* = 6.6Hz), 2.40 (s, 3H), 1.86 (t, 2H, *J* = 6.6 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.1, 161.5, 161.3, 158.8, 153.2 (2C), 152.3, 149.4, 139.1, 129.8, 127.1, 126.4, 122.2, 121.5, 119.3, 118.1, 117.9, 113.8, 113.3, 108.7 (2C), 104.1, 93.9, 84.0, 75.8, 61.2, 61.0, 56.2 (2C), 32.3, 26.9 (2C), 22.3, 9.0. MS ($APCI^+$) *m/z*: 600 $[M+H]^+$. IR (cm^{-1}): 1684, 1630, 1536, 1492, 1342, 1237, 1121, 943, 821, 752, 656, 600, 587.

*4.1.d.4. N-(4-hydroxy-7-methoxy-8-methyl-2-oxo-6-((trimethylsilyl)ethynyl)-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**4d**)*

After purification, **4d** was obtained in 73 % yield (146 mg, 0.29 mmol). R_f: 0.56 (*c*-hexane/Et_2O: 6/4). m.p.: 110-112 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 14.15 (s, 1H), 8.70 (s, 1H),7.98 (s, 1H), 7.71 (s, 1H), 7.71-7.68 (m, 1H), 6.87 (d, 1H, *J* = 8.3 Hz), 4.01 (s, 3H), 2.86 (t, 2H, *J* = 6.7 Hz), 2.36 (s, 3H), 1.86 (t, 2H, *J* = 6.6 Hz), 1.37 (s, 6H), 0.28 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.1, 162.0, 161.3, 158.7, 152.3, 149.4, 129.7, 127.1, 127.0, 122.3, 121.5, 119.2,

117.9, 113.7, 113.0, 104.0, 100.3, 99.4, 75.8, 60.9, 32.3, 26.9 (2C), 22.3, 8.9, -0.2 (3C). MS ($APCI^+$) *m/z*: 506.0 $[M+H]^+$. IR (cm^{-1}): 1689, 1626, 1489, 1327, 1157, 1120, 998, 838, 649, 567, 550.

*4.1.d.5. N-(6-(3-(dimethylamino)prop-1-ynyl)-4-hydroxy-7-methoxy-8-methyl-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**4e**)*

After purification, **4e** was obtained in 36 % yield (70 mg, 0.14 mmol). R_f: 0.22 (CH_2Cl_2/AcOEt : 8/2). m.p.: 141-143 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 8.69 (s, 1H), 7.94 (s, 1H), 7.70-7.64 (m, 2H), 6.86 (d, 1H, *J* = 8.3 Hz), 3.97 (s, 3H), 3.58 (s, 2H), 2.85 (t, 2H, *J* = 6.6 Hz), 2.42 (s, 6H), 2.36 (s, 3H), 1.85 (t, 2H, *J* = 6.7 Hz), 1.36 (s, 6H), the –OH proton was not observed. ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.1, 161.6, 161.3, 158.8, 152.3, 149.3, 129.7, 127.1, 126.7, 122.3, 121.5, 119.3, 117.9, 113.8, 113.2, 104.0, 88.8, 81.0, 75.7, 61.1, 48.7, 44.1 (2C), 32.3, 26.9 (2C), 22.3, 8.9. MS ($APCI^+$) *m/z*: 491 $[M+H]^+$. IR (cm^{-1}): 3325, 2966, 1650, 1600, 1519, 1493, 1402, 1261, 1159, 1019, 948, 801, 660, 568.

*4.1.d.6. N-(6-ethynyl-4-hydroxy-7-methoxy-8-methyl-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**4f**)*

An heterogeneous solution of **4d** (139 mg, 0.27 mmol, 1 eq) and potassium carbonate (76 mg, 0.54 mmol, 2 eq) in methanol (10 mL) was stirred at room temperature. After 2 hrs, the mixture was concentrated under reduced pressure. Purification of the residue by silica gel column chromatography gave the desired product **4f** in 93 % yield (111 mg, 0.25 mmol). R_f: 0.25 (*c*-hexane/Et_2O: 6/4). m.p.: 223 -225 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 14.16 (bs, 1H), 8.68 (s, 1H), 8.00 (s, 1H), 7.74-7.64 (m, 2H), 6.87 (d, 1H, *J* = 8.3 Hz), 4.00 (s, 3H), 3.31 (s, 1H), 2.86 (t, 2H, *J* = 6.7 Hz), 2.37 (s, 3H), 1.86 (t, 2H, *J* = 6.7 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 167.1, 162.1, 161.2, 158.8, 152.2, 149.7, 129.7, 127.3, 127.1, 122.2, 121.5, 119.4, 117.9, 113.2, 112.8, 104.1, 81.7, 79.1, 75.8, 61.2, 32.3, 26.9 (2C), 22.3, 8.9. MS ($APCI^-$) *m/z*: 432.0 $[M-H]^-$. IR (cm^{-1}): 3253, 1685, 1626, 1531, 1490, 1315, 1264, 1195, 1156, 1114, 945, 671, 592.

*4.1.e. General procedure for the tosylation of 4-hydroxycoumarin derivatives **4a-4f** and **6**.*

To a 10 mL round bottom flask, triethylamine (3 eq.) was added dropwise to a solution of 4-hydroxycoumarin (1 eq.) in dichloromethane (5 mL), under nitrogen atmosphere. The mixture was stirred for 5 minutes at room temperature, followed by addition of tosyl chloride (2 eq.). After 12 hrs, the reaction mixture was diluited with EtOAc (10 mL) and the organic phase washed with a 1N HCl solution (3 x 15 mL), filtered and concentrated under reduced pressure. Further purification on silica gel chromatography delivered desired 4-tolsyl coumarin derivative.

