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Eaton's Reagent-Mediated Domino π-Cationic Arylations of Aromatic Carboxylic Acids to Iasi-Red Polymethoxylated Polycyclic Aromatic Hydrocarbons: Products with Unprecedented Biological Activities as Tubulin Polymerization Inhibitors

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Abstract: A rapid domino π-cationic arylation of aromatic carboxylic acids, mediated by Eaton's reagent, has been developed for the synthesis of Iasi-red polymethoxylated polycyclic aromatic hydrocarbons (PAHs). This route is currently the easiest method to obtain such popular PAH compounds, which bear in addition numerous methoxy groups. The domino process was generalized, the structure of the obtained red products and the mechanism of their formations were elucidated, and some of their photophysical properties were determined. Newly synthesized polymethoxylated-PAHs were tested for their interaction with tubulin polymerization as well as for their cytotoxicity on a panel of NCI-60 human cancer cell lines. Interestingly, one of these rubicene derivatives exhibited remarkable cytotoxicity in vitro, including inhibition of leukemia, colon, melanoma, CNS, and ovarian cancer cell lines with GI_{50} values in the low nanomolar range ($GI_{50} < 10$ nM).

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are characterized by condensation of numerous (at least three) aromatic rings and they constitute a large, important family of persistent organic pollutants. PAHs are mainly formed during the incomplete combustion of fossil materials, but may also be formed to a lesser extent by microbes and plants. In addition, they are widely distributed in terrestrial, aquatic, and aerial areas.[1] Many of them are associated with carcinogenesis, mutagenesis, or teratogenesis. Consequently, they exert a high impact on nature and, as a result, on all the vital resources required for human health and life, including drinks and food.[2] Their metabolism in certain cell tissues, by the action of successive cytochrome P450 monooxygenases and epoxide hydrolase enzymes, results in the formation of various polyhydroxylated-PAHs (OH-PAHs) and vicinal-dihydroxy-PAHs. In many cases, they will often potentiate the expression of their biological effects as tumor activators[2a] compared with their parent PAHs.[3]

Importantly, alkoxy groups attached to a PAH platform, which are obtained by different chemical transformations, are reported to alter the electronic and photophysical properties of their parent PAHs essentially because of the extended π-conjugation that is gained by these π-electron materials. Certain examples of these products are used as dendritic polymers,[4] liquid-crystalline materials,[5] and electronic organic devices[6] including deep-blue organic light-emitting diodes (OLEDs),[7] fluorescent tags for labeling biomolecules,[8] and red-emitting electroluminescent materials.[9] On the other hand and in contrast to OH-PAHs, no examples of natural bioactive alkoxy-PAHs bearing one skeleton among PAHs family can be found in the literature. In the latter case, numerous natural products based on acephenanthrylene and benzo[*j*]fluoranthene PAH cores (Figure 1) with differing degrees of unsaturation have shown various and interesting biological properties. This is exemplified by laetevirenol A (**1b**)[10] and daldinone B (**2a**), daldinone A (**2b**), daldinone G (**2c**), and hypoxylonol F (**2d**).[11] These species, extracted from the roots and stems of

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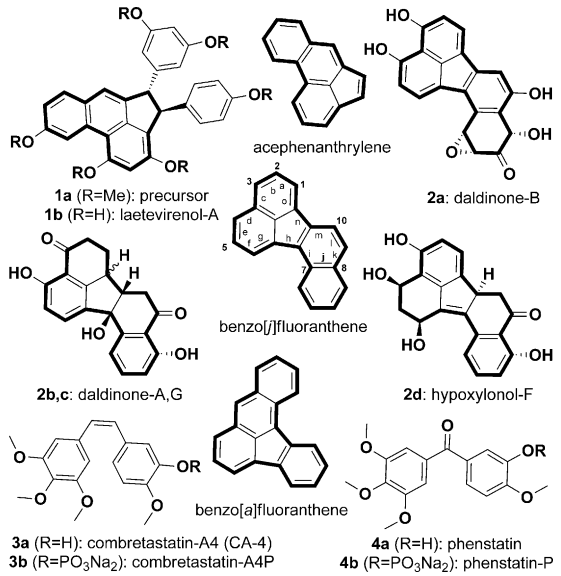


Figure 1. Naturally occurring compounds containing the acephenanthrylene and benzo[*j*]fluoranthene skeletons belonging to PHA family and combretastatins **3** and phenstatins **4** as the natural potent inhibitors of tubulin.

Parthenocissus laetevirens and the mushroom *Hypoxylon truncatum*, have displayed strong antioxidant activities and antiproliferative activity against human umbilical vein endothelial cells (HUVECs) and human umbilical artery endothelial cells (HUAECs), respectively (Figure 1). In parallel explorations on the biosynthetic pathways, determination of the immunosuppressive polyketides from the broth of mantis-associated *Daldinia eschscholzii*, identified 2,16-dihydroxybenzo[*j*]fluoranthene as a totally aromatic PAH substance, which has recently been isolated.[12] In addition, certain OH-PAH compounds containing one or more alkoxy groups (hybrid compounds), named hypoxylonols A–E, have been isolated from the same species as hypoxylonol F.[11c] Similar hybrid compounds were isolated as secondary metabolites[11c] from *Hypoxylon truncatum* (Xylariaceae), an endophytic fungus residing inside symptomless stems of *Artemisia annua*, which are the origin of potent cytotoxic substances.[13] A few examples of alkoxy-PAHs bearing the skeletons outlined in Scheme 1 have been used as valuable precursors during the total synthesis of laetevirenol A (**1b**)[10c] and benzo[*j*]fluoranthene derivatives.[11b]

