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ORIGINAL ARTICLE

Synthesis of novel xanthone and acridone carboxamides with potent antiproliferative activities

Antonios Georgakopoulos [a], Amalia D. Kalampaliki [a], Katerina Gioti [b], Sami Hamdoun [c,1], Aikaterini F. Giannopoulou [d], Thomas Efferth [c], Dimitrios J. Stravopodis [d], Roxane Tenta [b], Panagiotis Marakos [a], Nicole Pouli [a], Ioannis K. Kostakis [a,*]

[a] *Department of Pharmacy, Division of Pharmaceutical Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771 Athens, Greece*
[b] *Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University Athens, Greece*
[c] *Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Mainz, Germany*
[d] *Section of Cell Biology and Biophysics, Department of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771 Athens, Greece*

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Abstract Several new amino-substituted acridone and xanthone derivatives have been designed and synthesized, using an efficient methodology from suitable acridone- or xanthone-carboxylic acid intermediates. The antiproliferative activity of the target compounds has been evaluated against four cancer cell lines, namely breast adenocarcinoma MCF-7, acute lymphocytic leukemia CCRF-CEM, and its doxorubicin-resistant variant CEM/ADR5000 and prostate cancer PC-3 cell lines. Selected derivatives have also been tested against the urinary bladder T24 and metastatic melanoma WM266-4 cancer cell lines. Two nitro substituted acridones, bearing a basic side chain as well, were endowed with a remarkable profile against the majority of the cell lines tested, with

* Corresponding author.
E-mail address: ikkostakis@pharm.uoa.gr (I.K. Kostakis).
[1] Present address: Faculty of Pharmacy, University of Khartoum, Khartoum, Sudan.

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IC_{50} values in the low micromolar range. Both compounds cause accumulation at G0/G1 phase, induce apoptosis, and act as potent autophagy inhibitors in PC-3 cells, suggesting their further evaluation in various pathophysiological environments, conditions, and regimens.

1. Introduction

Among the many different classes of chemotherapeutic agents used against malignant diseases, acridone and xanthone derivatives have been extensively studied as anticancer compounds. Furthermore, both scaffolds, xanthone and its aza-analogue acridone, have been used as lead compounds for the synthesis of various analogues, which are endowed with a number of interesting, albeit diverse, biological properties, such as anti-inflammatory (Chen et al., 2002; Feng et al., 2020), anti-viral (Tonelli et al., 2011; Bernal and Coy-Barrera, 2015), anti-allergic (Chukaew et al., 2008; Shagufta and Ahmad, 2016), anti-malarial (Kumar et al., 2009; Tomar et al., 2010; Yu et al., 2012) and anti-parasitic (Shagufta and Ahmad, 2016) functions. They are characterized by a π-conjugated planar structure, which makes them considerably hydrophobic and allows their interaction with different bio-molecular targets. Due to this unique planar system, they intercalate in between base pairs in the double-stranded DNA, thereby inhibiting DNA replication in the rapidly growing cancer cells (Chiron and Galy, 2004; Demeunynck, 2004; Belmont et al., 2007; Belmont and Dorange, 2008; Kaur and Singh, 2011; Zhang et al., 2014; Shagufta and Ahmad, 2016). It is well established that various acridone and xanthone analogues exert their potent anticancer activities, through inhibition, among others, of topoisomerases, telomerase and cyclin-dependent kinases. The effectiveness of bioactive acridone derivatives is affected by their ability to exist in two tautomeric forms. The heterocyclic nitrogen adopts either an acceptor or a donor conformation, which has a radical effect on the binding properties of the molecule.

The most representative examples in this field include pyrazoloacridines (Zalupski et al., 1998; Bu et al., 2002; Ramaswamy et al., 2011), thiadiazinoacridines (Antonini et al., 2003), triazoloacridones (Lemke et al., 2004, 2005), imidazoloacridones (Mazerska et al., 2003; den Brok et al., 2005), pyrazoloxanthones (Kostakis et al., 2005, 2006), and aminoxanthones (Kostakis et al., 2005). Among them, PZA, an amino substituted 5-nitropyrazolo[3,4,5-*kl*]acridine derivative (Zalupski et al., 1998; Ramaswamy et al., 2011), imidazoloacridine C-1311 (Cholody et al., 1990; Wiśniewska et al., 2007), acridine carboxamide DACA (Atwell et al., 1987; Wolf et al., 2009) and acronycine (Elomri et al., 1996; Guilbaud et al., 2002) are of great biological interest (Fig. 1); hence, many analogues of them have been synthesized. Following extensive SAR studies in this class of compounds, it has been proposed that the substitution with one or two basic side chains, a 7-methoxyl, or an electron-deficient nitro-group, play important role in their activity and selectivity.

As part of an extensive research program concerning the synthesis and pharmacological evaluation of acridone and xanthone analogues (Kostakis et al., 2002, 2005, 2006, 2008; Giannouli et al., 2007, 2015), we have recently reported the synthesis of several amino-substituted xanthones and acridones (general formulas I–III, Fig. 1). These compounds share common structural features with the biologically active derivatives described above, bearing at least one basic side chain together with a nitro group, or a second amino substituted side chain. They exhibit strong cytotoxic activities against a panel of cancer cell lines, probably due to DNA binding and intercalation.

Prompted by these results, we, next, decided to examine these two scaffolds further and we present herein the synthesis and biological evaluation of two series of novel xanthone and acridone carboxamides, with direct similarity to PZA and other amino substituted acridones. In these derivatives, a carbonyl group has been inserted between the amino side chain and the chromophore, in order to gain a better insight of the structure–activity relationships regarding: a) the influence of the altered flexibility and spatial arrangement of the amide side chain, b) the effect of an additional substitution with either a nitro group, or a second basic side chain, as well as an α,β-unsaturated side chain, which could probably be susceptible to nucleophilic attack from DNA bases (i.e. guanine's N-7) and c) the contribution of the closely related acridone or xanthone core to the biological activity.

2. Results and discussion

2.1. Chemistry

The synthesis of the amino-substituted xanthones and acridones started from 4-nitro-9-oxo-9,10-dihydroacridine-1-carboxylic acid (**8**) (Scheme 1), and 4-nitro-9-oxo-9*H*-xanthene-1-carboxylic acid (**17**) (Scheme 2). For the synthesis of acridone carboxylic acid **8**, we have used the nitro derivative **1** which was prepared from the commercially available methyl 4-aminobenzoate following a previously described procedure (Decodts et al., 1983; Knepper et al., 2006). Bromide **1** was then reacted with methyl anthranilate (**3**), in the presence of $Pd[P(C_6H_5)_3]_4$ as catalyst and Cs_2CO_3 as base, to yield the intermediate methyl ester **4**. Saponification of compound **4** and ring closure of the intermediate dicarboxylic acid **5**, upon treatment with concentrated H_2SO_4, provided the acridone carboxylic acid **8** (Burdeska et al., 1972). Unfortunately, the overall yield of this procedure was only 35%, taking also under consideration the difficult purification of the last step (42%); therefore, a slightly different synthetic pathway was examined. This involves the initial preparation of methyl ester **6**, by reaction of bromide **1** with anthranilic acid (**2**). Acid **6** was then ring closed, upon treatment with a mixture of TFAA and TFA, and the resulting methyl ester **7** was saponified to afford the desired acid **8** (85%) in 65% overall yield.

For the preparation of xanthone carboxylic acid **17**, we followed a slightly different approach from the methodology

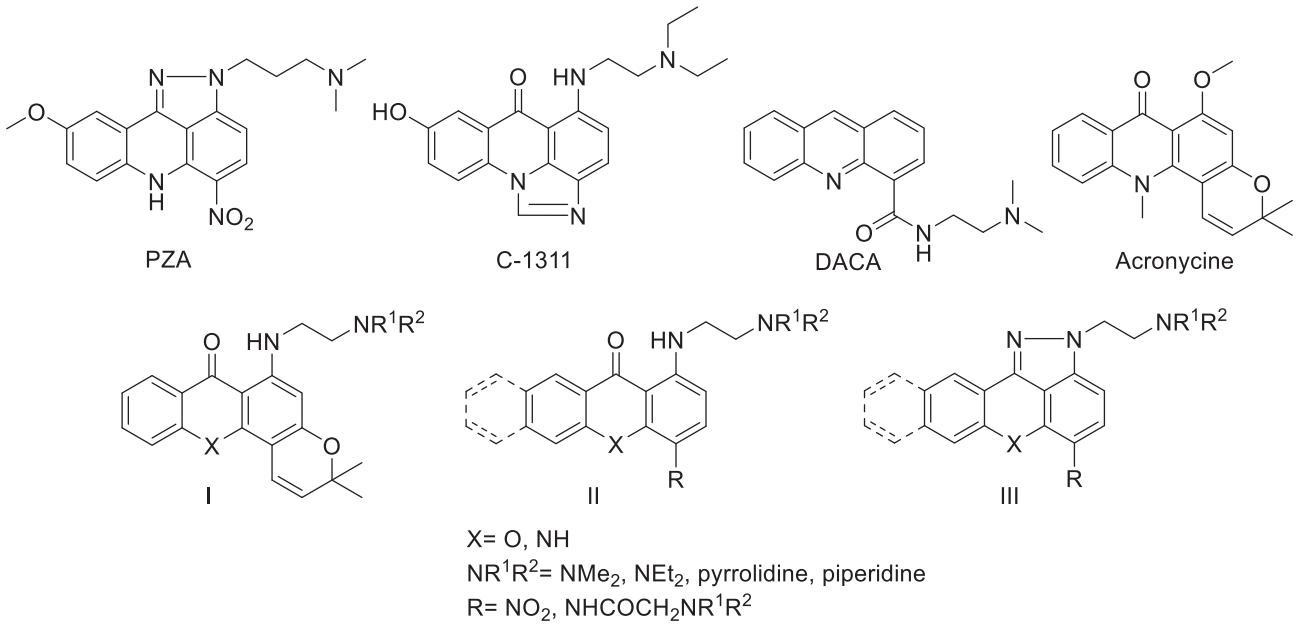
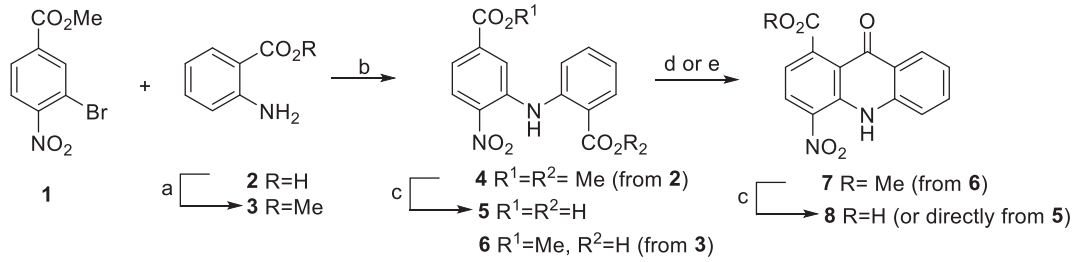
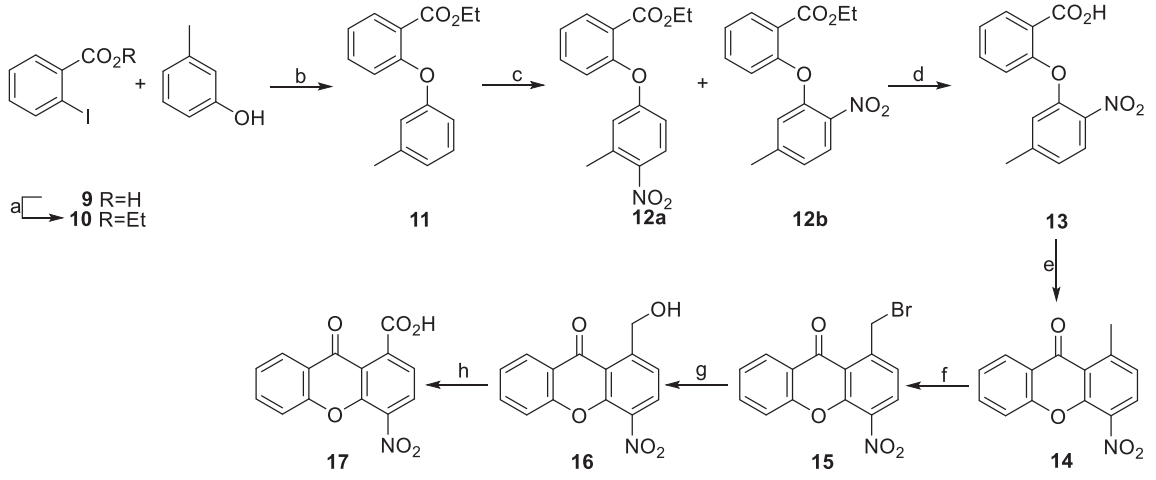


Fig. 1 Structures of PZA, C-1311, DACA, acronycine, and compounds prepared by our group (I – III).



Scheme 1 Reagents and conditions: a) c. H_2SO_4, abs. EtOH, reflux; b) Cs_2CO_3, $Pd[P(C_6H_5)_3]_4$, toluene (or toluene-DMA (3/1) for **6**), reflux; c) NaOH 15%, MeOH, rt; d) c. H_2SO_4, 90 °C; e) TFAA-TFA.



Scheme 2 Reagents and conditions: a) c. H_2SO_4, abs. EtOH, reflux; b) CuCl, K_2CO_3, Pyr, reflux; c) fum. HNO_3, 0 °C, rt; d) NaOH 40%, EtOH, rt; e) PPA, 110 °C; f) NBS, dibenzoyl peroxide, CCl_4, UV (140 Watt); g) $AgNO_3$, $(CH_3)_2CO$-H_2O, rt; h) Jones reagent, $(CH_3)_2CO$, rt.