*4.1.e.1. 3-(2,2-dimethylchroman-6-carboxamido)-7-methoxy-8-methyl-2-oxo-6-(phenylethynyl)-2H-chromen-4-yl 4-methylbenzenesulfonate (**5a**)*

Starting from the 4-hydroxycoumarin **4a** (50 mg, 0.1 mmol), after purification, **5a** was obtained in 82 % yield (53 mg, 0.08 mmol). R_f: 0.10 (CH_2Cl_2/*c*-hexane: 9/1). m.p.: 192-194 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.80 (d, 2H, *J* = 8.1 Hz), 7.62 (s, 1H), 7.56-7.53 (m, 3H), 7.47-7.44 (m, 2H), 7.39-7.37 (m, 3H), 7.18 (d, 2H, *J* = 8.2 Hz), 6.79 (d, 1H, *J* = 8.5 Hz), 4.06 (s, 3H), 2.82 (t, 2H, *J* = 6.5 Hz), 2.37 (s, 3H), 2.27 (s, 3H), 1.84 (t, 2H, *J* = 6.6 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 164.1, 161.9, 160.1, 157.9, 149.5, 147.6, 146.2, 133.1, 131.4 (2C), 130.0 (2C), 129.9, 128.6, 128.4 (2C), 128.1 (2C), 127.1, 126.0, 123.8, 122.8, 120.9, 119.8, 117.2, 115.6, 114.1, 113.0, 94.2, 84.4, 75.4, 61.2, 32.5, 26.8 (2C), 22.3, 21.6, 9.0. MS ($APCI^+$) *m/z*: 664.0 $[M+H]^+$. IR (cm^{-1}): 3310, 1726, 1677, 1607, 1483, 1371, 1255, 1176, 1119, 998, 939, 884, 791, 757, 733, 690, 646.

*4.1.e.2. 3-(2,2-dimethylchroman-6-carboxamido)-7-methoxy-6-((4-methoxyphenyl)ethynyl)-8-methyl-2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate (**5b**)*

Starting from the 4-hydroxycoumarin **4b** (50 mg, 0.09 mmol), after purification, **5b** was obtained in 84 % yield (54 mg, 0.08 mmol). R_f: 0.20 (CH_2Cl_2). m.p.: 187-189 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.80 (d, 2H, *J* = 7.8 Hz), 7.60 (s, 1H), 7.55 (s, 1H), 7.49-7.43 (m, 4H), 7.18 (d, 2H, *J* = 8.1 Hz), 6.91 (d, 2H, *J* = 8.0 Hz), 6.78 (d, 1H, *J* = 8.4 Hz), 4.05 (s, 3H), 3.84 (s, 3H), 2.82 (t, 2H, *J* = 6.2 Hz), 2.36 (s, 3H), 2.27 (s, 3H), 1.84 (t, 2H, *J* = 6.6 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 164.1, 161.7, 160.1, 159.9, 157.9, 149.3, 147.7, 146.2, 133.1, 132.9 (2C), 129.9 (3C), 128.1 (2C), 127.1, 125.8, 123.9, 120.9, 119.8, 117.2, 115.5, 114.9, 114.5, 114.1 (2C), 113.0, 94.3, 83.1, 75.4, 61.1, 55.3, 32.5, 26.8 (2C), 22.3, 21.6, 9.0. MS (APCI$^+$) *m/z*: 695.0 $[M+H]^+$. IR (cm^{-1}): 1721, 1606, 1482, 1373, 1248, 1172, 1033, 941, 829, 695.

*4.1.e.3. 3-(2,2-dimethylchroman-6-carboxamido)-7-methoxy-8-methyl-2-oxo-6-((3,4,5-trimethoxyphenyl)ethynyl) -2H-chromen-4-yl 4-methylbenzenesulfonate (**5c**)*

Starting from the 4-hydroxycoumarin **4c** (50 mg, 0.08 mmol), after purification, **5c** was obtained in 82 % yield (53 mg, 0.07 mmol). R_f: 0.48 (CH_2Cl_2/AcOEt: 95/5). m.p.: 151-153 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.79 (d, 2H, *J* = 8.2 Hz), 7.62 (s, 1H), 7.54 (s, 1H), 7.49 (s, 1H), 7.37 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.6 Hz), 7.13 (d, 2H, *J* = 8.1 Hz), 6.80 (s, 2H), 6.76 (d, 1H, *J* = 8.5 Hz), 4.05 (s, 3H), 3.91 (s, 6H), 3.89 (s, 3H), 2.80 (t, 2H, *J* = 6.4 Hz), 2.38 (s, 3H), 2.25 (s, 3H), 1.84 (t, 2H, *J* = 6.6 Hz), 1.36 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 163.9, 161.9, 160.2, 157.9, 153.1 (2C), 149.4, 147.4, 146.0, 139.2, 133.2, 129.9 (3C), 128.0 (2C), 127.0, 126.0, 123.6, 120.8, 119.9, 117.8, 117.1, 115.3, 114.2, 113.3, 108.8 (2C), 94.3, 83.4, 75.4, 61.2, 61.0, 56.2 (2C), 32.4, 26.8 (2C), 22.3, 21.6, 9.0. MS (APCI$^+$) *m/z*: 754.0 $[M+H]^+$. IR (cm^{-1}): 2974, 1722, 1604, 1482, 1370, 1240, 1177, 1156, 1119, 1091, 1031, 947, 758, 731, 691.