Given the interest in these polycondensed systems, several synthetic approaches for the elaboration of PAH skeletons as well as for their peripheral modifications have been developed.[14] The most generally used methodologies for the construction of the PAH framework include: 1) intramolecular and intermolecular Diels–Alder cycloaddition,[15] 2) selective radical cascade cyclization of polyalkynes,[16] 3) flash-vacuum pyrolysis (FVP) of vinyl compounds,[14] 4) intramolecular photocyclization of stilbene-type compounds,[14] 5) ring-closing olefin metathesis (RCM),[14] 6) acid-catalyzed benzannulation and electrophilic cyclization,[14] 7) Lewis-acid promoted oxidative cyclodehydrogenation,[14] 8) Pd-catalyzed cyclization in tandem or not with other transformations,[17] 9) decoration of PAHs by direct C–H arylation with alkyl and aryl systems,[18] and 10) the very popular and efficient Friedel–Crafts reaction.[11b,19]

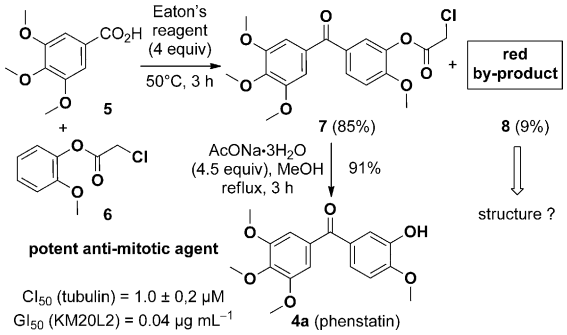
Our contribution to this area started from an interesting serendipitous observation we made during the execution of one of our research projects concerning the elaboration of new inhibitors of tubulin polymerization, analogous to the potent combretastatin A4[20] (CA-4; **3a**) and phenstatin (**4a**; Figure 1 and Scheme 1).[21] Our interest was aroused by the observation of a strong red-brown dark tint of the reaction media when some arylcarboxylic acids reacted with aryl compounds under Friedel–Crafts conditions with acid promoters such as polyphosphoric acid (PPA) or Eaton's reagent, which was generated from an mixture of P_2O_5 and $MeSO_3H$ (1:10 w/w).[22]

Results and Discussion

In this full account, we document our reinvestigation of the acid-catalyzed Friedel–Crafts reaction of arylcarboxylic acids with aromatic nucleophiles. Our attention is especially focused on the use of Eaton's reagent to promote cascade Friedel–Crafts type reactions producing hitherto unpublished structures belonging to the PAH family. During these investigations, the domino process was generalized, the structure of the obtained red products and the mechanism of their formations were elucidated, and some of their photophysical properties were determined. The results of their biological evaluation for the inhibition of tubulin and cancer cell growth are also reported.

Synthesis and characterization of the red by-product

A detailed view of the synthesis of the key intermediate, protected phenol-derivative **7**, starting from commercially available 3,4,5-trimethoxybenzoic acid (**5**) and 2-methoxyphenyl chloroacetate (**6**), was based on our previous work (Scheme 1).[21a] Following the well-known deprotection of chloroacetic ester in mild alkaline medium [e.g., $AcONa{\cdot}3H_2O$ (4.5 equiv), MeOH, reflux, 3 h],[23] ketone derivative **7** was separated from the dark red-brown mixture to afford the expected phenstatin (**4a**) in high yield (91%). It is worth mentioning that this two-step sequence represents a significant improvement over the first hemisynthesis of phenstatin (**4a**) from com-



Scheme 1. Synthetic pathway providing the bioactive phenstatin (**4a**) and red by-product **8** in the Friedel–Crafts domino reaction.

bretastatin A4 (**3a**) based on Jacobsen's oxidation.[24] The method constitutes a competitive approach to phenstatin and derivatives compared with the PPA-mediated Friedel–Crafts reaction.[23] Intrigued by the strong tint of the solution in numerous cases, additional purification procedures led to the isolation of a red pigment **8**, that we named "lasi-red", in 9% average yield. Interestingly, by repeating this reaction using PPA[23] at 100 °C for 4 h (instead of Eaton's reagent[25] at 60 °C; Scheme 1), the reaction presented the same profile. A dark red-brown coloration of the reaction media was also observed, and ketoester **7** was obtained albeit in lower yield (62%),[21a] together with the red pigment **8**, which was produced in comparable yield. However, the use of methanesulfonic acid alone, either with or without solvent, led to the formation of neither phenstatin (**4a**) nor the red by-product **8**. This result confirmed that an acidic dehydrating medium, such as PPA or, more conveniently, freshly prepared Eaton's reagent, is pivotal for the formation of red product **8**, due probably to the formation of mixed anhydride in situ. These results also confirmed that Eaton's reagent is more appropriate than PPA for this type of reaction, as mentioned elsewhere for related compounds.[22,26]

The structure of red by-product **8**, which was very puzzling, was established by aggregating numerous data and spectral characteristics from mono- and bidimensional NMR spectroscopy (Figure 2). However, the structural assignment was achieved without the aid of X-ray data because it was not possible to prepare single crystals of **8**. Its solubility was low in DMSO but very high in dichloromethane. The molecular weight, as obtained by LC-MS (Si-60/MeCN) analysis, was 612 g mol^{-1}, however, elemental analysis furnished a molecular composition formula of $C_{29}H_{27}ClO_{10}$, corresponding to MW = 571 g mol^{-1}. It was deduced that acetonitrile (MW = 41 g mol^{-1}), used as solvent, binds sufficiently strongly to **8** to lead to a combined MS peak.