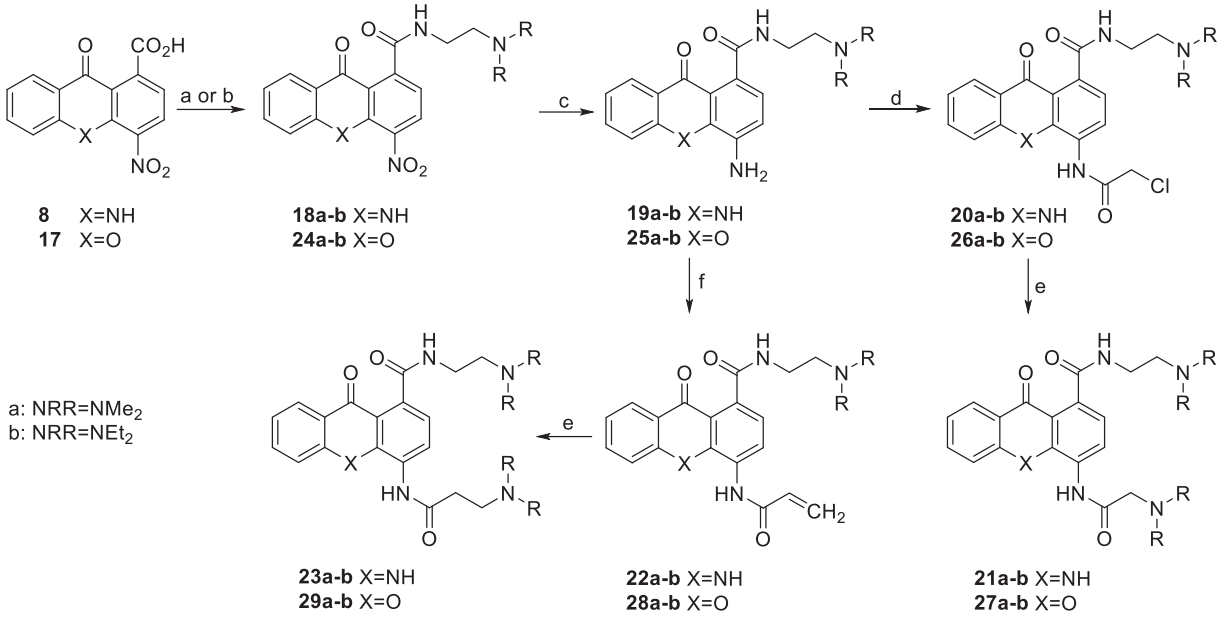
previously reported from our team (Hadjipavlou et al., 2006). Thus, commercially available 2-iodobenzoic acid was first esterified to the corresponding ethyl ester **10** (Baker et al., 1965; Hadjipavlou et al., 2006) and then reacted with m-cresol, in the presence of cuprous chloride and potassium carbonate, to provide the ether **11** (Hadjipavlou et al., 2006) (Scheme 2). Treatment of compound **11** with fuming nitric acid, in the presence of acetic anhydride, resulted in a mixture of isomeric nitro compounds **12a** and **12b**. Structural discrimination of compounds **12a** and **12b** was unambiguously established by NMR spectroscopy using both direct and long-range experiments. The nitration site resulted from the observation that for compound **12b**, two aromatic protons (namely H-4′ and H-6′, exhibit ^3J coupling with the methyl group, while in the case of compound **12a**, only H-2′ exhibits ^3J coupling with the methyl group. Then, ester **12b** was saponified under mild conditions, and the resulting carboxylic acid **13** was ring closed upon treatment with PPA providing xanthone **14** (Recanatini et al., 2001). Xanthone **14** was then brominated with NBS, in the presence of a catalytic amount of dibenzoyl peroxide applying UV irradiation, to give the bromomethyl analogue **15** (Recanatini et al., 2001), which upon hydrolysis in the presence of $AgNO_3$, provided the benzyl alcohol **16**. The target carboxylic acid **17** was then obtained from xanthone **16** upon oxidation with Jones reagent.

The nitro derivatives **18a-b** and **24a-b** were prepared upon amidation of acridone acid **8** and xanthone acid **17** respectively, with the appropriately substituted amines (Scheme 3). The ^{1}H NMR spectra of compounds **18a-b** display a characteristic doublet in the range of 8.15–8.28 ppm, integrating for 1 proton, attributed to H-8. On the other hand, H-3 appears downfield as a doublet at 8.44–8.60 ppm, while H-2 shifts upfield at 7.04–7.14 ppm. Next, the nitro group was reduced by hydrogenation over palladium on activated carbon, to afford the anilines **19a-b** and **25a-b**, which upon treatment with chloracetyl chloride afforded the amides **20a-b** and **26a-b**. Finally, reaction of these amides with the suitable amines resulted in the target diamines **21a-b** and **27a-b**. By analogy to compounds **18a-b**, the ^{1}H NMR spectrum of **21a-b** shows a doublet at 7.82–7.90 ppm for H-8. The most characteristic H-3 appears as a doublet at 7.30–7.36 ppm while the proton at position 2 observed as a doublet at 6.68–6.70 ppm.

For the synthesis of the acrylamides **22a-b** and **28a-b**, and the diamines **23a-b** and **29a-b**, we followed a similar strategy. Therefore, anilines **19a-b** and **25a-b** were converted to the corresponding acrylamides **22a-b** and **28a-b** respectively, by treatment with 3-chloropropionyl chloride, in the presence of potassium carbonate. Finally, the aforementioned acrylamides, underwent 1,4-Michael's addition using suitable secondary amines to provide the target diamines **23a-b** and **29a-b**.

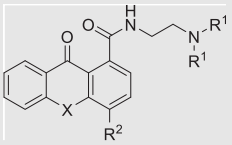
2.2. Biological activity

All acridone and xanthone analogues were evaluated for their antiproliferative activities against four human cancer cell lines, namely, MCF-7 (breast adenocarcinoma) (Seo et al., 2015), CCRF-CEM and its variant CEM/ADR5000, a doxorubicin-resistant sub-clone (acute lymphocytic leukemia: ALL) (Kimmig et al., 1990; Efferth et al., 2008; Kadioglu et al., 2016) and PC-3 (prostate cancer) (Vistica et al., 1991). For comparative reasons and in order to further evaluate the activity of the new compounds, four derivatives which emerged from the initial screening, namely **18a**, **18b**, **28b** and **29b**, were also examined against two additional human cancer cell lines, the T24 (urinary bladder cancer) (Giannopoulou et al., 2019b) and the MW266-4 (metastatic melanoma / skin cancer) (Giannopoulou et al., 2019a), which are being typified by high malignancy grades and strong metastatic capacities (Table 1). Furthermore, for compounds **18a** and **18b** flow cytometric analysis of DNA content was also conducted.



Scheme 3 Reagents and conditions: a) 1. CDI, dry DMF, rt; 2. suitable diamine, 50 °C; b) 1. NBS, PPh_3, dry CH_2Cl_2, 0 °C; 2. suitable diamine, rt; c) H_2, Pd/C, 50 psi, EtOH abs., rt; d) K_2CO_3, chloroacetyl chloride, dry CH_2Cl_2; e) suitable secondary amine, EtOH abs., 60 °C; f) 3-chloropropionyl chloride, K_2CO_3, dry CH_2Cl_2, rt.

Table 1 Accumulative results of the antiproliferative activities for all the synthesized compounds.



Cmp	X	R^1	R^2	MCF-7 % MCV[a] (30 μM)	CCRF/CEM % MCV[a] (10 μM)	CEM/ADR5000 % MCV[a] (10 μM)	PC3 IC_{50}[b] (μM)	T24 %MCV[c]	MW266-4 % MCV[c]
18a	NH	CH_3	NO_2	82.08 ± 0.83	**58.92 ± 3.42**	86.63 ± 3.38	**1.20 ± 0.06**	50.80 ± 1.30 (50 μM) 37.40 ± 2.37 (100 μM)	52.00 ± 2.14 (50 μM) 26.50 ± 0.82 (100 μM)
18b	NH	CH_2CH_3	NO_2	73.84 ± 1.40	**56.67 ± 2.32**	87.94 ± 1.85	**2.00 ± 0.05**	56.00 ± 0.90 (50 μM) 40.50 ± 1.03 (100 μM)	54.60 ± 0.77 (50 μM) 29.20 ± 1.09 (100 μM)
21a	NH	CH_3	$NHCOCH_2N(CH_3)_2$	86.42 ± 1.44	84.32 ± 3.48	84.27 ± 2.91	> 40		
21b	NH	CH_2CH_3	$NHCOCH_2N(CH_2CH_3)_2$	86.03 ± 1.19	86.71 ± 1.79	103.09 ± 1.82	40.00 ± 1.72		
22a	NH	CH_3	$NHCOCH = CH_2$	85.06 ± 2.04	80.40 ± 2.18	92.68 ± 2.86	> 40		
22b	NH	CH_2CH_3	$NHCOCH = CH_2$	82.87 ± 1.17	84.56 ± 2.53	92.42 ± 2.36	> 40		
23a	NH	CH_3	$NHCOCH_2CH_2N(CH_3)_2$	84.60 ± 1.64	87.77 ± 2.07	96.29 ± 3.14	> 40		
23b	NH	CH_2CH_3	$NHCOCH_2CH_2N(CH_2CH_3)_2$	85.43 ± 1.50	87.94 ± 1.61	94.20 ± 2.95	> 40		
24a	O	CH_3	NO_2	84.56 ± 1.23	75.79 ± 2.32	88.84 ± 2.31	24.20 ± 0.91		
24b	O	CH_2CH_3	NO_2	86.41 ± 1.35	75.37 ± 1.92	89.46 ± 0.78	33.30 ± 1.20		
27a	O	CH_3	$NHCOCH_2N(CH_3)_2$	83.76 ± 2.31	75.72 ± 3.84	86.17 ± 3.03	14.30 ± 0.41		
27b	O	CH_2CH_3	$NHCOCH_2N(CH_2CH_3)_2$	81.33 ± 1.16	73.54 ± 2.85	83.45 ± 3.80	15.30 ± 0.52		
28a	O	CH_3	$NHCOCH = CH_2$	83.84 ± 1.17	75.07 ± 2.57	82.02 ± 2.33	31.00 ± 0.94		
28b	O	CH_2CH_3	$NHCOCH = CH_2$	N/T	**61.09 ± 3.38**	**59.43 ± 4.01**	**7.20 ± 0.23**	60.60 ± 1.54 (50 μM) 14.40 ± 0.87 (100 μM)	2.20 ± 0.83 (50 μM) 3.00 ± 0.72 (100 μM)
29a	O	CH_3	$NHCOCH_2CH_2N(CH_3)_2$	85.85 ± 0.83	66.58 ± 3.73	86.77 ± 2.32	18.30 ± 0.65		
29b	O	CH_2CH_3	$NHCOCH_2CH_2N(CH_2CH_3)_2$	85.10 ± 0.93	67.81 ± 1.95	85.72 ± 3.00	**7.40 ± 0.23**	82.40 ± 1.95 (50 μM) 77.90 ± 3.35 (100 μM)	96.20 ± 0.57 (50 μM) 38.60 ± 1.33 (100 μM)
5-FU (Fluorouracil)							3.10 ± 0.09		

[a] The results represent means [± standard error of the mean (SEM)] of three independent experiments and are expressed as % mean cell viability (MCV). [b]The results represent means [± standard deviation (SD)] of three independent experiments and are expressed as IC_{50}. [c]The results represent means [± standard deviation (SD)] of three independent experiments and are expressed as % mean cell viability (MCV).

2.2.1. In vitro antiproliferative activity against MCF-7 cell line

The cytotoxicity of target compounds was tested using the Resazurin assay (O'Brien et al., 2000). The results, expressed as cell viability at 30 μM, can be visualized in Supplementary Figure S1. All the new compounds showed low activity and only the nitro-acridone **18b**, displayed an interesting activity against MCF-7 cell line with 73.84% ± 1.40 cell viability. It has been reported that certain quinone type derivatives are also endowed with cytotoxic effects against this cell line and this could be marginally related to the activity of the compounds presented herein (Mollica et al., 2014).

2.2.2. In vitro antiproliferative activity against CCRF-CEM and CEM/ADR5000 cell lines

The new compounds were assayed *in vitro* for their antiproliferative activities against CCRF-CEM and CEM/ADR5000 cell lines using the Resazurin assay (O'Brien et al., 2000). Results, expressed as % mean cell viability at 10 μM, are summarized in Supplementary Figure S2. The majority of the new compounds show improved cytotoxic properties against the two ALL cell lines, compared to the MCF-7 one. The cytotoxic potency of xanthone analogues is slightly higher than the one of corresponding acridones. However, it is noticeable

that acridones **18a** and **18b**, bearing a nitro substitution, are the most potent derivatives in this series and display mean cell viabilities of 58.92% ± 3.42 and 56.67% ± 2.32 respectively, in CCRF-CEM cell line. This is consistent with the observations concerning the MCF-7 cell line and unveils the beneficial effect of the nitro group in terms of cytotoxicity. Notably, from a direct comparison between the CCRF-CEM and CEM/ADR5000 respective responses to the administered compounds, it is clearly observed that the derivatives show comparable anticancer activities against both cell lines, with the exception of the nitro acridones **18a** and **18b**, which are less effective against the doxorubicin-resistant sub-clone CEM/ADR5000. These results strongly suggest the importance of the acridone core and the nitro substitution, thus, providing evidence regarding the favorable scaffold and substitution for the maximization of cytotoxic capacity of these classes of compounds.

It must be underlined that although the antiproliferative activity of the di-substituted xanthones **28a-b** and **29a-b** is moderate, their potency is enhanced, compared to the di-substituted acridones **22a-b** and **23a-b**. Compound **28b** exhibits the highest cytotoxicity among the xanthone analogues, with mean cell viability 61.09% ± 3.38, followed by compounds **29a-b**. Besides, compound **28b** showed high cytotoxicity against the doxorubicin-resistant CEM/ADR5000 cell sub-clone, with an inhibitory activity of approximately 40%, at 10 μM. On the contrary, the acridone analogue **22b** possesses negligible activity, whereas the most active derivative among the acridone analogues, **18b**, possesses a remarkable inhibitory activity of approximately 44% against the parental CCRF/CEM cells and only 13% against the resistant sub-clone. This could suggest that the di-substituted xanthones possessing an additional side chain could partly overcome multi-drug resistance in an ALL cellular setting.

2.2.3. *In vitro antiproliferative activity against PC-3 cell line*

The antiproliferative activity of the new compounds was evaluated *in vitro* against the PC-3 human prostate cancer cell line. The results of the MTT dye reduction assay, expressed as 50% inhibitory concentrations (IC_{50}) in μM, are depicted in Table 1. The majority of xanthone analogues show interesting anticancer activities, with IC_{50} values varying within the range of 7.20 to 33.3 μM. In general, the di-substituted compounds **27a-b**, **28a-b**, and **29a-b** seem considerably more active when compared with the nitro substituted analogues **24a-b**. These data indicate that the replacement of the nitro group with a second basic side chain affords higher cytotoxicity against prostate cancer cells.

On the contrary, we observe that the di-substituted acridone analogues **21a-b**, **22a-b**, and **23a-b**, appear to be considerably less active when compared with the corresponding di-substituted xanthones **27a-b**, **28a-b**, and **29a-b**. In consistence with the previous results, the nitro acridones **18a** and **18b** emerge as the most potent compounds, showing strong cytotoxicity with IC_{50} values of 1.20 and 2.00 μM, respectively. The data indicate that in the case of the acridone analogues, in contrast to the xanthone counterparts, the replacement of the nitro group significantly reduces the activity. This could probably be attributed to a different mechanism of action; nevertheless, the existence of the two tautomeric forms, which the acridone core can adopt, could have a radical effect on the activity. This issue remains to be clarified.

2.2.4. *In vitro antiproliferative activity against T24 human urinary bladder cancer cell line and WM266-4 human metastatic melanoma cancer cell line*

Through employment of an MTT-based protocol, a cancer-cell type-specific sensitivity to the four herein tested compounds is clearly revealed (see Supplementary Figures S3 and S4). An overall assessment typifies T24 (human) urinary bladder cancer cells (Stravopodis et al., 2009; Giannopoulou et al., 2019b) more refractory to each one of the four examined compounds, as compared to the WM266-4 (human) metastatic melanoma (skin cancer) cells (Giannopoulou et al., 2019a). Specifically, compounds **18a** and **18b** seem capable to significantly kill both T24 and WM266-4 cancer-cell types, at 50 and 100 μM compound doses, with T24 and WM266-4 retaining survival-percentage values of approximately 40% and 30%, respectively, at the highest compound concentration being added. Similarly, administration of compound **28b**, with a dose of 50 or 100 μM, results in complete eradication of WM266-4 cell populations, with the highest compound concentration remarkably reducing T24 viability, albeit rather incompletely, since an approximate 15% of T24 cells remains unaffected in the presence of 100 μM of **28b**. In contrast to **28b**, compound **29b** was effective against T24 or WM266-4 cells only in the highest concentration tested. Interestingly, although T24 are rather refractory to 100 μM of **29b**, WM266-4 cells are presented prominently vulnerable to the cytotoxic power of **29b** compound.

Since compound **29b** carries surprisingly decreased killing activity against T24 and WM266-4 cancer cells, when compared to the vinyl substituted compound **28b**, it could be suggested that the electrophilic character of **28b** is in favor of enhanced cytotoxicity regarding the specific cell lines.