*4.1.e.4. 6-(3-(dimethylamino)prop-1-ynyl)-3-(2,2-dimethylchroman-6-carboxamido)-7-methoxy-8-methyl-2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate (**5e**)*

Starting from the 4-hydroxycoumarin **4e** (30 mg, 0.06 mmol), after purification, **5e** was obtained in 55 % yield (22 mg, 0.03 mmol). R_f: 0.22 (CH_2Cl_2/MeOH: 9/1). m.p.: 116-118 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.78 (d, 2H, *J* = 8.2 Hz), 7.57 (s, 1H), 7.52-7.51 (m, 2H), 7.42 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.9 Hz), 7.16 (d, 2H, *J* = 8.2 Hz), 6.78 (d, 1H, *J* = 8.5 Hz), 3.97 (s, 3H), 3.55 (s, 2H), 2.81 (t, 2H, *J* = 6.6 Hz), 2.40 (s, 6H), 2.35 (s, 3H), 2.31 (s, 3H), 1.84 (t, 2H, *J* = 6.6 Hz), 1.37 (s, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 164.0, 162.0, 160.1, 157.9, 149.3, 147.4, 146.0, 133.1, 129.9 (3C), 128.0 (2C), 127.1, 126.3, 123.7, 120.8, 119.9, 117.2, 115.4, 114.2, 113.2, 89.1, 80.7, 75.4, 61.2, 48.6, 44.0 (2C), 32.4, 26.8 (2C), 22.3, 21.7, 9.0. MS (APCI$^+$) *m/z*: 645.0 $[M+H]^+$, 667.0 $[M+Na]^+$. IR (cm^{-1}): 1734, 1603, 1478, 1373, 1260, 1155, 1119, 1009, 946, 757, 729, 682, 629.

*4.1.e.5. 3-(2,2-dimethylchroman-6-carboxamido)-6-ethynyl-7-methoxy-8-methyl-2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate (**5f**)*

Starting from the 4-hydroxycoumarin **4f** (50 mg, 0.11 mmol), after purification, **5f** was obtained in 39 % yield (26 mg, 0.04 mmol). R_f: 0.50 (CH_2Cl_2/AcOEt: 95/5). m.p.: 164-166 °C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.78 (d, 2H, *J* = 7.8 Hz), 7.60 (s, 1H), 7.55 (s, 1H), 7.46-7.44 (m, 2H), 7.18 (d, 2H, *J* = 7.8 Hz), 6.79 (d, 1H, *J* = 8.4 Hz), 3.99 (s, 3H), 3.29 (s, 1H), 2.82 (t, 2H, *J* = 6.3 Hz), 2.35 (s, 3H), 2.33 (s, 3H), 1.85 (t, 2H, *J* = 6.3 Hz), 1.37 (s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ): 164.2, 162.5, 160.0, 158.0, 149.8, 147.3, 146.2, 133.0, 130.0 (2C), 129.9, 128.1 (2C), 127.1, 127.0, 123.8,

120.9, 120.0, 117.3, 115.7, 113.1 (2C), 82.1, 78.6, 75.4, 61.3, 32.5, 26.9 (2C), 22.3, 21.7, 9.0. MS ($APCI^+$) *m/z*: 588.0 $[M+H]^+$. IR (cm^{-1}): 1730, 1603, 1480, 1365, 1256, 1177, 1118, 997, 937, 756, 692.

*4.1.f. N-(4-hydroxy-7-methoxy-6-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**6**)*

A 10 mL round bottom flask was charged with compound **3** (50 mg, 0.10 mmol, 1 eq.), *p*-methoxyphenylboronique (23 mg, 0.15 mmol, 1,5 eq.), KF (18 mg, 0.30 mmol, 3 eq.), $Pd(OAc)_2$ (5 mol %) and Xphos (20 mol %). The flask was evacuated and backfilled with argon (three times). Then dry THF (2 mL) was added and the reaction mixture was heated to reflux for 24 hrs. The mixture was then filtered through a Celite pad with AcOEt, the solvent evaporated under reduced pressure and the resulting mixture transferred into a resealable Schlenk tube. Triethylamine (1 mL) and dry DMF (1 mL) were added, the Schlenk tube sealed and the mixture was stirred at 120 °C for 30 minutes. After cooling at room temperature the crude mixture was purified on silica gel chromatography delivered product **6** in 63 % yield (33 mg, 0.06 mmol). R_f: 0.80 (CH_2Cl_2). MS ($APCI^+$) *m/z*: 516.0 $[M+H]^+$. Compounds **6** was not enough soluble for NMR and was used directly in the tosylation step to get compound **7**.

*4.1.g. 3-(2,2-dimethylchroman-6-carboxamido)-7-methoxy-6-(4-methoxyphenyl)-8-methyl-2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate (**7**)*

Product **7** was prepared according to the general procedure for tosylation (*4.6.*). Starting from the 4-hydroxycoumarin **6** (30 mg, 0.040 mmol), after purification, **7** was obtained in 90 % yield (27 mg, 0.035 mmol).