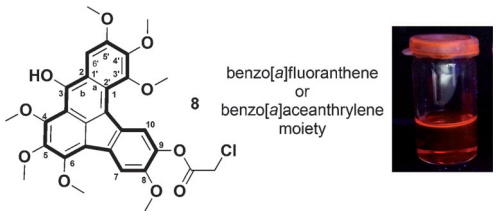
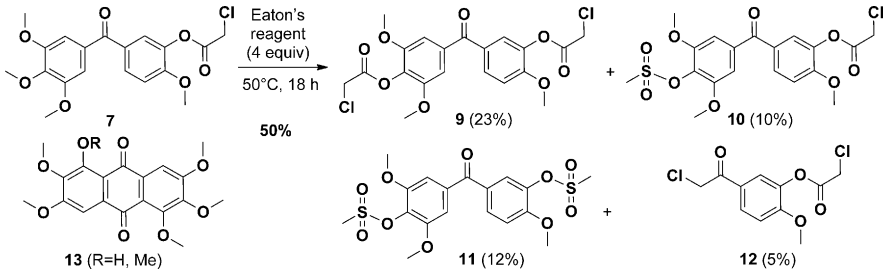


Figure 2. Established structure of lasi-red by-product **8**, namely 8-hydroxy-3,5,6,7,10,11,12-heptamethoxybenzo[*a*]aceanthrylen-2-yl chloroacetate.

Mechanistic aspects of the red-product formation

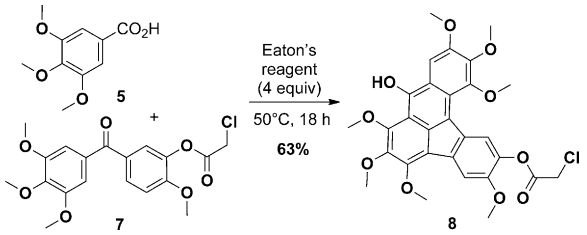
In our quest to understand the course of the reaction, especially that providing **8** (Figure 2), various investigations were planned. Taking into account that the structure of **8**, which could be obtained in an acid-mediated domino reaction, indicates the presence of three main motifs, presumably derived from two 3,4,5-trimethoxybenzoic acid (**5**) and one 2-methoxyphenyl chloroacetate (**6**). A plausible evolution of the protected phenstatin **7** in the reaction media was suggested as detailed below.

The stability of pure phenstatin derivative **7** was first examined in Eaton's reagent (4 equiv) medium at 50 °C under the optimized reaction conditions (Scheme 1). The results, shown in Scheme 2, indicate that the red by-product **8** was not formed. However, under these conditions extensive decomposition of the reaction medium occurred. The first events are cleavage and hydrolysis of the methoxy and ester functions, respectively, in the *para* position of ring A and the *meta* position of ring B of the starting phenstatin derivative **7**. Both new phenolic OH groups were then acylated by generating the chloroacetyl cation in the media and/or sulfonation with Eaton's reagent acid to provide chloroacetate diester **9** (23%), methanesulfonate ester **10** (10%) and diester **11** (12%).[27] These compounds, which were separable by flash chromatography on silica gel column, were accompanied by low amounts of chloroacetophenone derivative **12** (5%). The latter was probably formed by a tandem retro-Friedel–Crafts/Friedel–Crafts reaction sequence. More importantly, no traces of benzoquinone derivatives **13a** (R = H) or **13b** (R = Me) were detected in the reaction medium. It is worth noting that pentamethyl rufigallic acid (**13a**, R = H), lacking a methyl group, which could be considered as an intermediate for the production of benzo[*a*]aceanthrylene product **8**, was obtained as by-product in a related condensation of trimethoxybenzoic acid (**5**) with benzoxazolone.[21a] To date, such quinones have only been described from reactions using sulfuric acid at very high temperatures.[28] These results strongly suggested that a pathway starting from benzophenone **13** as intermediate in the mechanistic reaction for the formation of **8** should not be eliminated (Scheme 2).



Scheme 2. Results of the evolution of phenstatin precursor **7** in Eaton's reagent under the optimized reaction conditions.

We then considered the direct reaction of protected phenstatin **7** with 3,4,5-trimethoxybenzoic acid (**5**) catalyzed by



Scheme 3. Reaction of phenstatin precursor **7** with trimethoxybenzoic acid (**5**) leading solely to red product **8**.

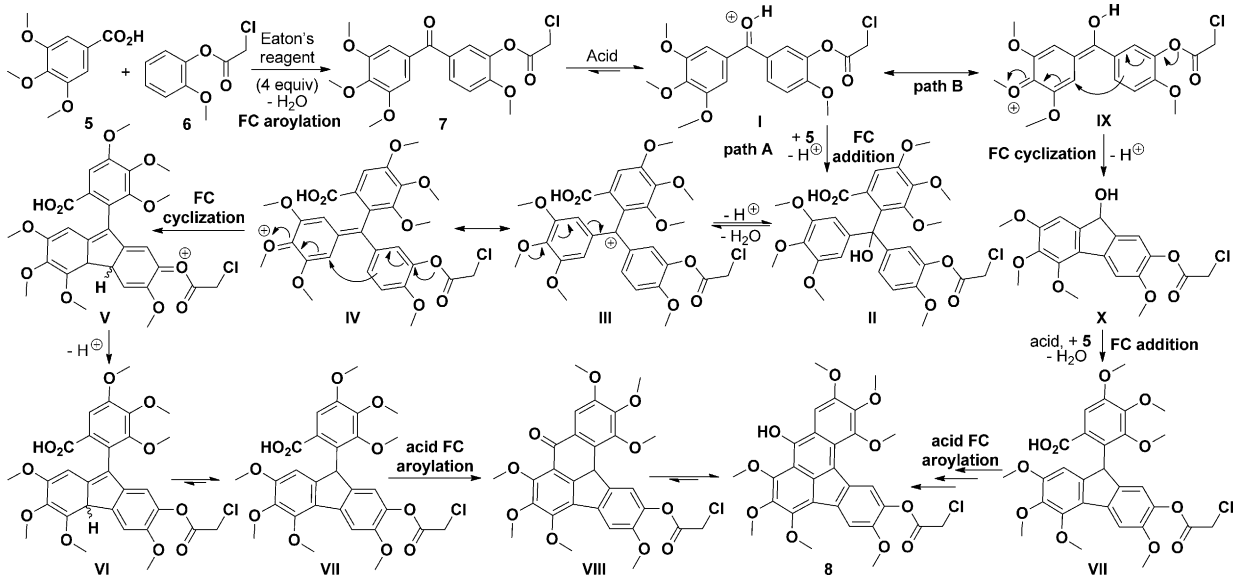
Eaton's reagent (Scheme 3). To our delight, heating carboxylic acid **5** with ketone **7** in Eaton's reagent for 18 h at 60 °C resulted in the formation of lasi-red **8** as the sole reaction product in 63% isolated yield. Considering that only one equivalent of carboxylic acid **5** was used, and that plausible decomposition of **7** to provide numerous derivatives as mentioned above is not a favored process (Scheme 2), this reaction confirms that benzoquinone **13** evoked in Scheme 2 was not an effective intermediate in the reaction. Moreover, this finding provides an elegant way to prepare benzo[*a*]aceanthrylene PAH **8** in significant yield.