2.2.5. *Flow cytometric analysis of DNA content*

Cell-cycle perturbation and arrest of exponentially growing PC-3 cells with compounds **18a** and **18b** for 24, 48 and 72 h are given in Table 2. Both compounds caused accumulation of PC-3 cells at G0/G1 phase, reducing in parallel the percentage of cells at G2/M and S phase of the cycle (though the reduction of S phase was marginally non-significant).

Table 2 PC-3 cell cycle distribution (%) after 24-, 48- and 72-hour treatment of the compounds **18a** and **18b** in concentration equal to the corresponding IC_{50} value.

Sample (Compound)	**Time** (h)	**G0/G1** (%)	**S** (%)	**G2/M** (%)
Control	24	56.4	17.1	26.5
18a	24	60.3	16.1	23.6
18b	24	61.1	18	20.9
Control	48	59	17.2	23.8
18a	48	67.9	15.3	16.8
18b	48	70	13.3	16.7
Control	72	54.1	15	30.9
18a	72	60.5	12.3	27.2
18b	72	60.6	17.3	22.1

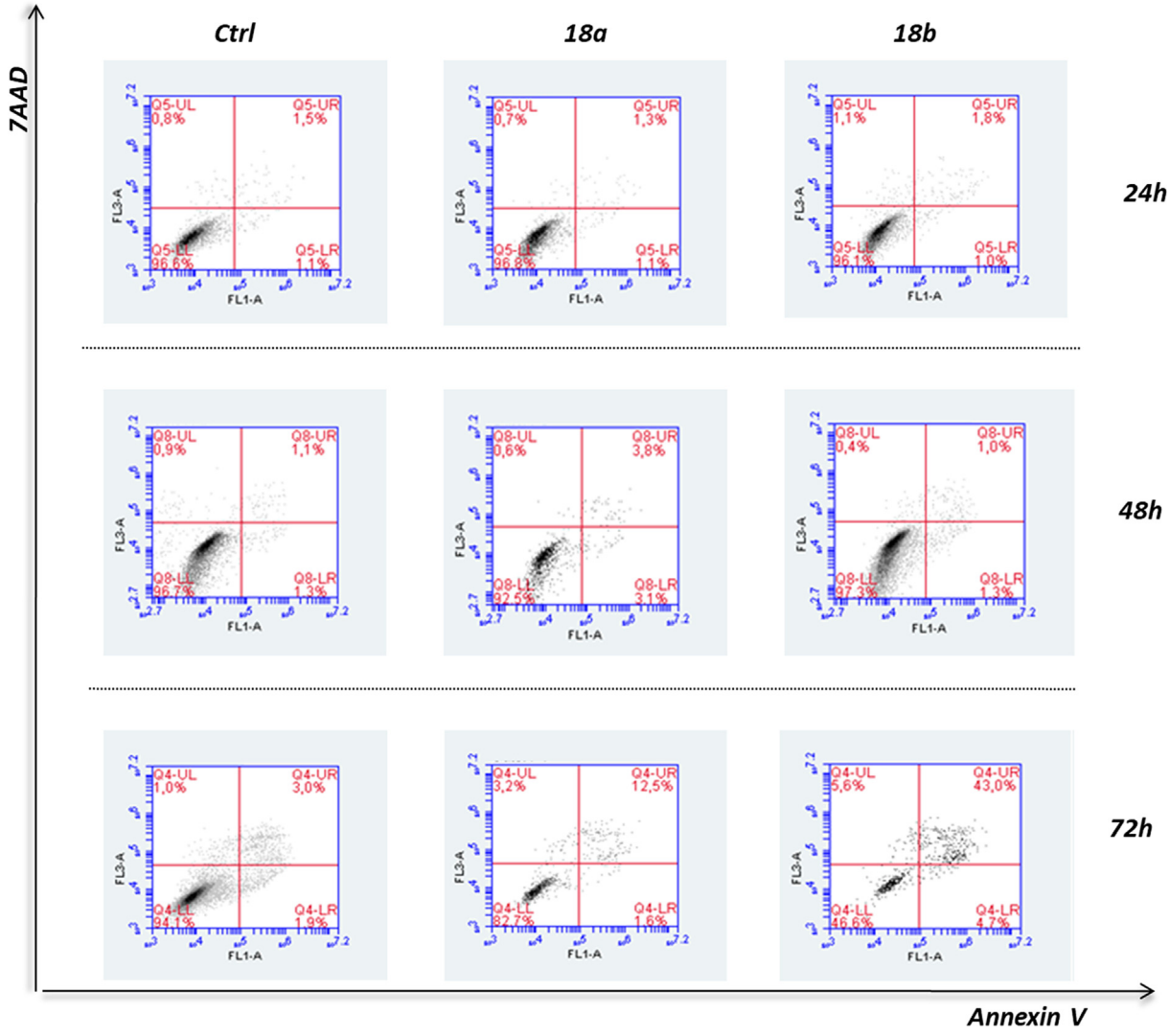


Fig. 2 Flow cytometric analysis of DNA content. Early or late apoptosis and necrosis were estimated in PC-3 cells after 24-, 48- and 72-h exposure to the compounds **18a** and **18b**, versus control cells (Ctrl), based on the AnnexinV − 7AAD staining. Representative graphs illustrate the overall apoptosis activated upon each treatment, on an incubation-time basis.

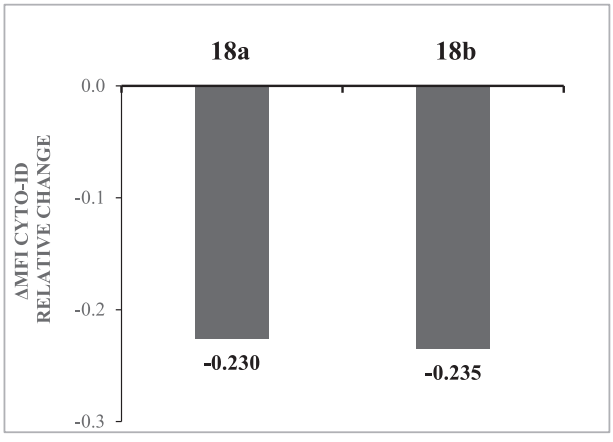


Fig. 3 Representative flow cytometry graphs showing ΔMFI Cyto-ID after 48-hour treatment of the compounds **18a** and **18b** in PC-3 cells, in concentration equal to the corresponding IC_{50} values. [ΔMFI Cyto-ID = MFI Cyto-ID (treated) - MFI Cyto-ID (control)].

Nevertheless, both compounds were found to significantly induce apoptosis of PC-3 cells at 72 h, with **18b** being comparatively the most potent. More specifically, **18b** induced at 72 h treatment the mobilization of late apoptosis at an elevated 40% ratio, as compared to control cells, whereas **18a** increased late apoptosis almost 10% at 72 h of exposure (Fig. 2).

The semi-quantification of autophagy engagement revealed that **18a** and **18b** showed a similar pattern compared to control cells, by decreasing the levels of CYTO ID fluorescence signal (ΔMFI = -0.230) and (ΔMFI = -0.235), respectively (Fig. 3). ΔMFI values for rapamycin (potent autophagy inducer) and chloroquine (potent autophagic flux inhibitor) were calculated as 0.800 and −0.420, respectively (Guo et al., 2011; Guo and White, 2013). This indicates that both **18a** and **18b** likely act as potent autophagy inhibitors in a PC-3 cellular environment (Fig. 3).

Since autophagy seems to facilitate the survival of hypoxic cells and hypoxia has been associated with resistance to anti-cancer therapy (Tan et al., 2016), novel compounds **18a** and **18b** (by inhibiting autophagy) may hold strong promise in

the prompt development of new drug cocktail-based regimens for the successful management of human prostate cancer in the clinic.

3. Conclusions

Two series of novel compounds possessing the acridone and xanthone scaffold were synthesized and evaluated for their antiproliferative activities against a panel of four tumor cell lines. Selected derivatives have been subsequently examined against two additional highly metastatic cancer cell lines. Two analogues with the most interesting cytotoxic properties have also been studied in terms of cell-cycle perturbation and arrest of exponentially growing PC-3 cells, as well as for their ability to affect apoptosis and autophagy. All new compounds possess moderate to good antiproliferative properties, with the most interesting results - among all inhibitors - being extracted from their profile against the human prostate cancer cells. Apart from the nitro substituted analogues, 18a and 18b, no other acridone-containing derivative possesses a considerably interesting profile against the tested cell lines, in contrast to the synthesized compounds belonging to the xanthone series. Compounds **18a** and **18b**, which also bear a basic side chain, emerged as the most efficient of the acridone series, with the best activities being demonstrated against the PC-3 (prostate cancer) cells. Interestingly, both compounds were found to act as potent autophagy inhibitors in a PC-3 cellular environment, causing accumulation of PC-3 cells at G0/G1 phase and significantly induce apoptosis. Regarding xanthone analogues, all of them showed interesting cytotoxicity profiles, although, in this case the nitro xanthones were not among the noteworthy analogues. Compounds 28b and 29b, bearing an additional side chain, appear very potent against the PC-3 cell line, with IC_{50} values estimated at the low micromolar range, especially the vinyl-substituted derivative **28b**, which in parallel showed substantial cytotoxic activity against T24 and mainly WM266-4 cancer-cells. When compared to the previously reported xanthone analogues which possess a direct amino substituent on the chromophore (Kostakis et. al. 2006), we could state that the carboxamides presented in this work appear overall less cytotoxic. This could indicate that in this scaffold the insertion of the carbonyl group is not beneficial for the biological activity.

In terms of their clinical relevance and therapeutic potential, **18a**, **18b**, **28b** and **29b** are suggested to be further optimized and investigated for prostate cancer treatment. Although the mechanisms for the tumor-suppressing functions of the herein described novel compounds are still unclear, our results indicate that in the case of xanthone analogues, the replacement of nitro group with a second side chain significantly improves the anticancer activity. Furthermore, the nitro acridones appear considerably more potent, when compared with the corresponding nitro xanthones, whereas the di-substituted compounds are inactive. These results strongly suggest the pivotal role of these "structural transformations" in the mechanism of action for the herein presented compounds. Since a number of tumors seem to require autophagy for their survival and growth, the **18a**- and **18b**- mediated suppression of autophagic flux may compel prostate cancer cells (PC-3) to apoptotic death; yet, more studies are needed to further clarify how these alterations of the autophagic potential may serve as death signals. On the basis of these results, the establishment of a preliminary Structure-Activity-Relationship (SAR) is feasible, in order to determine the structural features that possess a crucial role in enhanced and targeted inhibition of cancer cell survival, and growth

4. Experimental section

4.1. Chemistry

All commercially available chemicals were purchased from Alfa Aesar. Melting points were determined on a Büchi apparatus and were uncorrected. ^{1}H NMR, ^{13}C NMR and 2D spectra were recorded on a Bruker Avance III 600, 400 and 200 spectrometer (Bruker GmbH, Germany) using dimethyl sulfoxide (DMSO d_6), methanol (CD_3OD) and chloroform ($CDCl_3$) as deuterated solvents and were referenced to TMS (δ scale). The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: ^{1}H^{1}H COSY, HMQC, and HMBC. ^{1}H NMR and ^{13}C NMR of compounds **1** (Decodts et al., 1983; Knepper et al., 2006), **8** (Burdeska et al., 1972), **11** (Hadjipavlou et al., 2006), **14** (Recanatini et al., 2001), **15** (Recanatini et al., 2001) and **17** (Burdeska et al., 1972) were confirmed with those reported in the literature (See Supplementary Material). Flash chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Analytical thin layer chromatography (TLC) was carried out on pre-coated (0.25 mm) Merck KgaA (Darmstadt, Germany) silica gel F-254 plates. HRMS were obtained on an LTQ-Orbitrap Discovery Mass Spectrometer (Thermo Scientific, Brehmen, Germany).

4.1.1. Methyl 3-{[2-(methoxycarbonyl)phenyl]amino}-4-nitrobenzoate (4)

A suspension of methyl anthranilate (617 mg, 4.08 mmol, **2**), 3-bromo-4-nitrobenzoate (1.06 g, 4.08 mmol, **1**), Cs_2CO_3 (6.64 g, 18 mmol) and tetrakis(triphenylphosphine)palladium (0) (235 mg, 0.20 mmol) in dry toluene (100 mL), was heated at 110 °C, under argon, for 24 h. After completion of the reaction, the volatiles were removed under reduced pressure and the residue was extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated to dryness. Flash chromatography on silica gel, using a mixture of cyclohexane / EtOAc 10 / 1, as the eluent, afforded 1.2 g (89%) of the title compound **4**. M.p. 160–161 °C (CH_2Cl_2 - *n*-Pentane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.16 (s, D_2O exch., 1H, NH), 8.28 (d, J = 1.5 Hz, 1H, H-2), 8.21 (d, J = 8.7 Hz, 1H, H-5), 8.06 (d, J = 8.7, 1H, H-3′), 7.55–7.47 (m, 3H, H-6′, H-5′, H-6), 7.10 (td, J = 8.2 Hz, 2.0 Hz, 1H, H-4′), 3.97 (s, 3H, CH_3), 3.91 (s, 3H, CH_3′); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.5 ($COOCH_3$′), 165.4 ($COOCH_3$′), 141.9 (C-1′), 139.4 (C-4), 138.9 (C-3), 135.5 (C-1), 133.7 (C-5′), 132.2 (C-3′), 126.9 (C-5), 122.4 (C-4′), 120.3 (C-2), 120.0 (C-6), 118.9 (C-6′), 118.8 (C-2′), 52.8 ($COOCH_3$), 52.4 ($COOCH_3$′); HRMS (ESI$^-$) m/z 329.0779 (calcd for $C_{16}H_{13}N_2O_6^-$, 329.0771).

4.1.2. 3-[(2-Carboxyphenyl)amino]-4-nitrobenzoic acid (5)

To a solution of the diester **4** (1.2 g, 3.6 mmol) in methanol (150 mL), at room temperature, was added dropwise a cold

15% NaOH solution (10 mL) and the mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was poured into water, acidified with 18% HCl solution (pH ~ 2) and the resulting solid was filtered and dried over P_2O_5 to afford 1.05 g (96%) of the title compound **5**, which was used for the next step without any further purification. M.p. > 270 °C (MeOH - Et_2O); ^{1}H NMR (600 MHz, DMSO d_6) δ 11.12 (s, D_2O exch., 1H, NH), 8.26 (d, J = 7.6 Hz, 1H, H-5), 8.16 (s, 1H, H-2), 8.05 (d, J = 7.6 Hz, 1H, H-3′), 7.61 (m, 2H, H-5′, H-6′), 7.55 (d, J = 7.6 Hz, 1H, H-6), 7.19 (t, J = 8.7 Hz, 1H, H-4′); ^{13}C NMR (151 MHz, DMSO d_6) δ 168.6 (*C*OOH), 165.7 (*C*OOH), 141.6 (C-1′), 138.3 (C-3), 136.4 (C-1), 133.7 (C-5′), 131.9 (C-3′), 130.0 (C-4), 127.0 (C-5), 122.3 (C-4′), 120.2 (C-2), 119.8 (C-6), 119.0 (C-6′), 118.9 (C-2′).