R_f: 0.27 (CH_2Cl_2). m.p.: 201-203 °C. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.75 (d, 2H, *J* = 8.2 Hz), 7.66 (s, 1H), 7.56 (s, 1H), 7.47 (dd, 1H, J_1 = 8.6 Hz, J_2 = 2.0 Hz), 7.37 (d, 2H, *J* = 8.7 Hz), 7.09 (d, 2H, *J* = 8.2 Hz), 6.95 (d, 2H, *J* = 8.7 Hz), 6.77 (d, 1H, *J* = 8.6 Hz), 3.86 (s, 3H), 3.40 (s, 3H), 2.80 (t, 2H, *J* = 6.5 Hz), 2.41 (s, 3H), 2.24 (s, 3H), 1.83 (t, 2H, *J* = 6.6 Hz), 1.36 (s, 6H), one proton not observed. ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 164.2, 160.5, 159.1 (2C), 157.8, 148.9, 148.5, 145.9, 133.2, 131.8, 130.0 (2C), 129.8 (3C), 129.4, 128.0 (2C), 127.1, 123.9, 123.0, 120.8, 119.9, 117.1, 115.2, 113.8 (2C), 113.0, 75.3, 60.3, 55.2, 32.4, 26.8 (2C), 22.3, 21.5, 9.1. MS ($APCI^+$) *m/z*: 670.0 $[M+H]^+$. IR (cm^{-1}): 2939, 1734, 1604, 1480, 1370, 1240, 1176, 1155, 1119, 1089, 999, 947, 800, 757, 731, 683.

*4.1.h. N-(7-hydroxy-8-methyl-4-(2-morpholinoethylamino)-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**8**)*

A flame-dried resealable Schlenk tube under nitrogen atmosphere, was charged with **4TCNA** (100 mg, 0.18 mmol, 1 eq.), 2-morpholinoethanamine (47 mg, 0.36 mmol, 2 eq.) and dioxane (1 mL). The Schlenk tube sealed and the mixture was stirred at 100 °C for 14 hrs. The resulting suspension was then cooled to room temperature and filtred through Celite eluting with ethyl acetate and dichloromethane. The filtrate was then concentrated under reduced pressure. Purification of the residue by silica gel column chromatography gave the desired product **8** in 71 % yield (66 mg, 0.13 mmol).

R_f: 0.20 (CH_2Cl_2/MeOH: 95/5). m.p.: 186-188 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 10.26 (s, 1H), 9.13 (s, 1H), 7.78 (d, 1H, *J* = 1.9 Hz), 7.71 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz), 7.66 (d, 1H, *J* = 9.0 Hz), 6.84 (d, 1H, *J* = 9.0 Hz), 6.78 (d, 1H, *J* = 8.5 Hz), 6.74 (s, 1H), 3.53 (q, 2H, *J* = 6.1 Hz), 3.43-3.40 (m, 4H), 2.79 (t, 2H, *J* = 6.6 Hz), 2.50-2.47 (m, 2H), 2.28-2.25 (m, 4H), 2.15 (s, 3H), 1.81 (t, 2H, *J* = 6.6 Hz), 1.31 (s, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6, δ): 166.6, 160.7, 158.0, 156.3, 150.9, 149.4, 129.6, 127.0, 125.4, 120.4, 120.3, 116.2, 111.0, 110.6, 107.4, 93.6, 74.9, 66.0 (2C), 57.1,

52.8 (2C), 40.8, 31.8, 26.4 (2C), 21.7, 8.1. MS (APCI$^+$) *m/z*: 508.0 $[M+H]^+$. IR (cm^{-1}): 1658, 1607, 1548, 1515, 1454, 1343, 1263, 1158, 1114, 837, 653.

*4.1.i. 3-(2,2-dimethylchroman-6-carboxamido)-7-(2,3,4,6-tetra-O-acétyl-β-glucopyranos-1-yl)-8-methyl-2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate (**2a**)*

To a 100 mL round bottom flask, charged with 4TCNA (150 mg, 0.27 mmol, 1 eq.) and dry THF (10 mL) under nitrogen atmosphere were added a THF (10 mL) solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (190 mg, 0.54 mmol, 2 eq.) and triphenylphosphine (144 mg, 0.54 mmol, 2 eq.). The reaction mixture was cooled at -78 °C and DIAD (108 µL, 0.54 mmol, 2 eq.) was added. The reaction was allowed to warm at room temperature. After 24 hrs, the mixture was diluted with AcOEt (30 mL), the organic phase washed with a NaCl sat. (40 mL), filtered and concentrated under reduced pressure. Further purification on silica gel chromatography delivered desired product **2a** in 20 % yield (47 mg, 0.05 mmol).

R_f: 0.14 (Et_2O/*c*-hexane: 8/2). m.p.: 143-145 °C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.76 (d, 2H, *J* = 8.2 Hz), 7.61 (d, 1H, *J* = 8.9 Hz), 7.47 (s, 1H), 7.44 (s, 1H), 7.35 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.8 Hz), 7.12 (d, 2H, *J* = 8.2 Hz), 7.03 (d, 1H, *J* = 8.9 Hz), 6.76 (d, 1H, *J* = 8.6 Hz), 5.40-5.29 (m, 2H), 5.19 (t, 1H, *J* = 9.4 Hz), 5.14 (d, 1H, *J* = 7.4 Hz), 4.32 (dd, 1H, J_1 = 12.2 Hz, J_2 = 5.4 Hz), 4.18 (dd, 1H, J_1 = 12.2 Hz, J_2 = 1.9 Hz), 3.93-3.90 (m, 1H), 2.81 (t, 2H, *J* = 6.7 Hz), 2.28 (s, 3H), 2.27 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.85 (t, 2H, *J* = 6.7 Hz), 1.37 (s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ): 170.5 (C=O, -OAc), 170.1 (C=O, -OAc), 169.3 (C=O, -OAc), 169.1 (C=O, -OAc), 163.9, 160.5, 157.9, 157.5, 149.6, 147.9, 145.9, 133.3, 129.9 (2C), 129.8, 128.0 (2C), 127.1, 123.7, 122.5, 120.8, 117.1, 116.1, 114.4, 113.0, 111.9, 99.0, 75.4, 72.5, 72.3, 70.9, 68.2, 61.8, 32.5, 26.9 (2C), 22.3, 21.6, 20.7, 20.6 (3C), 8.4. MS (APCI$^+$) *m/z*: 880.2 $[M+H]^+$. IR (cm^{-1}): 2974, 1747, 1609, 1478, 1353, 1218, 1178, 1119, 1036, 947, 906, 811, 761, 736, 691.