A possible mechanism leading to the formation of benzo[*a*]aceanthrylene PAH **8** would proceed through domino π-cationic reactions including successive intermolecular and intramolecular Friedel–Crafts aroylation and arylation, respectively (Scheme 4). Thus, reaction of 3,4,5-trimethoxybenzoic acid (**5**) with 2-methoxyphenyl chloroacetate (**6**) under these conditions resulted in the formation of the standard Friedel–Crafts aroylation product **7**. Protonation of the ketone group furnished cation **I**, which is in equilibrium with **IX** (path B). Reaction of cationic species **I**, with a second equivalent of acid **5**, provided alcohol **II** through electrophilic addition. In the acidic medium this triaryl carbinol was never isolated and evolved into the more stable cation **III**, which undergoes, via the intermediary species **IV**, an intramolecular π-cationic cyclization related to the Clarkson and Gomberg approach.[29]

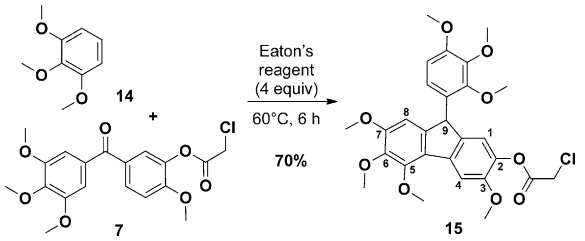
The highly activated arylcarboxylic acid intermediate **VII**, generated after deprotonation of cation **V** followed by tautomerism equilibration of **VI**, cyclizes to the pentacyclic benzo[*a*]aceanthrylene PAH core **VIII**, again, through intramolecular Friedel–Crafts aroylation mediated by Eaton's reagent. Ultimately, the keto-enolic equilibrium in **VIII** provided the expected red PAH product **8**. Interestingly, during this proposed domino process, four carbon–carbon bonds were formed, two intermolecular and two intramolecular, and the integrity of the chloroacetate group was maintained.

The domino reaction seems to proceed predominantly through the intermediacy of triaryl carbinol **II** (path A) rather than the mesomeric form **IX** of species **I**. Indeed, it could be considered that, upon protonation of **7**, benzophenone **IX** cyclized to give the corresponding substituted 9*H*-fluoren-9-ol **X** (Scheme 4).[30] This intermediate, with carboxylic acid **5** would give access to the intermolecular arylation product **VII**, which is also evoked in path A. However, we have already described that, in acid media ($CF_3SO_3H_{cat}$), dismutation of benzhydrols and 9*H*-fluoren-9-ol occurs rapidly, leading to diphenylmethanes and benzophenones derivatives.[31] A detailed investigation of the products formed in the synthesis of PAH compound **8** did not reveal any traces of these tricyclic compounds, rendering path B less probable. In addition, the high acidity of methanesulfonic acid used here ($pK_a = -0.6$) resulted in the significant stabilization of the tertiary carbocation **III** compared with that generated from 9*H*-fluoren-9-ol derivative **X**. This fact also supports the conclusion that the reaction follows path A (Scheme 4).[10c,32]

Another result that also supports mechanistic path A was obtained by the reaction of 1,2,3-trimethoxybenzene (**14**) and ketone **7** in Eaton's reagent. In this case, after 6 h heating at 60 °C, 9*H*-fluorene product **15** was isolated in 70% yield as the sole reaction product (Scheme 5). Here, the pathway seems to



Scheme 4. Mechanistic scheme leading to protected phenstatin **7** and benzo[*a*]aceanthrylene PAH compound **8** from 3,4,5-trimethoxybenzoic acid (**5**).



Scheme 5. Reaction of phenstatin precursor **7** with trimethoxybenzene (**14**) leading to 9*H*-fluorene product **15**.

proceed through ketone **7** protonation, arene addition, followed by intramolecular π-cationic cyclization of the unstable triaryl carbinol in acid media.[29] Consequently, the benzhydrol intermediates of type **II** (Scheme 4) emerged as the probable intermediate in the formation of PAH compound **8**.[32]

Having demonstrated that domino reaction based on Friedel–Crafts aroylation and arylation mediated by Eaton's reagent is effective to provide the benzo[*a*]aceanthrylene skeleton, we next examined this reaction using additional arylcarboxylic acids and arenes with the objective of generalizing the process. To measure the impact of benzoic acid substitution (mono-, di-, and tri-substituents) and the nature of the aromatic ring used as nucleophile (benzene, benzo[*d*]oxazol-2-ones) on the domino reaction, eight additional combinations were chosen (Table 1).