*4.1.3. 2-{[5-(Methoxycarbonyl)-2-nitrophenyl]amino}benzoic acid (**6**)*

A suspension of methyl 3-bromo-4-nitrobenzoate (1 g, 3.80 mmol, **1**), anthranilic acid (615 mg, 4.07 mmol, **3**), Cs_2CO_3 (7.2 g, 20.40 mmol) and tetrakis(triphenylphosphine)palladium (0) (235 mg, 0.20 mmol) in a 3 / 1 mixture of toluene / *N*, *N*-dimethylacetamide (100 mL), was stirred under reflux, for 24 h, under argon. After completion of the reaction, the volatiles were removed under reduced pressure and the residue was poured into water, acidified with 18% HCl solution (pH ~ 2) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. Flash chromatography on silica gel, using a mixture of cyclohexane / EtOAc 1 / 3, as the eluent, afforded 1.1 g (92%) of the title compound **6**. M.p. 230–231 °C (THF - *n*-Pentane); ^{1}H NMR (400 MHz, DMSO d_6) δ 11.10 (s, D_2O exch., 1H, NH), 8.23 (d, J = 7.6 Hz, 1H, H-5), 8.11 (d, J = 1.7 Hz, 1H, H-2), 7.99 (d, J = 7.6 Hz, 1H, H-3′), 7.58–7.49 (m, 3H, H-5′, H-6′, H-6), 7.15 (t, J = 8.7 Hz, 1H, H-4′), 3.84 (s, 3H, $COOCH_3$); ^{13}C NMR (151 MHz, DMSO d_6) δ 168.6 (*C*OOH), 164.7 (*C*$OOCH_3$), 141.6 (C-1′), 139.2 (C-4), 138.3 (C-3), 135.0 (C-1), 133.7 (C-5′), 131.9 (C-3′), 127.1 (C-5), 122.4 (C-4′), 120.0 (C-2), 119.8 (C-6), 119.0 (C-2′), 118.9 (C-6′), 53.3 ($COOCH_3$); HRMS (ESI$^-$) m/z 329.0623 (calcd for $C_{15}H_{11}N_2O_6^-$, 329.0627).

*4.1.4. Methyl 4-nitro-9-oxo-9,10-dihydroacridine-1-carboxylate (**7**)*

A suspension of acid **6** (3.25 g, 10.28 mmol) in a 2 / 1 mixture of trifluoroacetic acid - trifluoroacetic anhydride (12 mL) was stirred at room temperature for 14 h. After completion of the reaction, the mixture was poured into crushed ice, the precipitate was filtered, washed with water and air dried, to afford 2.54 g (83%) of the title compound, which was used for the next step without any further purification. M.p. > 270 °C (THF - Et_2O). ^{1}H NMR (600 MHz, DMSO d_6) δ 11.64 (s, D_2O exch., 1H, NH), 8.71 (d, J = 8.2 Hz, 1H, H-3), 8.20 (d, J = 7.4 Hz, 1H, H-8), 8.11 (d, J = 8.4 Hz, 1H, H-5), 7.84 (t, J = 7.5 Hz, 1H, H-6), 7.44 (t, J = 7.5 Hz, 1H, H-7), 7.36 (d, J = 8.4 Hz, 1H, H-2), 3.93 (s, 3H, CH_3); ^{13}C NMR (151 MHz, DMSO d_6) δ 175.0 (C-9), 168.7 (*C*OCH_3), 140.6 (C-4a), 139.8 (C-10a), 135.9 (C-4), 135.1 (C-1), 134.8 (C-6), 131.4 (C-3), 125.7 (C-8), 123.7 (C-7), 120.6 (C-8a), 119.4 (C-9a), 119.3 (C-2), 118.2 (C-5), 52.6 ($CO*C*H_3$); HRMS (ESI$^-$) m/z 297.0517 (calcd for $C_{15}H_9N_2O_5^-$, 297.0522).

*4.1.5. 4-Nitro-9-oxo-9,10-dihydroacridine-1-carboxylic acid (**8**)*

Method A: A solution of acid **5** (1 g, 3.31 mmol) in c. sulfuric acid (10 mL) was stirred at 110 °C for 2 h. After cooling, the mixture was poured into crushed ice, the precipitate was filtered, washed with water and dried (P_2O_5). Flash chromatography on silica gel, using a mixture of CH_2Cl_2 / MeOH 6 / 1, afforded the title compound **8** (400 mg, 42%). Method B: To a solution of ester **7** (1.5 g, 5.03 mmol) in methanol (60 mL), at room temperature, was added dropwise a cold 40% NaOH solution (2.5 mL) and the mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was poured into water, acidified with 18% HCl solution (pH ~ 2) and the resulting solid was filtered and dried over P_2O_5 to afford 1.35 g (85%) of the title compound **8**, which was used for the next step without any further purification.

*4.1.6. Ethyl 2-(5-methyl-2-nitrophenoxy)benzoate (**12b**)*

To a suspension of ester **11** (4.1 g, 16 mmol) in acetic anhydride (9.34 mL, 99 mmol) at 0 °C was added dropwise a solution of fuming HNO_3 (0.68 mL, 16 mmol) in acetic anhydride (2.36 mL, 25 mmol) and the mixture was stirred at room temperature for 24 h. After completion of the reaction, the mixture was poured into ice - water, basified with 15% NaOH solution (pH ~ 9) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over anh. Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (silica gel), using a mixture of petroleum ether / EtOAc (100 / 0 – 30 / 1), as the eluent, to afford 1.01 g (21%) of ester **12a** and 3.03 g (63%) of the ester **12b**.

Physicochemical data of ethyl 2-(5-methyl-2-nitrophenoxy) benzoate (**12a**): oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, J = 8.0 Hz, 2 Hz, 1H, H-6), 7.92 (d, J = 8.0 Hz, 1H, H-3′), 7.58 (td, J = 8.0 Hz, 2 Hz, 1H, H-4), 7.33 (td, J = 8.0 Hz, 2 Hz, 1H, H-5), 7.11 (d, J = 8.0 Hz, 1H, H-3), 6.95 (d, J = 8.0 Hz, 1H, H-4′), 6.58 (s, 1H, H-6′), 4.22 (q, J = 7.0 Hz, 2H, CH_2CH_3), 2.30 (s, 3H, CH_3) 1.16 (t, J = 7.0 Hz, 3H, CH_2CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 164.9 (*C*O), 153.8 (C-2), 151.7 (C-1′), 145.9 (C-5′), 137.8 (C-2′), 133.9 (C-4), 132.3 (C-6), 125.8 (C-3′),125.2 (C-5), 124.0 (C-1), 123.1 (C-4′), 122.1 (C-3), 118.5 (C-6′), 61.2 (CH_2CH_3), 21.6 (CH_3), 13.8 (CH_2CH_3).

Physicochemical data of ethyl 2-(3-methyl-4-nitrophenoxy) benzoate (**12b**): M.p. 63–64 °C (Et_2O - *n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.0 Hz, 1H, H-5′), 8.03 (d, J = 8.0 Hz, 1H, H-6), 7.62 (t, J = 8.0 Hz, 1H, H-4), 7.36 (t, J = 8.0 Hz, 1H, H-5), 7.15 (d, J = 8.0 Hz, 1H, H-3), 6.80 (s, 1H, H-2′), 6.75 (d, J = 8.0 Hz, 1H, H-6′), 4.20 (q, J = 7.0 Hz, 2H, CH_2CH_3), 2.56 (s, 3H, CH_3), 1.18 (t, J = 7.0 Hz, 3H, CH_2CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 164.7 (*C*O), 162.0 (C-1′), 153.4 (C-2), 143.1 (C-4′), 137.0 (C-3′), 134.1 (C-4), 132.3 (C-6), 127.4 (C-5′), 125.7 (C-5), 124.5 (C-1), 123.1 (C-3), 119.4 (C-2′), 113.9 (C-6′), 61.17 (CH_2CH_3), 21.5 (CH_3), 14.0 (CH_2CH_3).

*4.1.7. 2-(5-Methyl-2-nitrophenoxy)benzoic acid (**13**)*

To a solution of ester **12b** (3.01 g, 10 mmol) in ethanol (40 mL) was added a 20% sodium hydroxide solution (8 mL, 40 mmol) and the mixture was stirred at room temperature for 3 h. After completion of the reaction, the mixture was poured into water and acidified with a 36% HCl solution (pH ~ 2). The precipitate was filtered and dried over P_2O_5, to afford the title com-

pound **13** (2.62 g, 96%), which was used without any further purification for the next reaction. M.p. 144–145 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (dd, J = 8.0 Hz, 2 Hz, H-6), 8.04 (d, J = 8.0 Hz, 1H, H-3′), 7.57 (td, J = 8.0 Hz, 2 Hz, H-4), 7.26 (t, J = 8.0 Hz, 1H, H-5), 7.15 (d, J = 8.0 Hz, 1H, H-3), 6.94 (d, J = 8.0 Hz, 1H, H-4′), 6.90 (s, 1H, H-6′), 2.43 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.9 (*C*O), 155.6 (C-2), 150.1 (C-1′), 146.6 (C-5′), 138.6 (C-2′), 135.0 (C-4), 133.3 (C-6), 126.1 (C-3′), 124.8 (C-5), 124.7 (C-1), 121.4 (C-4′), 120.7 (C-3), 120.2 (C-6′), 21.6 (*C*H$_3$).

*4.1.8. 1-(Hydroxymethyl)-4-nitro-9H-xanthen-9-one (**16**)*

A suspension of bromide **15** (1.67 g, 5 mmol) and $AgNO_3$ (3.57 g, 21 mmol) in a 2 / 1 mixture of acetone / water (100 mL) was stirred in the dark, at room temperature, for 36 h. After completion of the reaction, most of the volatiles were removed under reduced pressure and the residue was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over anh. Na_2SO_4 and evaporated to dryness. Flash chromatography on silica gel, using a mixture of cyclohexane / EtOAc 1 / 1 as the eluent, afforded 1.23 g (91%) of the title compound **16**. M.p. 229–230 °C (EtOH); ^{1}H NMR (600 MHz, DMSO d_6) δ 8.50 (d, J = 8.0 Hz, 1H, H-3), 8.14 (dd, J = 8.0 Hz, 1 Hz, 1H, H-8), 7.89 (d, J = 8.0 Hz, 1H, H-2), 7.88 (td, J = 8.0 Hz, 1 Hz, 1H, H-6), 7.62 (d, J = 8.0 Hz, 1H, H-5), 7.51 (t, J = 8.0 Hz, 1H, H-7), 5.68 (t, J = 8.0 Hz, D_2O exch, 1H, OH), 5.21 (d, J = 5.0 Hz, 2H, C*H*$_2$OH); ^{13}C NMR (151 MHz, DMSO d_6) δ 177.0 (C-9), 154.3 (C-10a), 153.5 (C-1), 149.1 (C-4a), 137.6 (C-4), 136.3 (C-6), 130.2 (C-3), 126.4 (C-8), 125.7 (C-7), 122.1 (C-8a), 120.2 (C-2), 119.6 (C-9a), 118.2 (C-5), 62.6 (*C*H$_2$OH); HRMS (ESI$^-$) m/z 271.0481 (calcd for $C_{14}H_9NO_5^-$, 271.0488).

*4.1.9. 4-Nitro-9-oxo-9H-xanthene-1-carboxylic acid (**17**)*

A solution of Jones reagent (1 mmol / mL) was added dropwise at room temperature, to a solution of alcohol **16** (100 mg, 0.36 mmol) in acetone (8 mL). The addition is continued until the characteristic orange - red color persists for about 5 min. After completion of the reaction, isopropanol was added until color disappearance and the residual green salts were filtered off. The volatiles were removed under reduced pressure and the residue was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over anh. Na_2SO_4 and evaporated to dryness. Flash chromatography on silica gel, using a mixture of CH_2Cl_2 / MeOH (9 / 2) as the eluent, afforded 92.5 mg (88%) of the title compound **17** (Burdeska et al., 1972).

*4.1.10. N-[2-(Dimethylamino)ethyl]-4-nitro-9-oxo-9,10-dihydroacridine-1-carboxamide (**18a**)*

To a solution of acid **8** (300 mg, 1.05 mmol) in dry DMF (10 mL) was added 1,1′-carbonyldiimidazole (300 mg, 1.05 mmol) and the mixture was stirred under argon, at room temperature, for 25 min. *N,N*-dimethylethylenodiamine (572 μL, 5.25 mmol) was then added and the reaction mixture was heated at 50 °C for 12 h. After completion of the reaction, DMF was removed under reduced pressure and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anh. Na_2SO_4 and evaporated to dryness. Flash chromatography on silica gel, using a mixture of CH_2Cl_2 / MeOH 10 / 1–4 / 1 as the eluent, afforded 240 mg (63%) of the title compound **18a**. M.p. 224–225 °C (CH_2Cl_2 - *n*-Pentane); ^{1}H NMR (600 MHz $CDCl_3$) δ 11.37 (s, D_2O exch., 1H, NH), 8.60 (d, J = 8.3 Hz, 1H, H-3), 8.28 (dd, J = 8.0 Hz, 1.2 Hz, 1H, H-8), 7.68 (td, J = 8.0 Hz, 1.2 Hz, 1H, H-6), 7.38 (d, J = 8.0 Hz, 1H, H-5), 7.30 (t, J = 8.0 Hz, 1H, H-7), 7.14 (d, J = 8.3 Hz, 1H, H-2), 6.57 (brs, D_2O exch., 1H, CONH), 3.62 (q, J = 8.7 Hz, 2H, CONHC*H*$_2$), 2.67 (t, J = 8.7 Hz, 2H, CH$_2$C*H*$_2$N(CH$_3$)$_2$), 2.27 (s, 6H, N(C*H*$_3$)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.1 (C-9), 169.4 (*C*ONH), 146.6 (C-1), 138.9 (C 10a), 136.6 (C-4a), 134.8 (C-6), 134.2 (C-4), 131.2 (C-3), 127.3 (C-8), 124.0 (C-7), 122.1 (C-8a), 120.6 (C-9a), 119.3 (C-2), 117.7 (C-5), 57.4 (*C*H$_2$N(CH$_3$)$_2$), 45.0 (N(*C*H$_3$)$_2$), 37.12(CONH*C*H$_2$); HRMS (ESI$^+$) m/z 355.1401 (calcd for $C_{18}H_{19}N_4O_4^+$, 355.1406).

*4.1.11. N-[2-(Diethylamino)ethyl]-4-nitro-9-oxo-9,10-dihydroacridine-1-carboxamide (**18b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **18a**, using *N,N*-diethylethylenodiamine. Yield: 70%. M.p. 193–194 °C (CH_2Cl_2 - *n*-Pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 8.2 Hz, 1H, H-3), 8.15 (d, J = 7.9 Hz, 1H, H-8), 7.64 (t, J = 7.9 Hz, 1H, H-6), 7.37 (d, J = 7.9 Hz, 1H, H-5), 7.25 (t, J = 7.9 Hz, 1H, H-7), 7.04 (d, J = 8.2 Hz, 1H, H-2), 6.83 (brs, D_2O exch., 1H, CONH), 3.61 (q, J = 8.7 Hz, 2H, CONHC*H*$_2$), 2.78 (t, J = 8.7 Hz, 2H, C*H*$_2$N(CH$_2$CH$_3$)$_2$), 2.60 (t, J = 8.4 Hz, 4H, N(C*H*$_2$CH$_3$)$_2$), 0.99 (t, J = 8.4 Hz, 6H, N(CH$_2$C*H*$_3$)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.7 (C-9), 169.1 (CONH), 146.5 (C-1), 138.7 (C-10a), 136.1 (C-4a), 134.7 (C-6), 134.0 (C-4), 131.0 (C-3), 126.9 (C-8), 123.8 (C-7), 121.6 (C-8a), 120.1 (C-9a), 119.2 (C-2), 117.7 (C-5), 51.3 (*C*H$_2$N(CH$_2$CH$_3$)$_2$), 46.6 (N(*C*H$_2$CH$_3$)$_2$), 37.4 (CONH*C*H$_2$), 11.5 (N(CH$_2$*C*H$_3$)$_2$); HRMS (ESI$^+$) m/z 383.1714 (calcd for $C_{20}H_{23}N_4O_4^+$, 383.1718).