*4.1.j. N-(4-amino-7-(2,3,4,6-tetra-O-acetyl-β-glucopyranos-1-yl)-8-methyl-2-oxo-2H-chromen-3-yl)-2,2-dimethylchroman-6-carboxamide (**10**)*

A flame-dried resealable Schlenk tube was charged with **2a** (40 mg, 0.04 mmol, 1 eq.) and dry THF (2 mL), under nitrogen atmosphere. En excess of ammonia (gas) was bubbled into the Schlenk over 15 min, then the Schlenk tube was sealed and the reaction stirred at room temperature. After 24 hrs, the mixture was filtred through Celite eluting with ethyl acetate and dichloromethane and the filtrate was concentrated under reduced pressure. Purification of the residue by silica gel column chromatography gave the desired product **10** in 27 % yield (9 mg, 0.01 mmol). R_f: 0.17 (Et_2O/*c*-hexane: 8/2). ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 8.06 (s, 1H), 7.69 (m, 2H), 7.40 (d, 1H, *J* = 8.9 Hz), 6.93 (d, 1H, *J* = 8.9 Hz), 6.82 (d, 1H, *J* = 8.3 Hz), 6.02 (bs, 2H), 5.37-5.35 (m, 2H), 5.21-5.15 (m, 2H), 4.30-4.18 (m, 2H), 3.96-3.90 (m, 1H), 2.81 (t, 2H, *J* = 6.6 Hz), 2.16 (s, 3H), 2.10 (s, 3H), 2.05 (s, 6H), 2.04 (s, 3H), 1.82 (t, 2H, *J* = 6.6 Hz), 1.35 (s, 6H). MS (APCI$^+$) *m/z*: 725.2 $[M+H]^+$.

4.2 Biology

4.2.1. Cell Culture and Drug Treatment. Breast cancer MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) cells were grown in Dulbecco's modified eagle medium (DMEM, Lonza, Vervier, Belgium) supplemented with L-glutamine (2 mM), penicillin (5 IU/mL), streptomycin (50 IU/mL), 10% charcoal-treated fetal calf serum (FCS) (charcoal Norit A 1%, Dextran T70 0.1%, 30 min at room temperature). Multiple myeloma RPMI 8226, LP-1 and prostate PC-3 cancer cells were grown in RPMI 1640 medium supplemented in L-glutamine (2 mM), penicillin (5 IU/mL), streptomycin (50 IU/mL), 10% heat-inactivated fetal bovine serum (FBS)The monosodium novobiocin salt was obtained from Sigma (St. Louis, MO) and the proteasome inhibitor MG132 was from Calbiochem (La Jolla, CA). Nvb was diluted in water and

coumarin analogues (stock solutions at 10 mM) were diluted in DMSO. Drug treatments of cells were performed during different periods of time in the presence or not of 5 µM of the proteasome inhibitor MG132 (Sigma).

4.2.2. Quantification of Cell Survival/Proliferation. Cells were seeded in 96-well plates at 2500-5000 cells/well, and after 24 h, serial dilutions of drugs (in complete culture medium) were added. Control condition correspond to the highest DMSO dilution. After 72 h, for MCF-7 (wild type or resistant) and MDA-MB-231 cells, 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT, Sigma) (500 μg/mL) was added to each well during 3 h at 37 °C. Medium was removed and MTT formazan crystals were dissolved in 100 μL DMSO followed by gentle agitation for 10 min. The absorbance of converted dye which directly correlates with the number of viable cells was measured at 570 nm with background substraction at 650 nm using a spectrophotometric microtiter reader (Metertech, Σ960, Fisher-Bioblock, Illkirch, France). All determinations were carried out in sextuplate, and each experiment was repeated three times. For myeloma cells and prostate cancer cells, MTS assay was used (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega France, Charbonnières, France). The medium soluble reagent was added after 72h of treatment in each well. Absorbance was read at 492 nm after 2 or 3 h. All determinations were carried out in triplicate, and each experiment was repeated at least two times The percentage of survival was calculated as the absorbance ratio of treated to untreated cells. The IC_{50} values were determined as the drug concentrations that inhibit cell growth by 50% compared with growth of vehicle-treated cells.