Generalization of the domino reaction was first realized by using the optimized reaction conditions [2-methoxyphenyl chloroacetate (**6**; 1 equiv), Eaton's reagent (P_2O_5/$MeSO_3H$, 4 equiv), 50 °C, 4–12 h], and changing the arylcarboxylic acid used (Table 1, entries 1–5). Conducting the same reaction with carboxylic acid **16** resulted in the formation of ketone **17** accompanied by trace amounts of **18** (entry 2). In this case, the yield of ketone **17** was superior to that obtained for ketone **7** (91 vs. 85%; Table 1). 2-Methoxybenzoic acid (**19**) also proved to be less active towards the domino process, with ketone **20**

Table 1. Protected phenstatin derivatives and PAH type compounds produced through the domino reaction based on Friedel–Crafts aroylation and arylation of arylcarboxylic acids.

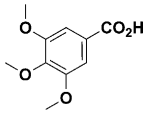
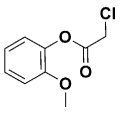
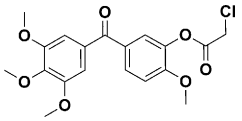
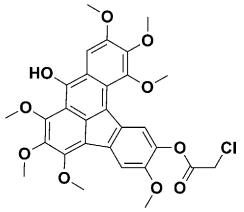
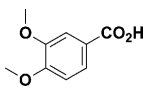
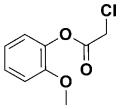
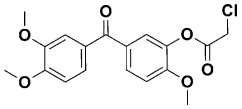
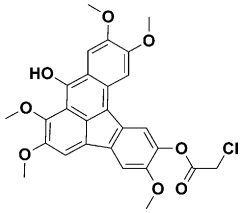
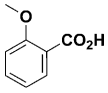
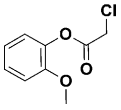
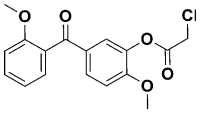
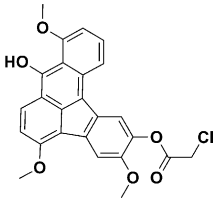
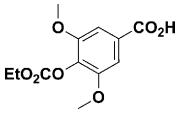
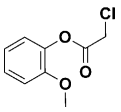
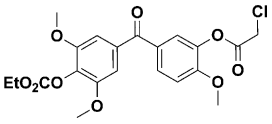
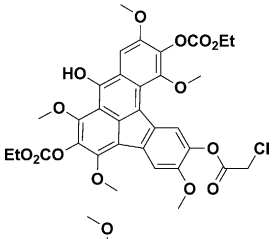
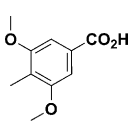
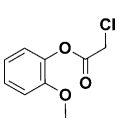
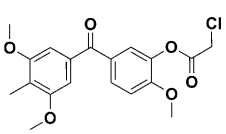
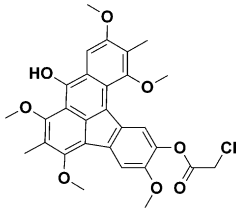
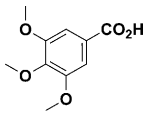
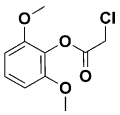
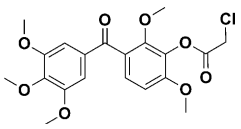
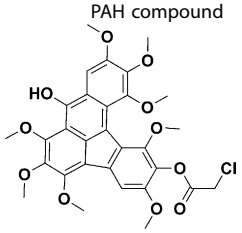
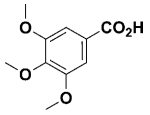
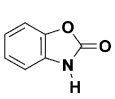
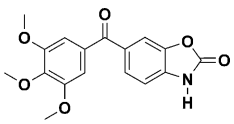
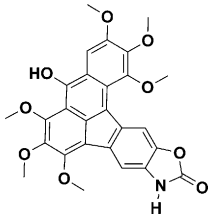
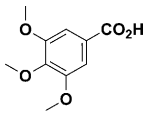
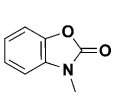
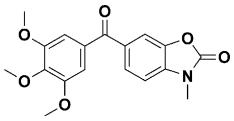
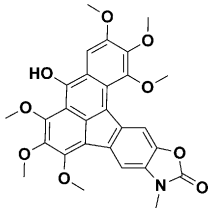
Entry	Aryl carboxylic acid	Arene	Phenstatin analogue	Yield [%][a]	PAH compound	Yield [%][a]
1		**5** 	**6** 	**7**[21a] 85		**8** 9
2		**16** 	**6** 	**17** 91		**18** trace
3		**19** 	**6** 	**20** 76		**21** trace
4		**22** 	**6** 	**23**[21a] 77		**24** 0
5		**25** 	**6** 	**26** 38		**27** 10

Table 1. (Continued)

Entry	Aryl carboxylic acid	Arene	Phenstatin analogue	Yield [%][a]	PAH compound	Yield [%][a]
6		**5** 	**28** 	**29**[21a] 80		**30** 0
7		**5** 	**31** 	**32**[21a] 60		**33** 11
8		**5** 	**34** 	**35** 60		**36** 7