*4.1.12. 4-[(Chloroacetyl)amino]-N-[2-(dimethylamino)ethyl]-9-oxo-9,10-dihydroacridine-1-carboxamide (**20a**)*

A suspension of **18a** (240 mg, 0.67 mmol) in absolute ethanol (50 mL) was hydrogenated in the presence of 10% Pd / C (30 mg) under a pressure of 50 psi, at room temperature, for 7 h. The resulting mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness, to afford amine **19a**. Without any further purification, the amine was dissolved under argon in dry THF (5 mL) and to this solution were added K_2CO_3 (166 mg, 1.20 mmol) and chloracetyl chloride (34 μL, 0.40 mmol), at 0 °C and the mixture was stirred at room temperature for 8 h. After completion of the reaction, THF was vacuum evaporated and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anh. Na_2SO_4 and evaporated to dryness. Flash chromatography on silica gel, using a mixture of CH_2Cl_2 / MeOH 10 / 1 to 4 / 1 as the eluent, afforded 70 mg (43%) of the title compound **20a**. M.p. > 270 °C (MeOH - Et$_2$O); ^{1}H NMR (600 MHz, CD_3OD) δ 8.35 (d, J = 8.4 Hz, 1H, H-8), 7.85 (d, J = 8.4 Hz, 1H, H-5), 7.83–7.77 (m, 2H, H-3, H-6), 7.37 (t, J = 8.4 Hz, 1H, H-7), 7.22 (d, J = 7.9 Hz, 1H, H-2), 4.50 (s, 2H, COCH$_2$), 3.89 (t, J = 8.7 Hz, 2H, CONHC*H*$_2$), 3.56 (t, J = 8.7 Hz, 2H, C*H*$_2$N(CH$_3$)$_2$), 3.27 (s, 6H, N(C*H*$_3$)$_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 179.1 (C-9), 175.2 (*C*ONHCH$_2$), 169.7 (NH*C*OCH$_2$), 142.0 (C-10a), 138.9 (C-

1), 136.5 (C-4a), 135.8 (C-6), 132.3 (C-3), 127.6 (C-4), 127.3 (C-8), 123.8 (C-7), 122.1 (C-8a), 121.0 (C-2), 119.0 (C-5), 118.9 (C-9a), 60.4 ($CH_2N(CH_3)_2$), 44.6 ($N(CH_3)_2$), 44.0 ($COCH_2$), 35.8 ($CONHCH_2$); HRMS (ESI^+) m/z 401.1375 (calcd for $C_{20}H_{22}ClN_4O_3^+$, 401.1367).

*4.1.13. 4-[(Chloroacetyl)amino]-N-[2-(diethylamino)ethyl]-9-oxo-9,10-dihydroacridine-1-carboxamide (**20b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **20a**, starting from compound **18b**. Yield: 45%. M.p. 210–211 °C (MeOH - Et_2O); ^1H NMR (600 MHz, CD_3OD) δ 8.28 (dd, J = 8.1 Hz, 1.8 Hz, 1H, H-8), 7.90 (d, J = 8.1 Hz, 1H, H-5), 7.83–7.78 (m, 2H, H-3, H-6), 7.36 (t, J = 8.1 Hz, 1H, H-7), 7.20 (d, J = 7.9 Hz, 1H, H-2), 4.53 (s, 2H, $COCH_2$), 3.91 (t, J = 8.7 Hz, 2H, $CONHCH_2$), 3.60 (q, J = 7.3 Hz, 4H, $N(CH_2CH_3)_2$), 3.55 (t, J = 8.7 Hz, 2H, $CH_2N(CH_2CH_3)_2$), 1.49 (t, J = 7.3 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 178.7 (C-9), 174.8 ($CONHCH_2$), 169.5 ($NHCOCH_2$), 142.0 (C-10a), 138.5 (C-1), 136.2 (C-4a), 135.7 (C-6), 132.0 (C-3), 127.6 (C-4), 127.0 (C-8), 123.8 (C-7), 122.0 (C-8a), 121.1 (C-2), 119.0 (C-5), 118.8 (C-9a), 54.2 ($CH_2N(CH_2CH_3)_2$), 48.8 ($N(CH_2CH_3)_2$), 44.1 ($COCH_2$), 35.3 ($CONHCH_2$), 9.2 ($N(CH_2CH_3)_2$); HRMS (ESI^+) m/z 429.1688 (calcd for $C_{22}H_{26}ClN_4O_3^+$, 429.1693).

*4.1.14. 4-[2-(Dimethylamino)acetamido]-N-[2-(dimethylamino)ethyl]-9-oxo-9,10-dihydroacridine-1-carboxamide (**21a**)*

To a solution of compound **20a** (70 mg, 0.17 mmol) in absolute ethanol (25 mL) was added dimethylamine (0.32 mL, 1.80 mmol, 5.6 M in ethanol) and the mixture was refluxed for 8 h. Upon cooling, the mixture was vacuum-evaporated, extracted with EtOAc / water, the organic layer was dried (anh. Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2 / MeOH 10 / 1 – 4 /1) to afford the title compound (40 mg, 57%). M.p. 220–222 °C (MeOH - Et_2O); ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.0 Hz, 1H, H-8), 7.59 (d, J = 8.0 Hz, 1H, H-5), 7.41 (t, J = 8.0 Hz, 1H, H-6), 7.36 (d, J = 7.9 Hz, 1H, H-3), 6.94 (t, J = 8.0 Hz, 1H, H-7), 6.68 (d, J = 7.9 Hz, 1H, H-2), 3.67 (q, J = 8.3 Hz, 2H, $CONHCH_2$), 3.45 (s, 2H, $NHCOCH_2$), 3.06 (t, J = 8.3 Hz, 2H, $CONHCH_2CH_2$), 2.68 (s, 6H, $COCH_2N(CH_3)_2$), 2.49 (s, 6H, $CH_2CH_2N(CH_3)_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.02 (C-9), 172.28 ($CONHCH_2$), 170.40 ($NHCOCH_2$), 140.1 (C-10a), 135.0 (C-1), 133.5 (C-4a), 133.2 (C-6), 127.9 (C-4), 126.1 (C-3), 125.5 (C-8), 121.7 (C-7), 120.3 (C-8a), 119.6 (C-2), 118.0 (C-5), 117.5 (C-9a), 63.5 ($NHCOCH_2$), 60.4 ($CONHCH_2CH_2$), 45.8 ($COCH_2N(CH_3)_2$), 44.7 ($CH_2CH_2N(CH_3)_2$), *35.9* ($CONHCH_2$); HRMS (ESI^+) m/z 410.2187 (calcd for $C_{22}H_{28}N_5O_3^+$, 410.2189).

*4.1.15. 4-[2-(Diethylamino)acetamido]-N-[2-(diethylamino)ethyl]-9-oxo-9,10-dihydroacridine-1-carboxamide (**21b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **21a**, using diethylamine. Yield: 62%. M.p. 219–221 °C (MeOH - Et_2O); ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.1, 1.4 Hz, 1H, H-8), 7.45 (m, 2H, H-5, H-6), 7.30 (d, J = 8.1 Hz, 1H, H-3), 6.90 (td, J = 8.1 Hz, 1.4 Hz, 1H, H-7), 6.70 (d, J = 8.1 Hz, 1H, H-2), 3.60 (t, J = 7.0 Hz, 2H, $CONHCH_2$), 3.45 (s, 1H, $NHCOCH_2$), 2.90 (t, J = 7.0 Hz, 2H, $CONHCH_2CH_2$), 2.80 (m, 8H, $N(CH_2CH_3)_2$, $N(CH_2CH_3)_2$), 1.06 (t, J = 7.0 Hz, 6H, $CH_2CH_2N(CH_2CH_3)_2$), 1.19 (t, J = 7.3 Hz, 6H, $COCH_2N(CH_2CH_3)_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.6 (C-9), 172.1 ($CONHCH_2$), 171.7 ($NHCOCH_2$), 140.2 (C-10a), 135.1 (C-1), 134.0 (C-4a) 133.1 (C-6), 126.5 (C-4), 125.9 (C-3), 125.9 (C-8), 121.7 (C-7), 120.1 (C-8a), 119.7 (C-2), 118.1 (C-5), 118.1 (C-9a), 57.7 ($NHCOCH_2$), 51.9 ($CONHCH_2CH_2$), 48.3 ($COCH_2N(CH_2CH_3)_2$), 45.6 ($CH_2CH_2N(CH_2CH_3)_2$), 36.6 ($CONHCH_2$), 12.0 ($COCH_2N(CH_2CH_3)_2$), 10.6 ($CH_2CH_2N(CH_2CH_3)_2$); HRMS (ESI^+) m/z 466.2813 (calcd for $C_{26}H_{36}N_5O_3^+$, 466.2816).

*4.1.16. 4-(Acryloylamino)-N-[2-(dimethylamino)ethyl]-9-oxo-9,10-dihydroacridine-1-carboxamide (**22a**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **20a**, using 3-chloropropionyl chloride. Yield: 75%. M.p. 206–207 °C (MeOH - Et_2O); ^1H NMR (600 MHz, CD_3OD) δ 8.22 (d, J = 8.1 Hz, 1H, H-8), 7.74–7.68 (m, 3H, H-3, H-5, H-6), 7.27 (t, J = 8.1 Hz, 1H, H-7), 7.10 (d, J = 7.9 Hz, 1H, H-2), 6.69 (dd, J = 16.0 Hz, 10.1 Hz, 1H, $CH = CH_2$), 6.47 (dd, J = 16.0, 1.0 Hz, 1H, $CH = CH_2$), 5.92 (d, J = 10.1 Hz, 1H, $CH = CH_2$), 3.73 (t, J = 7.1 Hz, 2H, $CONHCH_2$), 3.03 (t, J = 7.1 Hz, 2H, $CONHCH_2CH_2$), 2.70 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 178.7 (C-9), 174.5 ($CONHCH_2$), 167.8 ($NHCOCH = CH_2$), 141.9 (C-10a), 138.2 (C-1), 136.5 (C-4a), 135.2 (C-6), 132.2 ($CH = CH_2$), 131.2 (C-3), 128.4 ($CH = CH_2$), 127.6 (C-4), 127.1 (C-8), 123.4 (C-7), 122.1 (C-8a), 121.1 (C-2), 119.2 (C-9a), 118.9 (C-5), 59.4 ($CONHCH_2CH_2$), 45.2 ($N(CH_3)_2$), 37.7 ($CONHCH_2$); HRMS (ESI^+) m/z 379.1765 (calcd for $C_{21}H_{23}N_4O_3^+$, 379.1768).

*4.1.17. 4-(Acryloylamino)-N-[2-(diethylamino)ethyl]-9-oxo-9,10-dihydroacridine-1-carboxamide (**22b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **20a**, using 3-chloropropionyl chloride. Yield: 70%. M.p. 160–162 °C (MeOH - Et_2O); ^1H NMR (600 MHz, CD_3OD) δ 8.31 (d, J = 8.0 Hz, 1H, H-8), 7.87–7.76 (m, 3H, H-3, H-5, H-6), 7.35 (t, J = 8.0 Hz, 1H, H-7), 7.19 (d, J = 7.9 Hz, 1H, H-2), 6.68 (dd, J = 16.0, 10.1 Hz, 1H, $CH = CH_2$), 6.48 (dd, J = 16.0, 1.1 Hz, 1H, $CH = CH_2$), 5.93 (d, J = 10.1 Hz, 1H, $CH = CH_2$), 3.84 (t, J = 7.6 Hz, 2H, $CONHCH_2$), 3.39 (m, 6H, $CONHCH_2CH_2$), 1.42 (t, J = 7.7 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 178.7 (C-9), 174.8 ($CONHCH_2$), 167.8 ($NHCOCH = CH_2$), 142.1 (C-10a), 138.5 (C-1), 136.2 (C-4a), 135.5 (C-6), 132.2 ($CH = CH_2$), 131.4 (C-3), 128.5 ($CH = CH_2$), 128.0 (C-4), 127.1 (C-8), 123.7 (C-7), 122.1 (C-8a), 121.1(C-2), 119.1 (C-5), 119.0 (C-9a), 53.9 ($CONHCH_2CH_2$), 48.6 ($N(CH_2CH_3)_2$), 36.0 ($CONHCH_2$), 9.8 ($N(CH_2CH_3)_2$); HRMS (ESI^+) m/z 407.2078 (calcd for $C_{23}H_{27}N_4O_3^+$, 407.2081).

*4.1.18. N-[2-(Dimethylamino)ethyl]-4-[3-(dimethylamino)propanamido]-9-oxo-9,10-dihydroacridine-1-carboxamide (**23a**)*

To a solution of compound **22a** (60 mg, 0.16 mmol) in absolute ethanol (25 mL) was added dimethylamine (0.30 mL,

1.68 mmol, 5.6 M in ethanol) and the mixture was refluxed for 8 h. Upon cooling, the mixture was vacuum - evaporated, extracted with EtOAc - water, the organic layer was dried (anh. Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2 - MeOH 10 / 1– 4 /1) to afford the title compound (55 mg, 81%). M.p. 183–184 °C (CH_2Cl_2 - Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.38 (s, D_2O exch., 1H, NH), 7.85 (d, J = 8.0 Hz, 1H, H-8), 7.44 (d, J = 8.0 Hz, 1H, H-5), 7.39 (t, J = 8.0 Hz, 1H, H-6), 7.29 (d, J = 7.5 Hz, 1H, H-3), 6.92 (t, J = 8.0 Hz, 1H, H-7), 6.66 (d, J = 7.5 Hz, 1H, H-2), 3.64 (q, J = 8.0 Hz, 2H, CONHCH_2), 2.90 (m, 6H, CONHCH$_2$CH_2, NHCOCH_2CH_2), 2.55 (s, 6H, COCH$_2$CH$_2$-N(CH_3)$_2$), 2.49 (s, 6H, NHCH$_2$CH$_2$N(CH_3)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.8 (C-9), 172.6 (*C*ONHCH$_2$), 172.1 (NH*C*OCH$_2$), 139.8 (C-10a), 135.2 (C-1), 133.4 (C-4a), 133.1 (C-6), 128.2 (C-4), 126.6 (C-3), 125.7 (C-8), 121.6 (C-7), 120.4 (C-8a), 119.6 (C-2), 117.7 (C-5), 117.5 (C-9a), 65.8 (CONHCH$_2$*C*H$_2$), 57.8 (NHCOCH$_2$*C*H$_2$), 45.5 (COCH$_2$CH$_2$-N(*C*H$_3$)$_2$), 44.9 (NHCH$_2$CH$_2$N(*C*H$_3$)$_2$), 36.6 (CONH*C*H$_2$-CH$_2$), 34.7 (NHCO*C*H$_2$CH$_2$); HRMS (ESI$^+$) m/z 424.2343 (calcd for $C_{23}H_{30}N_5O_3^+$, 424.2346).