4.2.3. Cell Extracts and Western Blots. Cells were grown to 50% confluence in 60-mm dishes before exposure to various agents as indicated in the text and figure legends. Cells were rinsed in PBS, scraped into PBS, collected by centrifugation, and resuspended in ice-cold lysis buffer (Tris-HCl 50 mM (pH 7.5), NaCl 150 mM, EGTA 1 mM, glycerol 10% (v/v), Triton X-100 1%, $MgCl_2$ 1.5 mM, NaF 10 mM, Na pyrophosphate 10 mM, Na_3VO_4 1 mM) plus protease inhibitors (Complete reagent, Roche Diagnostics, Indianapolis, IN) and kept on ice for 15 min with occasional vortexing. Insoluble debris were removed by centrifugation at 15 000 *g* for 5 min at 4 °C, and cell lysates were boiled in Laemmli sample buffer for 3 min. TCEs were obtained from pelleted cells by resuspension in lysis buffer for 30 min at 4 °C and boiling for 5 min in Laemmli sample buffer. Protein concentration was determined by the Bio-Rad Assay. Equal amounts of protein (20 μg) were fractionated by 8% or 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto Immobilon-P membranes (Millipore, Saint Quentin en Yvelines, France). Membranes were blocked for 1 h at 37 °C with 10% dry nonfat milk in PBS containing 0.1% Tween 20. ER was detected with the D12 (ER epitope: amino acids 2-185) (Santa Cruz, CA) mouse monoclonal anti-ER antibody used at 1 μg/mL in PBST-2% milk overnight at 4 °C. The antigen/antibody complexes were detected by incubation with a biotinylated anti-mouse antibody followed by revelation with the avidin/peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories, Inc., Burlingame, CA. Blots were developed using the Immobilon Western Detection Reagent (Millipore). Depending on the mobility of the proteins, membranes were either stripped (1 h at 50 °C in a medium containing 62.5 mM Tris-HCl pH 6.8, 2% SDS, and 100 mM 2-mercaptoethanol) or extensively washed before reprobing with different primary antibodies. Equal protein loading was assessed by examination of the intensities of nonspecific (NS) signals elicited by the commercial antibodies used and unresponsive to treatments.

4.2.4. Flow Cytometry Cell Cycle Analysis. Cells (1.3 x 10^5 cells/mL) were cultured in the presence or not of novobiocin analogues at 100 μM. Nvb at the same concentration served as reference inhibitor. After treatment for 48 and 72 h, cells were washed and fixed in PBS/ethanol (30/70). For cytofluorometric examination, cells (10^4) were incubated for 30 min in PBS/Triton X100 0.2% /EDTA 1 mM, and propidium iodide (PI) (50 μg/mL) in PBS

supplemented by RNase (0.5 mg/mL). The number of cells in the different phases of the cell cycle was determined, and the percentage of apoptotic cells was quantified. Analyses were performed with a FACS Calibur (Becton Dickinson, Le Pont de Claix, France). Cell Quest software was used for data acquisition and analysis.

5. Acknowledgments

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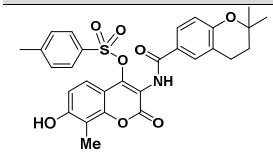
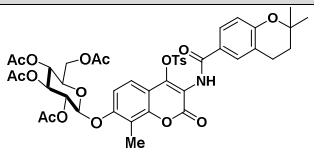
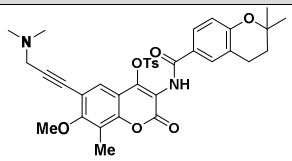
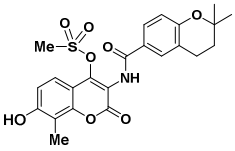
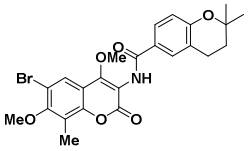
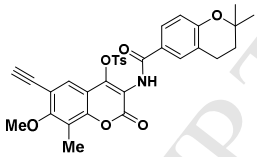
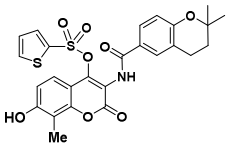
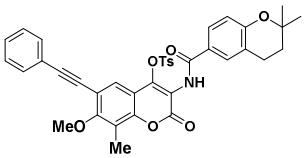
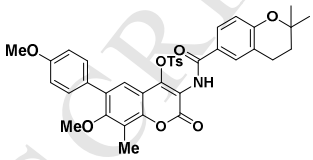
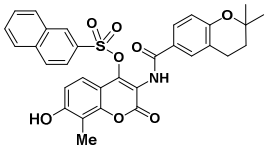
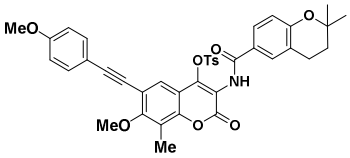
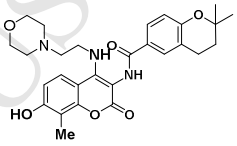
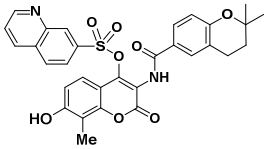
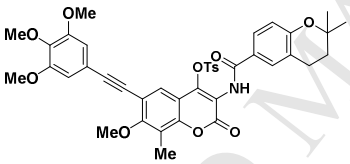
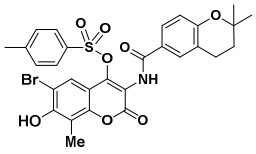
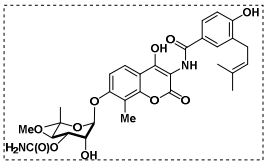
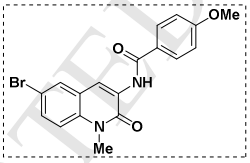
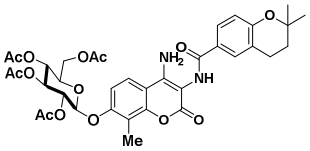
Table 1. Cytotoxic effect of **1**, **3**, **5** and **7-10** derivatives against MCF-7 cell line

Compounds	Viab. [%][a]	Compounds	Viab. [%][a]	Compounds	Viab. [%][a]
 **1a (4TCNA)**	**36**	 **2a**	**10**	 **5e**	**96**
 **1b**	**61**	 **3**	**90**	 **5f**	**82**
 **1c**	**62**	 **5a**	**90**	 **7**	**89**
 **1d**	**33**	 **5b**	**89**	 **8**	**87**
 **1e**	**22**	 **5c**	**92**	 **9**	**65**
 **Novobiocin**	**ND**	 **6BrCaQ**	**10**	 **10**	**71**