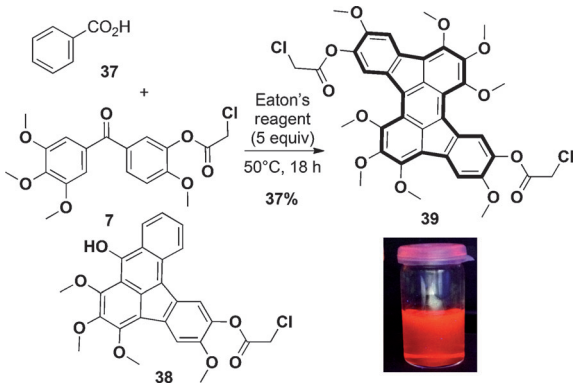
[a] Isolated yield after flash chromatography purification on silica gel column (EtOAc/*n*-heptane).

being isolated in 76% yield accompanied by only trace amounts of product **21**, which was not isolated (entry 3). In the same line, conducting the domino process with 4-(ethoxycarbonyloxy)-3,5-dimethoxybenzoic acid (**22**) was inoperative, and only product **23** was isolated (77%).[21a] Furthermore, replacement of one methoxy group with a methyl group at the C4-position on the benzene nuclei (entry 5) did not unfavorably affect the domino reaction, demonstrating that the pK_a and/or the nucleophilicity of the arylcarboxylic acids are important parameters for the domino process. Under these conditions (entry 5), acid **25** provided ketone **26** in only 38% and the PAH derivative **27** in 10% yield.

Another series, using 3,4,5-dimethoxybenzoic acid (**5**) with 2,6-dimethoxyphenyl 2-chloroacetate (**28**), benzo[*d*]oxazol-2(3*H*)-one (**31**), and 3-methylbenzo[d]oxazol-2(3*H*)-one (**34**) was investigated. Surprisingly, next to the expected ketones **29**,[21a] **32**,[21a] and **35**, respectively, no domino product **30** (Table 1, entry 6) was obtained with **28**, and in the other cases, PAH derivatives **33** and **36** were isolated in 11 and 7% yield, respectively (entries 7 and 8). From these results, it seems that the nucleophilicity of the aryl carboxylic acids is also important for the domino process.

Reaction with benzoic acid

To further validate the proposed domino mechanistic scheme depicted in Scheme 4, the reaction of benzoic acid (**37**) with protected phenstatin derivative **7** was then investigated as a complement to the reaction reported in Scheme 5. Under the established conditions, using 5 equivalents of Eaton's reagent (Scheme 6), the reaction after 18 h heating at 50 °C furnished only one isolated product with the characteristics of lasi-red dye in 37% yield after chromatography purification on silica gel column.



Scheme 6. Reaction of the protected phenstatin **7** with benzoic acid (**37**).

The structure of this product was established on the basis of an array of spectroscopic analyses including 1D and 2D NMR spectroscopy, MS as well as microanalysis, and seems to be, to our surprise, symmetrical polysubstituted PAH rubicene **39** (Scheme 6). This structure was secured without ambiguity on the basis of X-ray crystallographic analysis of crystals of lasi-red product **39**, as shown by the ORTEP drawing in Figure 3.[33] These crystals, which were difficult to obtain, were grown from anhydrous chloroform and cyclohexane (5:2) by slow evaporation during two weeks after a large screening of gradients of all the available solvents. Interestingly, the application of similar conditions to the other lasi-red products reported in this

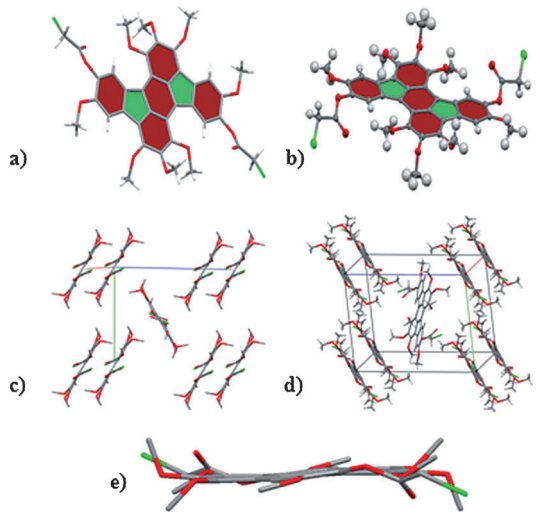


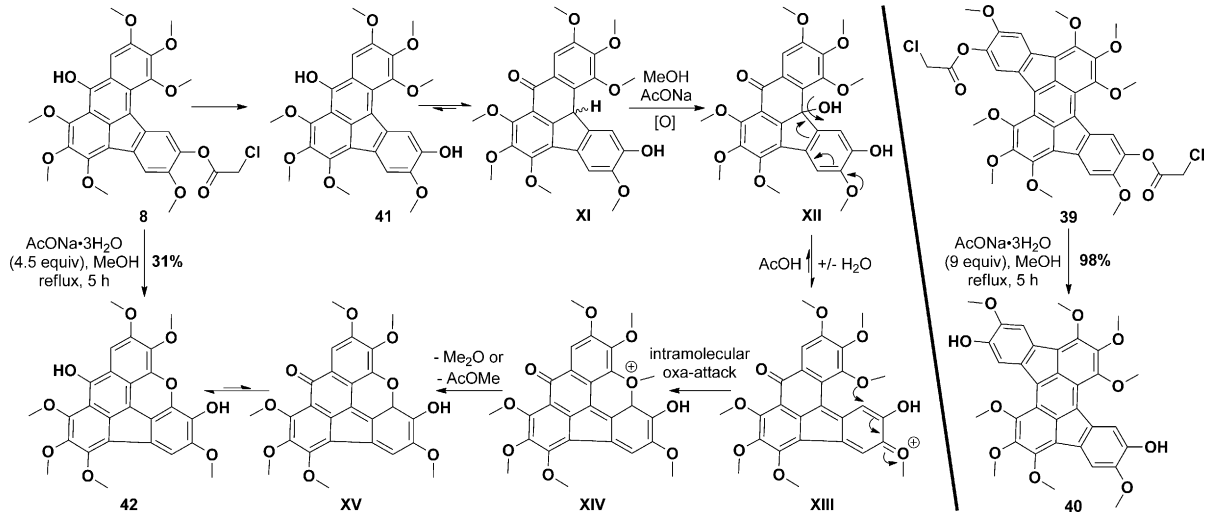
Figure 3. X-Ray crystal structure of rubicene derivative **39**. a), b) Top view with thermal ellipsoids at 30% probability, c), d) packing structure in the crystal, together with the crystal unit cell, and e) side view.

paper did not result in crystallization, and no X-ray crystal structure could be obtained.