*4.1.19. N-[2-(Diethylamino)ethyl]-4-[3-(diethylamino) propanamido]-9-oxo-9,10-dihydroacridine-1-carboxamide (**23b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **23a**, using diethylamine. Yield: 85%. M.p. 188–190 °C (CH_2Cl_2 - Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, D_2O exch., 1H, NH), 7.84 (d, J = 8.0 Hz, 1H, H-8), 7.62 (d, J = 8.0 Hz, 1H, H-5), 7.45 (t, J = 8.0 Hz, 1H, H-6), 7.38 (d, J = 7.9 Hz, 1H, H-3), 6.99 (t, J = 8.0 Hz, 1H, H-7), 6.69 (d, J = 7.9 Hz, 1H, H-2), 3.59 (q, J = 8.2 Hz, 2H, CONHCH_2), 3.10 (m, 8H, CONHCH$_2$CH_2, NHCOCH$_2$CH_2, NHCH$_2$CH$_2$N(CH_2-CH$_3$)$_2$), 2.98 (t, J = 8.2 Hz, 2H, NHCOCH_2), 2.81 (t, J = 7.8 Hz, 4H, COCH$_2$CH$_2$N(CH_2CH$_3$)$_2$), 1.08 (m, 12H, N(CH$_2$CH_3)$_2$, N(CH$_2$CH_3)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.8 (C-9), 172.4 (*C*ONHCH$_2$), 172.0 (NH*C*OCH$_2$), 140.0 (C-10a), 134.6 (C-1), 133.2 (C-6), 133.0 (C-4a), 127.5 (C-4), 126.6 (C-3), 125.6 (C-8), 121.7 (C-7), 120.4 (C-8a), 119.6 (C-2), 117.9 (C-5), 117.3 (C-9a), 52.2 (CONHCH$_2$*C*H$_2$), 48.5 (NHCOCH$_2$*C*H$_2$), 46.9 (COCH$_2$CH$_2$N(*C*H$_2$CH$_3$)$_2$), 46.6 (NHCH$_2$CH$_2$N(*C*H$_2$CH$_3$)$_2$, 33.9 (CONH*C*H$_2$), 29.7 (NHCO*C*H$_2$), 11.3 (COCH$_2$CH$_2$N(CH$_2$*C*H$_3$)$_2$), 10.1 (NHCH$_2$-CH$_2$N(CH$_2$*C*H$_3$)$_2$); HRMS (ESI$^+$) m/z 480.2969 (calcd for $C_{27}H_{38}N_5O_3^+$, 480.2973).

*4.1.20. N-[2-(Dimethylamino)ethyl]-4-nitro-9-oxo-9H-xanthene-1-carboxamide (**24a**)*

A mixture of acid **17** (500 mg, 1.75 mmol), triphenylphosphine (917 mg, 3.5 mmol) and NBS (717 mg, 4.03 mmol) in dry CH_2Cl_2 (50 mL) was stirred under argon, at 0 °C. After 25 min, *N,N*-dimethylethylenediamine (668 μL, 6.13 mmol) was added and stirring was continued at room temperature for 25 more minutes. Upon completion of the reaction, the mixture was washed with 5% sodium bicarbonate solution and water, dried over anh. Na_2SO_4 and evaporated to dryness. Flash chromatography on silica gel, using a mixture of CH_2Cl_2 / MeOH (10 / 1–7 / 1) as the eluent, afforded 570 mg (91%) of the title compound **24a**. M.p. 116 – 118 °C (EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (d, J = 8.0 Hz, 1H, H-3), 8.25 (dd, J = 8.0 Hz, 1.3 Hz, 1H, H-8), 7.91 (td, J = 8.0 Hz, 1.3 Hz, 1H, H-6), 7.65 (d, J = 8.0 Hz, 1H, H-5), 7.54 (t, J = 8.0 Hz, 1H, H-7), 7.49 (d, J = 8.0 Hz, 1H, H-2), 3.66 (t, J = 8.0 Hz, 2H, CONHCH_2), 2.86 (t, J = 8.0 Hz, 2H, CH_2N(CH$_3$)$_2$), 2.50 (s, 6H, N(CH_3)$_2$); ^{13}C NMR (50 MHz, CD_3OD) δ 174.6 (C-9), 169.7 (*C*ONH), 155.0 (C-10a), 148.4 (C-4a), 141.8 (C-1, C-4), 136.1 (C-6), 130.0 (C-3), 126.0 (C-8), 125.3 (C-7), 122.3 (C-2), 121.3 (C-8a), 120.0 (C-9a), 117.8 (C-5), 57.5 (CH$_2$*C*H$_2$N(CH$_3$)$_2$), 44.0 (N(CH$_3$)$_2$), 36.9 (CONH*C*H$_2$); HRMS (ESI$^+$) m/z 356.1241 (calcd for $C_{18}H_{18}N_3O_5^+$, 356.1246).

*4.1.21. N-[2-(Diethylamino)ethyl]-4-nitro-9-oxo-9H-xanthene-1-carboxamide (**24b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **24a**, using *N,N*-diethylethylenediamine. Yield: 94%. M.p. 175 – 177 °C (EtOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.30 (d, J = 8.4 Hz, 1H, H-3), 8.25 (dd, J = 8.4 Hz, 1.7 Hz, 1H, H-8), 7.82 (td, J = 8.4 Hz, 1.7 Hz, 1H, H-6), 7.60 (d, J = 8.4 Hz, 1H, H-5), 7.47 (td, J = 8.4 Hz, 1.7 Hz, 1H, H-7), 7.40 (d, J = 8.4 Hz, 1H, H-2), 6.93 (s, D_2O exch., 1H, CONH), 3.68 (t, J = 7.1 Hz, 2H, CONHCH_2), 2.87 (t, J = 7.1 Hz, 2H, CH_2N(CH$_2$CH$_3$)$_2$), 2.67 (q, J = 7.0 Hz, 4H, N(CH_2CH$_3$)$_2$), 1.04 (t, J = 7.1 Hz, 6H, N(CH$_2$CH_3)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5 (C-9), 168.0 (*C*ONHCH$_2$), 154.9 (C-10a), 148.8 (C-4a), 143.0 (C-1), 139.3 (C-4), 135.9 (C-6), 130.0 (C-3), 126.7 (C-8), 125.5 (C-7), 122.3 (C-2), 121.6 (C-8a), 120.5 (C-9a), 118.3 (C-5), 51.3 (*C*H$_2$N(CH$_2$CH$_3$)$_2$), 46.5 (N(*C*H$_2$CH$_3$)$_2$), 37.1 (CONH*C*H$_2$), 11.1 (N(CH$_2$*C*H$_3$)$_2$); HRMS (ESI$^+$) m/z 384.1554 (calcd for $C_{20}H_{22}N_3O_5^+$, 384.1558).

*4.1.22. 4-[(Chloroacetyl)amino]-N-[2-(dimethylamino)ethyl]-9-oxo-9H-xanthene-1-carboxamide (**26a**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **20a**, starting from **24a**. Yield: 25%. M.p. > 270 °C (EtOH); ^{1}H NMR (600 MHz, CD_3OD) δ 8.55 (d, J = 8.1 Hz, 1H, H-3), 8.25 (d, J = 8.0 Hz, 1H, H-8), 7.92 (t, J = 8.0 Hz, 1H, H-6), 7.76 (d, J = 8.0 Hz, 1H, H-5), 7.51 (t, J = 8.0 Hz, 1H, H-7), 7.35 (d, J = 8.1 Hz, 1H, H-2), 4.47 (s, 2H, NHCOCH_2) 3.85 (t, J = 7.4 Hz, 2H, CONHCH_2), 3.56 (t, J = 7.4 Hz, 2H, CH_2N(CH$_3$)$_2$), 3.20 (s, 6H, N(CH_3)$_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 178.4 (C-9), 173.7 (*C*ONHCH$_2$), 168.0 (NH*C*OCH$_2$), 156.7 (C-10a), 148.7 (C-1), 137.5 (C-6), 132.1 (C-4a), 129.6 (C-4), 128.0 (C-3), 127.5 (C-8), 126.3 (C-7), 123.7 (C-2), 122.4 (C-8a), 119.4 (C-9a), 119.3 (C-5), 60.1 (*C*H$_2$N(CH$_3$)$_2$), 44.6 (N(*C*H$_3$)$_2$), 44.0 (NHCO*C*H$_2$), 36.1 (CONH*C*H$_2$); HRMS (ESI$^+$) m/z 402.1215 (calcd for $C_{20}H_{21}ClN_3O_4^+$, 402.1219).

*4.1.23. 4-[(Chloroacetyl)amino]-N-[2-(diethylamino)ethyl]-9-oxo-9H-xanthene-1-carboxamide (**26b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **20a**, starting from **24b**. Yield: 25%. M.p. 260–262 °C (EtOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.46 (s, D_2O exch., 1H, NHCO), 8.52 (s, D_2O exch., 1H, CON*H*CH$_2$), 8.41 (d, J = 8.1 Hz, 1H, H-3), 8.06 (d, J = 8.1 Hz, 1H, H-8), 7.66 (t, J = 8.1 Hz, 1H, H-6), 7.50 (d, J = 8.1 Hz, 1H, H-5), 7.32 (t, J = 8.1 Hz,

1H, H-7), 7.09 (d, J = 8.1 Hz, 1H, H-2), 4.40 (s, 2H, NHCOCH_2), 3.82 (q, J = 7.0 Hz, 2H, CONHCH_2), 3.41 (t, J = 7.1 Hz, 2H, CH_2N(CH_2CH_3)$_2$), 3.24 (q, J = 8.1 Hz, 4H, N(CH_2CH_3)$_2$), 1.34 (t, J = 8.1 Hz, 6H, N(CH_2CH_3)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.0 (C-9), 170.6 (*C*ONHCH$_2$), 164.8 (NH*C*OCH$_2$), 154.6 (C-10a), 145.6 (C-1), 135.4 (C-6), 131.7 (C-4a), 128.7 (C-4), 126.3 (C-8), 124.7 (C-7), 124.2 (C-3), 123.0 (C-2), 121.3 (C-8a), 118.1 (C-9a), 117.8 (C-5), 52.3 (*C*H$_2$N(CH_2CH_3)$_2$), 46.9 (N(*C*H$_2$CH$_3$)$_2$), 43.4 (NHCO*C*H$_2$), 35.3 (CONH*C*H$_2$), 8.9 (N(CH_2*C*H$_3$)$_2$); HRMS (ESI$^+$) m/z 430.1528 (calcd for $C_{22}H_{25}ClN_3O_4^+$, 430.1533).

4.1.24. N-[2-(Dimethylamino)ethyl]-4-[2-(dimethylamino) acetamido]-9-oxo-9H-xanthene-1-carboxamide (**27a**)

This compound was synthesized by an analogous procedure as described for the preparation of compound **21a**, starting from **26a**. Yield: 84%. M.p. 149–150 °C (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.75 (d, J = 8.1 Hz, 1H, H-3), 8.22 (dd, J = 8.1 Hz, 1.5 Hz, 1H, H-8), 7.74 (td, J = 8.1 Hz, 1.5 Hz, 1H, H-6), 7.46 (d, J = 8.1 Hz, 1H, H-5), 7.39 (td, J = 8.1 Hz, 1.5 Hz, 1H, H-7), 7.29 (d, J = 8.1 Hz, 1H, H-2), 3.75 (q, J = 7.0 Hz, 2H, CONHCH_2), 3.22–3.16 (m, 4H, CH_2CH_2N(CH_3)$_2$, NHCOCH_2), 2.68 (s, 6H, CH_2CH_2-N(CH_3)$_2$), 2.51 (s, 6H, $COCH_2$N(CH_3)$_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.5 (C-9), 170.4 (*C*ONHCH$_2$), 169.3 (NH*C*OCH$_2$), 154.9 (C-10a), 145.8 (C-1), 135.1 (C-6), 131.8 (C-4a), 128.3 (C-4), 126.1 (C-8), 124.8(C-7), 123.9 (C-3), 123.4 (C-2), 122.0 (C-8a), 118.7 (C-9a), 117.6 (C-5), 63.8 (NHCO*C*H$_2$), 58.0 (CH_2*C*H$_2$N(CH_3)$_2$), 46.3 ($COCH_2$N(*C*H$_3$)$_2$), 44.7 (CH_2CH_2N(*C*H$_3$)$_2$), 36.2 (CONH*C*H$_2$); HRMS (ESI$^+$) m/z 411.2027 (calcd for $C_{22}H_{27}N_4O_4^+$, 411.2029).

4.1.25. N-[2-(Diethylamino)ethyl]-4-[2-(diethylamino) acetamido]-9-oxo-9H-xanthene-1-carboxamide (**27b**)

This compound was synthesized by an analogous procedure as described for the preparation of compound **27a**, using diethylamine. Yield: 89%. M.p. 123–125 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, J = 8.1 Hz, 1H, H-3), 8.25 (d, J = 8.1, 1.5 Hz, 1H, H-8), 7.77 (t, J = 8.1 Hz, 1.5 Hz, 1H, H-6), 7.49 (d, J = 8.1 Hz, 1H, H-5), 7.42 (t, J = 8.1 Hz, 1.5 Hz, 1H, H-7), 7.33 (d, J = 8.1 Hz, 1H, H-2), 3.82 (q, J = 7.1 Hz, 2H, CONHCH_2), 3.30 (m, 8H, CH_2CH_2N(CH_2-CH_3)$_2$, NHCOCH_2, CH_2CH_2N(CH_2CH$_3$)$_2$), 2.78 (q, J = 7.0 Hz, 4H, $COCH_2$N(CH_2CH$_3$)$_2$), 1.29 (t, J = 7.1 Hz, 6H, CH_2CH_2N(CH_2CH_3)$_2$), 1.23 (t, J = 7.1 Hz, 6H, $COCH_2$-N(CH_2CH_3)$_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.6 (C-9), 171.9 (*C*ONHCH$_2$), 171.8 (NH*C*OCH$_2$), 155.1 (C-10a), 146.2 (C-1), 136.0 (C-6), 130.8 (C-4a), 128.4 (C-4), 126.2 (C-8), 125.0 (C-7), 123.8 (C-3), 122.8 (C-2), 121.2 (C-8a), 118.0 (C-9a), 117.4 (C-5), 58.2 (NHCO*C*H$_2$), 52.3 (CH_2*C*H$_2$N(CH_2-CH_3)$_2$), 48.6 ($COCH_2$N(*C*H$_2$CH$_3$)$_2$), 47.4 (CH_2CH_2N(*C*H$_2$-CH_3)$_2$), 35.1 (CONH*C*H$_2$), 11.8 ($COCH_2$N(CH_2*C*H$_3$)$_2$), 8.5 (CH_2CH_2N(CH_2*C*H$_3$)$_2$); HRMS (ESI$^+$) m/z 467.2653 (calcd for $C_{26}H_{35}N_4O_4^+$, 467.2658).

4.1.26. 4-(Acryloylamino)-N-[2-(dimethylamino)ethyl]-9-oxo-9H-xanthene-1-carboxamide (**28a**)

This compound was synthesized by an analogous procedure as described for the preparation of compound **22a**, starting from **24a**. Yield: 80%. M.p. 155–157 °C (EtOH); ^{1}H NMR (600 MHz, CD_3OD) δ 8.54 (d, J = 8.0 Hz, 1H, H-3), 8.18 (dd, J = 8.1 Hz, 1.3 Hz, 1H, H-8), 7.86 (td, J = 8.1 Hz, 1.3 Hz, 1H, H-6), 7.73 (d, J = 8.1 Hz, 1H, H-5), 7.46 (t, J = 8.1 Hz, 1H, H-7), 7.28 (d, J = 8.0 Hz, 1H, H-2), 6.76 (dd, J = 16.1, 10.0 Hz, 1H, CH = CH_2), 6.51 (dd, J = 16.1, 1.3 Hz, 1H, CH = CH_2), 5.92 (dd, J = 10.0 Hz, 1.3 Hz, 1H, CH = CH_2), 3.67 (t, J = 7.6 Hz, 2H, CONHCH_2), 2.92 (t, J = 7.6 Hz, 2H, CH_2N(CH_3)$_2$), 2.56 (s, 6H, N(CH_3)$_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 175.9 (C-9), 171.3 (*C*ONHCH$_2$), 165.1 (NH*C*OCH = CH_2), 155.0 (C-10a), 147.1 (C-4a), 135.3 (C-6), 132.1 (C-1), 130.6 (*C*H = CH_2), 128.3 (C-9a), 127.6 (CH = *C*H$_2$), 126.3 (C-3), 125.8 (C-8), 124.5 (C-7), 122.4 (C-2), 121.2 (C-8a), 118.2 (C-4), 117.8 (C-5), 57.6 (*C*H$_2$N(CH_3)$_2$), 43.9 (N(*C*H$_3$)$_2$), 36.7 (CONH*C*H$_2$); HRMS (ESI$^+$) m/z 380.1605 (calcd for $C_{21}H_{22}N_3O_4^+$, 380.1608).