[a] Value of the anti-proliferative effect (% of viable cells compared to untreated cells 100%) of analogues **1**, **3**, **5** and **7-10** in MCF-7 cell line at a concentration of 10^{-4} M (MTT method). ND: Not determined

Table 2. GI_{50} (µM) values for anti-proliferative effects of selected compounds **1a**, **1d-e** and **2a**[a] against breast cancer cell lines (MCF-7)[b]

Compound	MCF-7 (µM)
1a (**4TCNA**)	50
1d	67
1e	64
2a	6
6BrCaQ	7
Novobiocin (Nvb)	260

[a] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). Values are means of three experiments, carried out in sextuplate.[b] MCF-7, human breast cancer cells.

Table 3. Cytotoxicity (GI_{50} µM, 72 h)[a]

Compounds	MCF-7	MDA-MB-231[c]	MCF-7 Tamoxifen^R	MCF-7 Faslodex^R	RPMI 8226	LP-1	PC-3
2a	**6**	**10**	**30**	**25**	**2.5**	**5**	**25**
6BrCaQ	**7**	**2**	**ND**	**ND**	**5**	**6.2**	**10**

[a] GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). Values are means of three experiments, carried out in triplicate or sextuplate. [b] MCF-7: human breast cancer cells. MCF7 Tamoxifen^R human breast cancer cells resistant to Tamoxifen. MCF7 Faslodex^R: human breast cancer cells resistant to Foslodex. MDA-MB231: hormone-independent breast cancer. RPMI 8226 and LP-1 : human myeloma cells. PC-3: human prostate cancer cells. ND. not determined

Table 4. Cell cycle and apoptosis analysis of MCF-7 cells treated with analogue **4TCNA**, **6BrCaQ** and **2a** (100 µM) for 48 h and 72 h.[a]

	Sub G1		G0/G1 (%)		S (%)		G2/M (%)	
compound	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
DMSO	5	9.5	87	60.5	6	20.5	7	19.0
4TCNA	6	36.0	44.0	37.0	30.5	35.5	25.5	27.5
6BrCaQ	29.5	47.0	34.81	40.43	17.14	18.09	48.0	41.5
2a	13	27	40	30.0	5.5	9.5	55	60.0

[a] Data represent percentage of cells in Sub G1 and G2/M phases of the cell cycle. The results are the mean of two independent experiments in which no more than 2.5 variations were measured.

Figure 1. Structures of novobiocin, synthetic derivatives, 4TCNA, 6BrCaQ and targeted molecules.

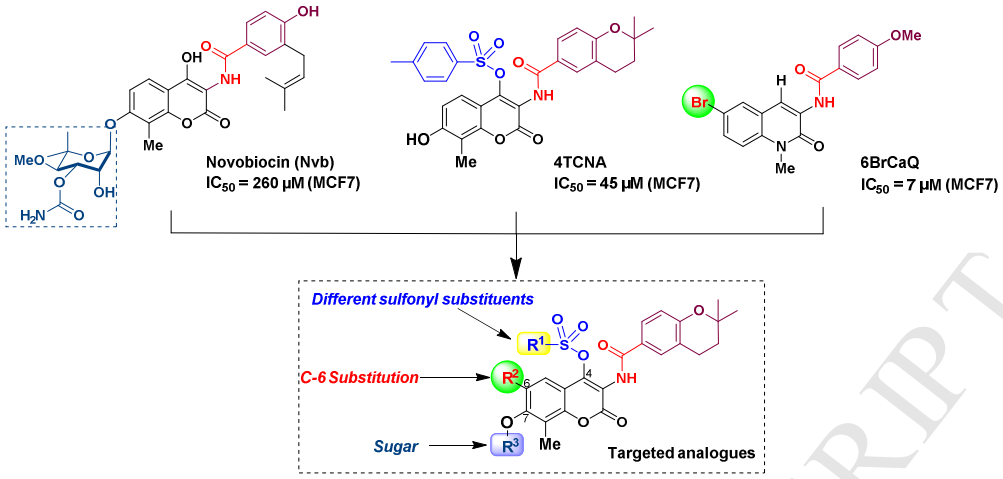
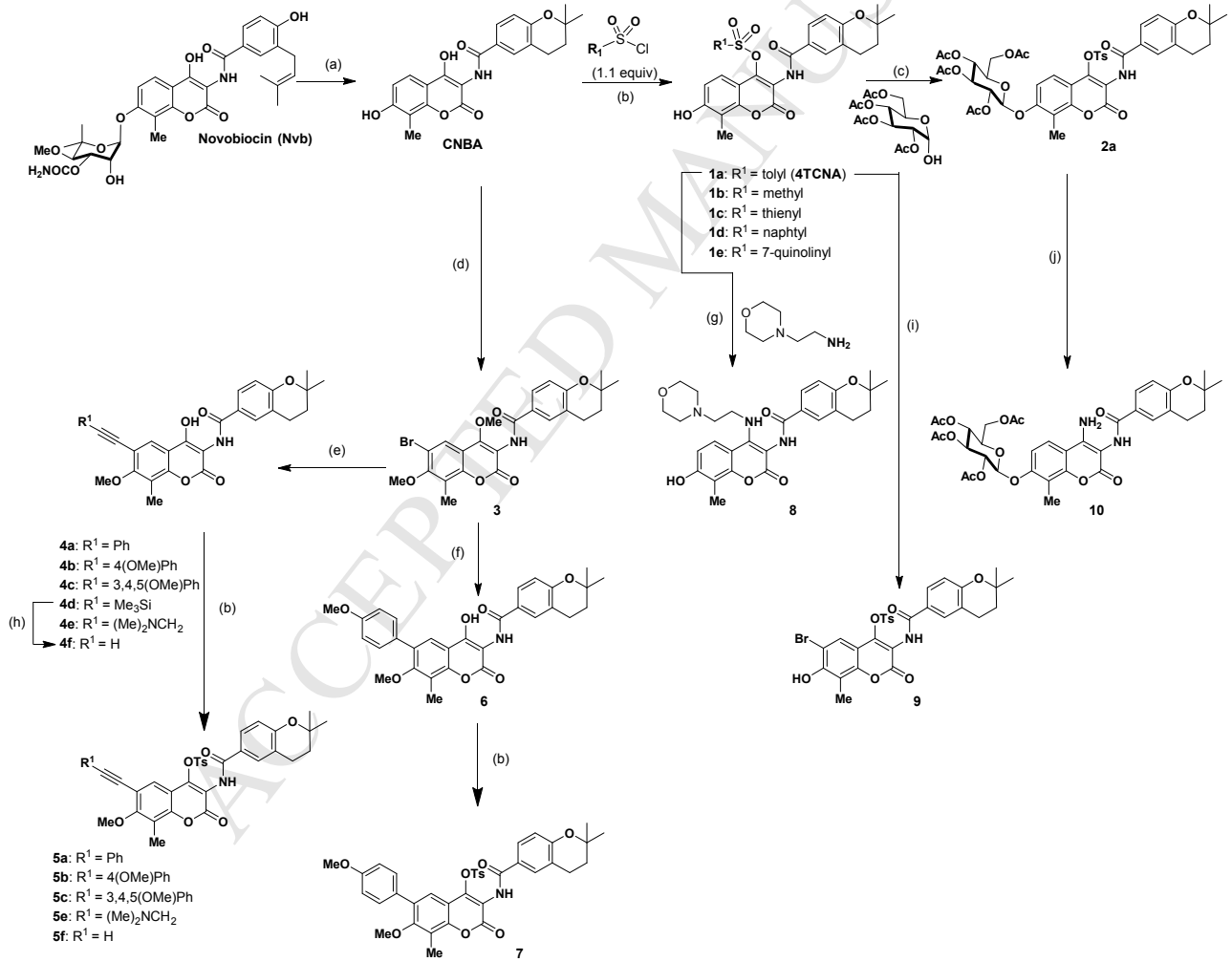