In addition to the information collected from the X-ray analysis of crystals of **39** (Figure 3), another important point is that the formation of rubicene **39** does not involve benzoic acid (**37**) as a reactant partner. Indeed, red compound **39** seems to be produced by dimerization through intramolecular domino Friedel–Crafts reaction initiated by the formation of triaryl carbinol of type **II**, as shown in Scheme 4. This is the result of nucleophilic attack by the more nucleophilic trimethoxyphenyl part of ketone **7**, on the carbonyl group of a second molecule of the same substrate **7**. It seems that benzoic acid (**37**), in contrast to trimethoxybenzoic acid (**5**; Table 1, entry 1), is not sufficiently nucleophilic to attack the carbonyl group of ketone **7**, and thus PAH compound **38** is not formed. This also lends supports to the conclusion that the mechanistic pathway proposed in Scheme 4 is plausible. However, the role of benzoic acid in the course of this reaction is unclear. As a consequence, this protocol merits serious and full examination with respect to the development of approaches that can be used to access large PAH compounds, which are often labor-intensive and associated with low yields.[34]

Chloroester hydrolysis of PAHs compounds

To increase the number of PAH products available for biological testing considerations and establish structure-activity relationships, benzo[*a*]aceanthrylene derivative **8** and rubicene **39**, were submitted separately to cleavage of the chloroacetyl protecting group. The reaction with AcONa·3H$_2$O (4.5 equiv) in MeOH heated to reflux for 3 h, according to our reports,[21a] led to two different reaction profiles. Whereas hydrolysis of rubicene **39** provided the symmetrical dihydroxy-rubicene **40** in near quantitative isolated yield (98%) as expected, benzo[*a*]-aceanthrylene **8** led to 2,3,6,8,9,10-hexamethoxyacenaphtho[1,2,3,4-*klmn*]xanthene-5,11-diol (**42**) in 31% yield after recrystallization from ethyl acetate/*n*-heptane (Scheme 7). The mechanism of the formation of this compound was tentatively proposed to start from sodium acetate mediated removal of the chloroacetyl protective group to give the expected product **41**, which was in equilibrium in the media with ketone **XI**. Oxidation of the anion formed by sodium acetate with oxygen dissolved in MeOH and protonation then provided alcohol **XII**.[35] Dehydration under the influence of acetic acid produced in the medium led to the stable cationic species **XIII**, which underwent an intramolecular oxa-cyclization to **XIV**. After demethylation of this oxonium species, the resulting derivative **XV** ultimately furnished acenaphthoxanthene product **42** through keto-enol equilibrium. Interestingly, no other products with definite structure rising from this hydrolysis reaction were detected in the reaction medium; however, large amounts of starting material were recovered. Ultimately, this behavior towards the hydrolysis reaction seems to be due to the existence



Scheme 7. Cleavage of the chloroacetyl protecting group of benzo[*a*]aceanthrylene **8** and rubicene **39** PAH derivatives and a corresponding plausible mechanistic scheme.

of the hydroxyl phenolic group in the benzo[*a*]aceanthrylene structure of **8**, which is capable of equilibrating with the ketone function. This phenomenon does not exist in the structure of PAH-rubicene derivative **39** and, consequently, only a classical hydrolysis reaction occurred.

UV/Vis measurements

The optical absorption of benzo[*a*]aceanthrylene **27**, rubicenes **39** and **40**, and xanthene **42** are shown in Figure 4. All compounds absorb in the blue (380–401 nm) and green (504–570 nm) regions, which is in accordance with their red color. However, xanthene **42** absorbs much less in the green region compared with other studied compounds. This may be explained by the length of conjugation, which, in this case, is shorter because of the newly formed oxygen-containing ring. Moreover, its red color is less intense relative to other compounds.

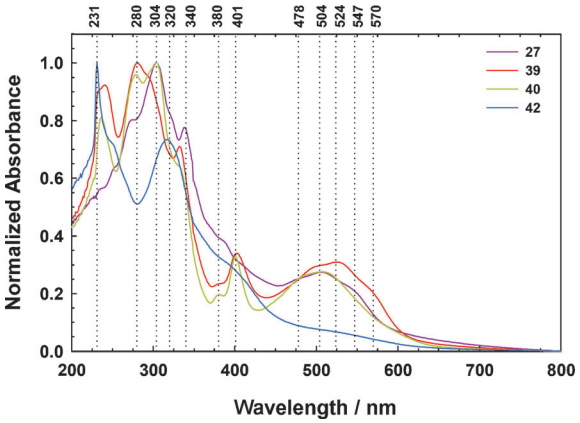


Figure 4. UV/Vis absorption spectra of compounds **27**, **39**, **40**, and **42** (10 μM) in CH_2Cl_2 as solvent.

The effect of the pH on the UV/Vis spectra of the studied compounds was explored, but no influence was observed (spectra available in the Supporting Information).

Table 2. In vitro growth inhibition ratio [%] caused by the selected compounds against tumor cell lines in the single-dose assay.