4.1.27. 4-(Acryloylamino)-N-[2-(diethylamino)ethyl]-9-oxo-9H-xanthene-1-carboxamide (**28b**)

This compound was synthesized by an analogous procedure as described for the preparation of compound **28a**, starting from **24b**. Yield: 83%. M.p. 265–267 °C (EtOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.68 (d, J = 8.1 Hz, 1H, H-3), 8.26 (d, J = 8.1 Hz, 1H, H-8), 7.96 (t, J = 8.1 Hz, 1H, H-6), 7.86 (d, J = 8.1 Hz, 1H, H-5), 7.55 (t, J = 8.1 Hz, 1H, H-7), 7.39 (d, J = 8.1 Hz, 1H, H-2), 6.83 (dd, J = 16.0, 10.1 Hz, 1H, CH = CH_2), 6.51 (d, J = 16.0 Hz, 1H, CH = CH_2), 5.93 (d, J = 10.1 Hz, 1H, CH = CH_2), 3.90 (t, J = 8.1 Hz, 2H, CONHCH_2), 3.65–3.51 (m, 6H, CH_2N(CH_2CH_3)$_2$, N(CH_2CH$_3$)$_2$), 1.49 (t, J = 8.1 Hz, 6H, N(CH_2-CH_3)$_2$); ^{13}C NMR (50 MHz, CD_3OD) δ 177.0 (C-9), 172.2 (*C*ONHCH$_2$), 165.2 (NH*C*OCH = CH_2), 155.3 (C-10a), 147.3 (C-4a), 136.0 (C-6), 131.2 (C-1), 130.6 (*C*H = CH_2), 128.8 (C-9a), 127.8 (CH = *C*H$_2$), 126.7 (C-3), 125.8 (C-8), 124.9 (C-7), 122.4 (C-2), 120.9 (C-8a), 118.2 (C-5), 117.9 (C-4), 52.8 (*C*H$_2$N(CH_2CH_3)$_2$), 46.6 (N(*C*H$_2$CH$_3$)$_2$) 34.3 (CONH*C*H$_2$), 7.9 (N(CH_2*C*H$_3$)$_2$); HRMS (ESI$^+$) m/z 408.1918 (calcd for $C_{23}H_{26}N_3O_4^+$, 408.1920).

4.1.28. N-[2-(Dimethylamino)ethyl]-4-[3-(dimethylamino) propanamido]-9-oxo-9H-xanthene-1-carboxamide (**29a**)

This compound was synthesized by an analogous procedure as described for the preparation of compound **23a**, starting from **28a**. Yield: 91%. M.p. 213–215 °C (MeOH - Et_2O); ^{1}H NMR (600 MHz, CD_3OD) δ 8.64 (d, J = 8.1 Hz, 1H, H-3), 8.25 (d, J = 8.1 Hz, 1H, H-8), 7.93 (t, J = 8.1 Hz, 1H, H-6), 7.84 (d, J = 8.1 Hz, 1H, H-5), 7.51 (t, J = 8.1 Hz, 1H, H-7), 7.34 (d, J = 8.1 Hz, 1H, H-2), 3.85 (t, J = 7.4 Hz, 2H, CONHCH_2), 3.52 (t, J = 7.4 Hz, 2H, $NHCH_2$CH_2N(CH_3)$_2$), 3.35 (t, J = 7.4 Hz, 2H, $NHCOCH_2$CH_2N(CH_3)$_2$), 3.14 (s, 6H, $NHCH_2CH_2$N(CH_3)$_2$), 3.12 (t, J = 7.4 Hz, 2H, $COCH_2$CH$_2$-N(CH_3)$_2$), 2.84 (s, 6H, $COCH_2CH_2$N(CH_3)$_2$); ^{13}C NMR (151 MHz, CD_3OD) δ 178.4 (C-9), 173.6 (*C*ONHCH$_2$), 171.9 (NH*C*OCH$_2$), 156.7 (C-10a), 148.3 (C-1), 137.3 (C-6), 132.5 (C-4a), 130.2 (C-4), 127.5 (C-8), 127.5 (C-3), 126.2 (C-7), 123.8 (C-2), 122.5 (C-8a), 119.4 (C-9a), 119.4 (C-5) 59.8 ($NHCH_2$*C*H$_2$N(CH_3)$_2$), 55.3 ($COCH_2$*C*H$_2$N(CH_3)$_2$), 44.7 ($NHCH_2CH_2$N(*C*H$_3$)$_2$), 44.4 ($COCH_2CH_2$N(*C*H$_3$)$_2$), 36.4 (CONH*C*H$_2$), 33.0 (NHCO*C*H$_2$); HRMS (ESI$^+$) m/z 425.2183 (calcd for $C_{23}H_{29}N_4O_4^+$, 425.2185).

4.1.29. *N-[2-(Diethylamino)ethyl]-4-[3-(diethylamino) propanamido]-9-oxo-9H-xanthene-1-carboxamide (**29b**)*

This compound was synthesized by an analogous procedure as described for the preparation of compound **29a**, using diethylamine. Yield: 85%. M.p. 167–169 °C (MeOH - Et_2O); ^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (d, J = 8.1 Hz, 1H, H-3), 8.21 (d, J = 8.1 Hz, 1H, H-8), 7.87 (t, J = 8.1 Hz 1H, H-6), 7.67 (d, J = 8.1 Hz, 1H, H-5), 7.47 (t, J = 8.1 Hz, 1H, H-7), 7.25 (d, J = 8.1 Hz, 1H, H-2), 3.57 (t, J = 7.5 Hz, 2H, $CONHCH_2$), 2.94 (t, J = 7.5 Hz, 2H, $COCH_2CH_2N(CH_2CH_3)_2$), 2.88 (t, J = 7.5 Hz, 2H, $NHCH_2CH_2N(CH_2CH_3)_2$), 2.74 (m, 10*H*, $NHCH_2CH_2N(CH_2CH_3)_2$), $NHCOCH_2CH_2N(CH_2CH_3)_2$), 1.16 (s, 6H, $COCH_2CH_2N(CH_2CH_3)_2$), 1.14 (s, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$); ^{13}C NMR (50 MHz, CD_3OD) δ 175.7 (C-9), 172.6 ($CONHCH_2$), 171.2 ($NHCOCH_2$), 155.1 (C-10a), 146.5 (C-1), 135.3 (C-6), 131.9 (C-4a), 128.6 (C-4), 126.0 (C-8), 125.4 (C-3), 124.5 (C-7), 122.8 (C-2), 121.4 (C-8a), 118.3 (C-9a), 117.5 (C-5) 50.7 ($NHCH_2CH_2N(CH_2CH_3)_2$), 47.9 ($COCH_2CH_2N(CH_2CH_3)_2$), 47.3 ($NHCH_2CH_2N(CH_2CH_3)_2$), 46.9 ($COCH_2CH_2N(CH_2CH_3)_2$), 36.8 ($NHCH_2CH_2N(CH_2CH_3)_2$), 33.0 ($COCH_2CH_2N(CH_2CH_3)_2$), 10.2 ($COCH_2CH_2N(CH_2CH_3)_2$); HRMS (ESI^+) m/z 481.2809 (calcd for $C_{27}H_{37}N_4O_4^+$, 481.2813).

4.2. Biological assays and experiments

4.2.1. Cell viability Resazurin assays

For the maintenance and culturing of the MCF-7 human breast cancer cells, which were provided by ATCC-LGC Standards GmbH (Wesel, Germany), EMEM, containing EBSS supplemented with 2 mM Glutamine, 1% Non-Essential Amino Acids (NEAA) and 10% Fetal Bovine Serum (FBS), was used as growth medium. Sub-culturing routine was performed via splitting of sub-confluent cultures (70–80%) 1:2 to 1:6 times, for succeeding an optimal seeding of 2–4 $\times$ 10^4 cells / cm^2, using 0.25% Trypsin (or Trypsin / EDTA), as detachment agent. Cells were grown in constant chamber conditions of 5% CO_2, 37 °C and $\geq$ 95% relative humidity.

The drug-sensitive CCRF-CEM and doxorubicin-resistant CEM/ADR5000 ALL cells were grown in RPMI medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml). CCRF-CEM and CEM/ADR5000 cells being obtained from exponential-phase cultures were counted and seeded into 96-well plates. The seeding density was ~ 100 cells per well for both cell lines. Cells were, then, exposed to tested agents at a single 30 μM concentration. After a 72 h incubation period in a 5% CO_2 incubator, under > 95% humidified environment, 20 μL of Resazurin (0.01% w/v) were added to each well and the plates were further incubated at 37 °C for 4 h. Fluorescence was measured on an Infinite M2000 Pro plate reader (Tecan, Crailsheim, Germany). Compounds were defined as active if the mean cell viability was < 32% in one or both cell lines. Viability was evaluated based on comparison with untreated cells. For active compounds, the same procedure was repeated using 0.03, 0.1, 0.3, 1, 3, 10 and 30 μM dose against the sensitive and resistant cell line. Dose-response curves were generated by plotting the mean cell viability (%) against the concentration of each examined compound (μM). IC_{50} values were calculated from a calibration curve by linear regression using Microsoft Excel.

4.2.2. Cell viability MTT assays

PC-3 is an androgen insensitive, p53-negative and *K-Ras* mutated human prostate cancer cell line, which was obtained from ATCC-LGC Standards GmbH (Wesel, Germany). PC-3 cells were cultured in DMEM media containing 10% FBS. To test the inhibitory activities of compounds using a cell-based protocol, MTT assay was performed for cell viability. Briefly, cells (750 / well) were seeded in 96-well plates, incubated overnight at 37 °C in 5% CO_2 and treated with the compounds in a dose-dependent manner for 96 h. Dimethyl sulfoxide (DMSO) was used as the vehicle control. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added at a concentration of 5 mg/ml directly to each well for 4 h at 37 °C. The medium was aspirated and the blue MTT formazan precipitate was dissolved in DMSO. Absorbance was determined in a Powerwave microplate spectrophotometer (Biotek Instruments, Inc., Vermont, USA) at 540 nm. Viable cell numbers were determined by tetrazolium conversion to its formazan dye. Each experiment was performed in triplicate.

The T24 human urinary bladder cancer cell line (major mutation signature: $HRAS^{G12V}$; $p53^{\Delta Y126}$) was provided by ATCC-LGC Standards GmbH (Wesel, Germany), while the WM2664 human metastatic melanoma (skin cancer) cell line (major mutation signature: $BRAF^{V600D}$) was purchased from ECACC-Sigma-Aldrich (Missouri, USA). Cells were cultured and exponentially grown in complete DMEM medium supplemented with 10% FBS, in a 5% CO_2 environment and at 37 °C. For the MTT assay, cells were seeded onto 48-well plates at approximately 80% confluency and subsequently treated with different doses of the herein examined four compounds, for 24 h. Next, cells were incubated with MTT solution for 4 h and the formazan crystals being produced were carefully dissolved in isopropanol. Spectrophotometric absorbance was measured via engagement of a Dynatech MR5000 Elisa microtiter-plate reader (Dynatech Laboratories, Virginia, USA), at 550 nm, using 630 nm as the wavelength of reference. Each MTT assay was repeated three times, using 3 wells per tested compound and cell line. All four compounds were dissolved in DMSO. Statistical significance of differences being observed in compound-treated versus control (DMSO) cell-survival values was determined using the unpaired, two-sided Student's t-test. Data are herein being reported as mean $\pm$ SD (standard deviation) of the mean value. $P < 0.001$ was considered statistically significant.

4.2.3. DNA staining and flow cytometric analysis of apoptosis and autophagy

Exponentially growing PC-3 human prostate cancer cells were treated with the IC_{50} values of the compounds **18a** and **18b** or the corresponding DMSO concentration (vehicle) for 24, 48 and 72 h. For cell cycle analysis, cell culture supernatants and attached cells were collected, centrifuged, washed in PBS, fixed in 50% ethanol and stained with an RNase-containing propidium iodide solution (50 μg/ml). For cell apoptosis assay, cells were harvested and stained with Annexin V binding buffer, Annexin V-FITC and PI (Annexin V-FITC Apoptosis Detection Kit, BD Systems) and were kept in the dark at room temperature for 15 min before being analyzed. DNA content was analyzed on a BD Accuri C6 Flow Cytometer, using the BD CSampler software (BD Biosciences, USA).

Non-apoptotic events were used to calculate the percentage of cells distributed in each phase. A P value < 0.05 was considered to be statistically significant (Student's t-test). Finally, quantification of autophagy was conducted using Cyto-ID Autophagy Detection Kit (Enzo Life Sciences, USA), according to manufacturer's instructions. ΔMFI Cyto-ID values were calculated for each treatment relative to control, as values > 0 indicate autophagy activation, whereas values < 0 indicate inhibition of autophagic flux.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary material

Synthetic procedures for compounds **1**, **8**, **10**, **11**, **14** and **15**, ^{1}H and ^{13}C NMR spectra can be found online at https://doi.org/10.1016/j.arabjc.2020.09.025.

References

Antonini, I., Polucci, P., Magnano, A., Cacciamani, D., Konieczny, M.T., Paradziej-Łukowicz, J., Martelli, S., 2003. Rational design, synthesis and biological evaluation of thiadiazinoacridines: a new class of antitumor agents. Bioorg. Med. Chem. 11, 399–405. https://doi.org/10.1016/S0968-0896(02)00442-X.

Atwell, G.J., Rewcastle, G.W., Baguley, B.C., Denny, W.A., 1987. Potential antitumor agents. 50. In vivo solid-tumor activity of derivatives of N-[2-(dimethylamino)ethyl]acridine-4-carboxamide. J. Med. Chem. 30, 664–669. https://doi.org/10.1021/jm00387a014.

Baker, G.P., Mann, F.G., Sheppard, N., Tetlow, A.J., 1965. 681. The structure of o-iodosobenzoic acid and of certain derivatives. J. Chem. Soc. 3721–3728. 10.1039/JR9650003721.

Belmont, P., Bosson, J., Godet, T., Tiano, M., 2007. Acridine and acridone derivatives, anticancer properties and synthetic methods: where are we now? Anticancer agents. Med. Chem. 7, 139–169. https://doi.org/10.2174/187152007780058669.

Belmont, P., Dorange, I., 2008. Acridine/acridone: a simple scaffold with a wide range of application in oncology. Expert Opin. Ther. Pat. 18, 1211–1224. https://doi.org/10.1517/13543776.18.11.1211.

Bernal, F.A., Coy-Barrera, E.D., 2015. Searching for antimicrobial and antiviral xanthones: a molecular docking study coupled to multivariate analysis. Planta Med. 81, PM_58. https://doi.org/10.1055/s-0035-1565435.

Bu, X., Chen, J., Deady, L.W., Denny, W.A., 2002. Synthesis and cytotoxicity of potential anticancer derivatives of pyrazolo[3,4,5-kl]acridine and indolo[2,3-a]acridine. Tetrahedron 58, 175–181. https://doi.org/10.1016/S0040-4020(01)01119-X.