Figure 1.

Scheme 1. Synthesis of compounds **1-10**.



[a]Reagents and conditions: (a) HCl (12 M), EtOH, reflux, 1 h (b) Pyridine, 0° C to RT, 2.5 h. (c), DIAD (2 equiv), PPh_3 (2 equiv), THF, -78 °C to RT, 24 h (d) (*i*) MPHT (2 equiv), MeCN, 80 °C, 16 h (*ii*) K_2CO_3 (6 equiv), Me_2SO_4 (6 equiv), DMF, RT, 12 h, (e) $PdCl_2(PPh_3)_2$ (6 mol%), CuI (6 mol%), alkyne (1 equiv), PPh_3 (20 mol%), DMF/Et_2NH (1:3), 120 °C, 30 min; (f) (*i*) $Pd(OAc)_2$ (5 mol%), Xphos (20 mol%), KF (3 equiv), THF, reflux, 24 h (*ii*) DMF/Et_2NH (1:3), 120 °C, 30 min. (g) Dioxane, sealed tube, 100 °C. (h) K_2CO_3 (2 equiv), MeOH, RT, 2 h. (i) Br_2, AcOH, 24 h, RT. (j) NH_3 (gas), THF, RT, 24 h.

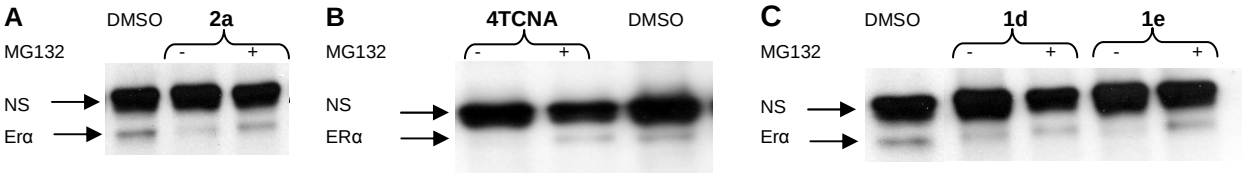


Figure 2 Effects of quinolone analogues **1d**, **1e**, **2a** and **4TCNA** on ERα stability. MCF-7 cells were grown and exposed to hsp90 inhibitors (**1d**, **1e**, **2a** and **4TCNA**, 100 μM) as described in Experimental Section in the presence (+) or absence (−) of the proteasome inhibitor MG132 (5 μM) for 24 h and cell lysates were analyzed by Western blotting with regard to the levels of ERα. DMSO was used as a control, NS = nonspecific protein band detected in these conditions and serving as a control of constant protein loading.

Highlights

A new series of novobiocin analogues was designed and synthesized.

Compound **2b** showed GI_{50} values at a micromolar level in various human cancer cells.

2b is a potent inducer of G2/M arrest as well as apoptosis.

2b is able to induce proteasome-mediated degradation of ERα.

Supporting online material for

Synthesis and Antiproliferative Activity of Novobiocin Analogues as Potential Hsp90 Inhibitors

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In memory of Dr. Christine Radanyi

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