Burdeska, K., Pugin, A., 1972. Über die synthese von in 1-stellung substituierten 4-nitro. Helv. Chim. Acta 55, 1948–1958. https://doi.org/10.1002/hlca.19720550612.

Chen, Y.-L., Lu, C.-M., Chen, I.-L., Tsao, L.-T., Wang, J.-P., 2002. Synthesis and antiinflammatory evaluation of 9-Anilinoacridine and 9-Phenoxyacridine derivatives. J. Med. Chem. 45, 4689–4694. https://doi.org/10.1021/jm020102v.

Chiron, J., Galy, J.-P., 2004. Reactivity of the acridine ring: A review. Synthesis 2004, 313–325. https://doi.org/10.1055/s-2003-44379.

Cholody, W.M., Martelli, S., Paradziej-Lukowicz, J., Konopa, J., 1990. 5-[(Aminoalkyl)amino]imidazo[4,5,1-de]acridin-6-ones as a novel class of antineoplastic agents. Synthesis and biological activity. J. Med. Chem. 33, 49–52. https://doi.org/10.1021/jm00163a009.

Chukaew, A., Ponglimanont, C., Karalai, C., Tewtrakul, S., 2008. Potential anti-allergic acridone alkaloids from the roots of Atalantia monophylla. Phytochemistry 69, 2616–2620. https://doi.org/10.1016/j.phytochem.2008.08.007.

Decodts, G., Wakselman, M., 1983. Suicide inhibitors of proteases. 1983. Lack of activity of halomethyl derivatives of some aromatic lactams. Eur. J. Med. Chem. 18, 107–111.

Demeunynck, M., 2004. Antitumour acridines. Expert Opin. Ther. Pat. 14, 55–70. https://doi.org/10.1517/13543776.14.1.55.

den Brok, M.W.J., Nuijen, B., Hillebrand, M.J.X., Grieshaber, C.K., Harvey, M.D., Beijnen, J.H., 2005. Development and validation of an LC–UV method for the quantification and purity determination of the novel anticancer agent C1311 and its pharmaceutical dosage form. J. Pharm. Biomed. Anal. 39, 46–53. https://doi.org/10.1016/j.jpba.2005.02.038.

Efferth, T., Konkimalla, V.B., Wang, Y.-F., Sauerbrey, A., Meinhardt, S., Zintl, F., Mattern, J., Volm, M., 2008. Prediction of broad spectrum resistance of tumors towards anticancer drugs. Clin. Cancer Res. 14, 2405–2412. https://doi.org/10.1158/1078-0432.CCR-07-4525.

Elomri, A., Skaltsounis, A.L., Michel, S., Tillequin, F., Koch, M., Rolland, Y., Pierre, A., Atassi, G., 1996. Synthesis and cytotoxic activity of acronycine derivatives modified at the pyran ring. Chem. Pharm. Bull. 44, 2165–2168. https://doi.org/10.1248/cpb.44.2165.

Feng, Z., Lu, X., Gan, L., Zhang, Q., Lin, L., 2020. Xanthones, a promising anti-inflammatory scaffold: structure, activity, and drug likeness analysis. Molecules 25, 598. https://doi.org/10.3390/molecules25030598.

Giannopoulou, A.F., Konstantakou, E.G., Velentzas, A.D., Avgeris, S.N., Avgeris, M., Papandreou, N.C., Zoi, I., Filippa, V., Katarachia, S., Lampidonis, A.D., Prombona, A., Syntichaki, P., Piperi, C., Basdra, E.K., Iconomidou, V., Papadavid, E., Anastasiadou, E., Papassideri, I.S., Papavassiliou, A.G., Voutsinas, G.E., Scorilas, A., Stravopodis, D.J., 2019a. Gene-specific intron retention serves as molecular signature that distinguishes melanoma from non-melanoma cancer cells in Greek patients. Int. J. Mol. Sci. 20, 937. https://doi.org/10.3390/ijms20040937.

Giannopoulou, A.F., Velentzas, A.D., Konstantakou, E.G., Avgeris, M., Katarachia, S.A., Papandreou, N.C., Kalavros, N.I., Mpakou, V.E., Iconomidou, V., Anastasiadou, E., Kostakis, I.K., Papassideri, I.S., Voutsinas, G.E., Scorilas, A., Stravopodis, D.J., 2019b. Revisiting histone deacetylases in human tumorigenesis: the paradigm of urothelial bladder cancer. Int. J. Mol. Sci. 20, 1291. https://doi.org/10.3390/ijms20061291.

Giannouli, V., Kostakis, I.K., Pouli, N., Marakos, P., Kousidou, O. Ch., Tzanakakis, G.N., Karamanos, N.K., 2007. Design, synthesis, and evaluation of the antiproliferative activity of a series of novel fused xanthenone aminoderivatives in human breast cancer cells. J. Med. Chem. 50, 1716–1719. https://doi.org/10.1021/jm061410m.

Giannouli, V., Kostakis, I.K., Marakos, P., Samara, P., Tsitsilonis, O., 2015. Synthesis and antiproliferative activity of some novel benzo-fused imidazo[1,8]naphthyridinones. Bioorg. Med. Chem. Lett. 25, 2621–2623. https://doi.org/10.1016/j.bmcl.2015.04.093.

Guilbaud, N., Léonce, S., Tillequin, F., Koch, M., Hickman, J.A., Pierré, A., 2002. Acronycine derivatives as promising antitumor agents. Anticancer Drugs 13, 445–449. https://doi.org/10.1097/00001813-200206000-00002.

Guo, J.Y., Chen, H.-Y., Mathew, R., Fan, J., Strohecker, A.M., Karsli-Uzunbas, G., Kamphorst, J.J., Chen, G., Lemons, J.M.S., Karantza, V., Coller, H.A., Dipaola, R.S., Gelinas, C., Rabinowitz, J.D., White, E., 2011. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev. 25, 460–470. https://doi.org/10.1101/gad.2016311.

Guo, J.Y., White, E., 2013. Autophagy is required for mitochondrial function, lipid metabolism, growth, and fate of KRAS(G12D)-driven lung tumors. Autophagy 9, 1636–1638.

Hadjipavlou, C., Kostakis, I.K., Pouli, N., Marakos, P., Mikros, E., 2006. 1-Ethyl-1H-3-nitrobenzopyrano[4,3,2-cd]isoindole: a novel heterocyclic ring system bearing an unusually labile deuterium-exchangeable aromatic proton. Tetrahedron Lett. 47, 3681–3684. https://doi.org/10.1016/j.tetlet.2006.03.130.

Kadioglu, O., Cao, J., Kosyakova, N., Mrasek, K., Liehr, T., Efferth, T., 2016. Genomic and transcriptomic profiling of resistant CEM/ADR-5000 and sensitive CCRF-CEM leukaemia cells for unravelling the full complexity of multi-factorial multidrug resistance. Sci. Rep. 6, 36754. https://doi.org/10.1038/srep36754.

Kaur, J., Singh, P., 2011. Acridine derivatives: a patent review (2009–2010). Expert Opin. Ther. Pat. 21, 437–454. https://doi.org/10.1517/13543776.2011.560570.

Kimmig, A., Gekeler, V., Neumann, M., Frese, G., Handgretinger, R., Kardos, G., Diddens, H., Niethammer, D., 1990. Susceptibility of multidrug-resistant human leukemia cell lines to human interleukin 2-activated killer cells. Cancer Res. 50, 6793–6799.

Knepper, K., Vanderheiden, S., Bräse, S., 2006. Nitrogen functionalities in palladium-catalyzed reactions on solid supports: a case study. Eur. J. Org. Chem. 2006, 1886–1898. https://doi.org/10.1002/ejoc.200500779.

Kostakis, I.K., Magiatis, P., Pouli, N., Marakos, P., Skaltsounis, A.-L., Pratsinis, H., Léonce, S., Pierré, A., 2002. Design, synthesis, and antiproliferative activity of some new pyrazole-fused amino derivatives of the pyranoxanthenone, pyranothioxanthenone, and pyranoacridone ring systems: a new class of cytotoxic agents. J. Med. Chem. 45, 2599–2609. https://doi.org/10.1021/jm011117g.

Kostakis, I.K., Tenta, R., Pouli, N., Marakos, P., Skaltsounis, A.L., Pratsinis, H., Kletsas, D., 2005. Design, synthesis, and antiproliferative activity of some novel aminosubstituted xanthenones, able to overcome multidrug resistance toward MES-SA/Dx5 cells. Bioorg. Med. Chem. Lett. 15, 5057–5060. https://doi.org/10.1016/j.bmcl.2005.07.079.

Kostakis, I.K., Pouli, N., Marakos, P., Skaltsounis, A.L., Pratsinis, H., Kletsas, D., 2006. Design and synthesis of novel aminosubstituted xanthenones and benzo[b]xanthenones: evaluation of their antiproliferative activity and their ability to overcome multidrug resistance toward MES-SA/D x 5 cells. Bioorg. Med. Chem. 14, 2910–2934. https://doi.org/10.1016/j.bmc.2005.12.003.

Kostakis, I.K., Pouli, N., Marakos, P., Kousidou, O.Ch., Roussidis, A., Tzanakakis, G.N., Karamanos, N.K., 2008. Design, synthesis and cell growth inhibitory activity of a series of novel aminosubstituted xantheno[1,2-d]imidazoles in breast cancer cells. Bioorg. Med. Chem. 16, 3445–3455. https://doi.org/10.1016/j.bmc.2007.03.003.

Kumar, A., Kumkum, S., Kumar, S.R., Puri, S.K., Chauhan, P.M.S., 2009. Synthesis of 9-anilinoacridine triazines as new class of hybrid antimalarial agents. Bioorg. Med. Chem. Lett. 10, 6996–6999. https://doi.org/10.1016/j.bmcl.2009.10.010.

Lemke, K., Poindessous, V., Skladanowski, A., Larsen, A.K., 2004. The antitumor triazoloacridone C-1305 is a topoisomerase II poison with unusual properties. Pharmacol. Mol. https://doi.org/10.1124/mol.104.000703.

Lemke, K., Wojciechowski, M., Laine, W., Bailly, C., Colson, P., Baginski, M., Larsen, A.K., Skladanowski, A., 2005. Induction of unique structural changes in guanine-rich DNA regions by the triazoloacridone C-1305, a topoisomerase II inhibitor with antitumor activities. Nucleic Acids Res. 33, 6034–6047. https://doi.org/10.1093/nar/gki904.

Mazerska, Z., Sowiński, P., Konopa, J., 2003. Molecular mechanism of the enzymatic oxidation investigated for imidazoacridinone antitumor drug, C-1311. Biochem. Pharmacol. 66, 1727–1736. https://doi.org/10.1016/S0006-2952(03)00477-5.

Mollica, A., Costante, R., Fiorito, S., Genovese, S., Stefanucci, A., Mathieu, V., Kiss, R., Epifano, F., 2014. Synthesis and anti-cancer activity of naturally occurring 2,5-diketopiperazines. Fitoterapia 98, 91–97. https://doi.org/10.1016/j.fitote.2014.07.010.

O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267, 5421–5426. https://doi.org/10.1046/j.1432-1327.2000.01606.x.

Ramaswamy, B., Mrozek, E., Kuebler, J.P., Bekaii-Saab, T., Kraut, E. H., 2011. Phase II trial of pyrazoloacridine (NSC#366140) in patients with metastatic breast cancer. Invest. New Drugs 29, 347–351. https://doi.org/10.1007/s10637-009-9338-1.

Recanatini, M., Bisi, A., Cavalli, A., Belluti, F., Gobbi, S., Rampa, A., Valenti, P., Palzer, M., Palusczak, A., Hartmann, R.W., 2001. A new class of nonsteroidal aromatase inhibitors: design and synthesis of chromone and xanthone derivatives and inhibition of the P450 enzymes aromatase and 17α-Hydroxylase/C17,20-Lyase. J. Med. Chem. 44, 672–680. https://doi.org/10.1021/jm000955s.

Seo, E.-J., Wiench, B., Hamm, R., Paulsen, M., Zu, Y., Fu, Y., Efferth, T., 2015. Cytotoxicity of natural products and derivatives toward MCF-7 cell monolayers and cancer stem-like mammospheres. Phytomedicine 22, 438–443. https://doi.org/10.1016/j.phymed.2015.01.012.

Shagufta, Ahmad I., 2016. Recent insight into the biological activities of synthetic xanthone derivatives. Eur. J. Med. Chem. 116, 267–280. https://doi.org/10.1016/j.ejmech.2016.03.058.

Stravopodis, D.J., Karkoulis, P.K., Konstantakou, E.G., Melachroinou, S., Lampidonis, A.D., Anastasiou, D., Kachrilas, S., Messini-Nikolaki, N., Papassideri, I.S., Aravantinos, G., 2009. Grade-dependent effects on cell cycle progression and apoptosis in response to doxorubicin in human bladder cancer cell lines. Int. J. Oncol. 34, 137–160. https://doi.org/10.3892/ijo_00000137.

Tan, Q., Wang, M., Yu, M., Zhang, J., Bristow, R.G., Hill, R.P., Tannock, I.F., 2016. Role of autophagy as a survival mechanism for hypoxic cells in tumors. Neoplasia 18, 347–355. https://doi.org/10.1016/j.neo.2016.04.003.

Tomar, V. et al, 2010. Synthesis of new chalcone derivatives containing acridinyl moiety with potential antimalarial activity. Eur. J. Med. Chem. 45, 745–751. https://doi.org/10.1016/j.ejmech.2009.11.022.

Tonelli, M., Vettoretti, G., Tasso, B., Novelli, F., Boido, V., Sparatore, F., Busonera, B., Ouhtit, A., Farci, P., Blois, S., Giliberti, G., La Colla, P., 2011. Acridine derivatives as anti-BVDV agents. Antiviral Res. 91, 133–141. https://doi.org/10.1016/j.antiviral.2011.05.005.

Vistica, D.T., Skehan, P., Scudiero, D., Monks, A., Pittman, A., Boyd, M.R., 1991. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. Cancer Res. 51, 2515–2520.

Wiśniewska, A., Chrapkowska, A., Kot-Wasik, A., Konopa, J., Mazerska, Z., 2007. Metabolic transformations of antitumor imidazoacridinone, C-1311, with microsomal fractions of rat and human liver. Acta Biochim. Pol. 54. 831–838. 10.18388/abp.2007_3179.

Wolf, S.J., Wakelin, L.P.G., He, Z., Stewart, B.W., Catchpoole, D.R., 2009. In vitro assessment of novel transcription inhibitors and topoisomerase poisons in rhabdomyosarcoma cell lines. Cancer Chemother. Pharmacol. 64, 1059–1069. https://doi.org/10.1007/s00280-009-0962-4.

Yu, X.-M., Ramiandrasoa, F., Guetzoyan, L., Pradines, B., Quintino, E., Gadelle, D., Forterre, P., Cresteil, T., Mahy, J.-P., Pethe, S.,

2012. Synthesis and biological evaluation of Acridine derivatives as antimalarial agents. Chem. Med. Chem. 7, 587–605. https://doi.org/10.1002/cmdc.201100554.

Zalupski, M.M., Shields, A.F., Philip, P.A., Kraut, M., LoRusso, P., Heilbrun, L.K., Vaitkevicius, V., 1998. Evaluation of pyrazoloacridine in patients with advanced pancreatic carcinoma. Invest. New Drugs 16, 93–96. https://doi.org/10.1023/A:1006087114621.

Zhang, B., Li, X., Li, B., Gao, C., Jiang, Y., 2014. Acridine and its derivatives: a patent review (2009–2013). Expert Opin. Ther. Pat. 24, 647–664. https://doi.org/10.1517/13543776.2014.902052.