Cell type	Cell line	Inhibition ratio at 10 μM [%][a]					
		8	**27**	**33**	**36**	**39**	**40**
leukemia	CCRF-CEM	20	57	n.d.[b]	41	n.d.	n.d.
	HL-60 (TB)	11	34	9	33	n.d.	n.d.
	MOLT-4	35	48	11	24	n.d.	n.d.
	K-562	9	34	10	26	n.d.	n.d.
	RPMI-8226	18	32	–[c]	58	n.d.	n.d.
CNS cancer	SNB-75	–	16	n.d.	42	74	32
colon cancer	HCT-15	–	29	8	10	87	62
	HT29	14	–	–	–	100[d]	100
	KM12	13	19	–	17	100[e]	56
non-small cell lung cancer	HOP-92	15	5	23	n.d.	n.d.	n.d.
melanoma	LOX IMVI	–	32	5	7	83	40
	SK-MEL-5	9	31	13	31	100[f]	27
ovarian cancer	OVCAR-5	–	–	–	35	57	3
	OVCAR-8	–	52	13	21	87	71
renal cancer	ACHN	–	11	6	12	72	43
	UO-31	14	29	11	52	72	6
prostate cancer	PC-3	26	34	42	77	80	22
breast cancer	MDA-MB-231/ ATCC	22	27	16	37	96	56
	T-47D	38	37	33	40	42	79
Mean [%][g]		0	18	1	18	81	22

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen at 10 μM concentration.[37] [b] Not determined. [c] Inactive. [d] Positive cytotoxic effect: cell growth percent of −10%. [e] Positive cytotoxic effect: cell growth percent of −4%. [f] Positive cytotoxic effect: cell growth percent of −17%. [g] Average percentage of inhibition of all cell lines for the tested compounds.

Biological activity

Six PAH type compounds, **8**, **27**, **33**, **36**, **39** and **40**, were selected by NCI for screening against 60 human tumor cell lines.[36] The compounds were tested initially at a single high dose (10 μM) in the full 60-cell panel. The representative results are summarized in Table 2 and Figure 5. In the pentacyclic PAH series, compounds **27** and **36** exhibited the most interesting cell growth inhibition among the tested compounds: 57% inhibition on leukemia CCRF-CEM and 52% inhibition on ovarian OVCAR-8 cell lines for pentamethoxy derivative **27** and 58% inhibition on leukemia RPMI-8226 and 77% inhibition on prostate PC-3 cell lines for *N*-methyl-benzoxazolone **36**. Their analogues, heptamethoxy derivative **8** and benzoxazolone **33**, lost their cytostatic potential. In the rubicene series, chloroacetic ester **39** and phenol **40** showed significant cytostatic activity, especially on colon cancer cell lines (total inhibition of the growth of HT29 cell lines).

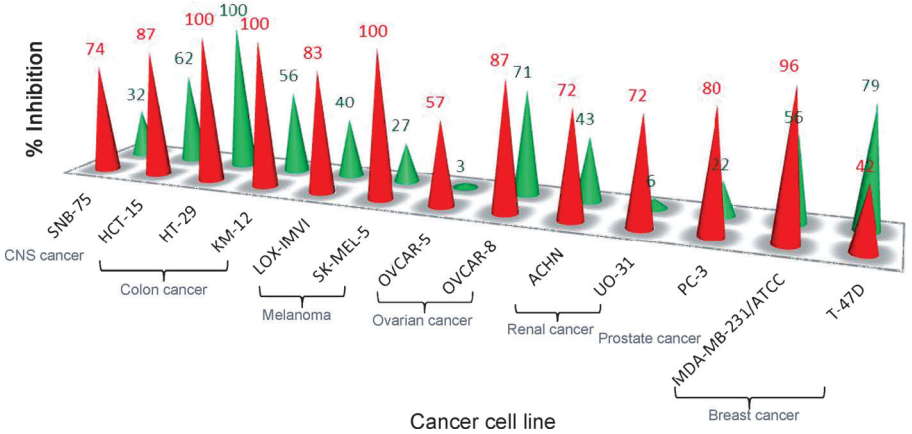


Figure 5. Representative biological activity of dichloroacetyl rubicene **39** (front row) versus deprotected rubicene **40** (back row), tested at a single high dose (10^{-5} M) in the full NCI 60 cell panel.

Generally, the deprotected compounds were more active than their precursors; the free hydroxyl group may be involved in interactions with different amino acids in the binding site of the biological target, which we assumed to be, in this case, the tubulin. Unexpectedly, deprotected rubicene **40** exhibited significantly decreased cellular activity on most tested cancer cell lines compared with protected precursor **39** (Figure 5). Therefore, only protected rubicene **39** satisfied predetermined threshold inhibition criteria and progressed to the 5-dose screen to evaluate its GI_{50} values (Table 2). This rubicene derivative exhibited the most important in vitro cytotoxicity among the studied compounds: inhibition of HL-60(TB), K-562, SR, HCT-15, KM-12, SW-620, M14, MDA-MB-435, MALME-3M, NCI-ADR/RES, and SF-539 cell lines with GI_{50} values in the low nanomolar range ($GI_{50} < 10$ nM; Table 3). It is important to note that the same compound **39** proved to be inactive on microtubules assembly (Table 4). Together, these results suggest a different mode of action for rubicene **39** that does not involve the tubulin. However, given its remarkable cytotoxicity and simple synthesis, this compound deserves further investigation, both in terms of biological mechanism and biotransformation.

From the results highlighted in Table 3, compounds with significant cytostatic and cytotoxic potency in the single-dose cellular assays were further tested for their ability to interact with tubulin[37] (Table 4). Interestingly and to our total surprise, the newly synthesized PAHs such as benzo[*a*]aceanthrylenes **8** and **27** showed antitubulin properties. Their biological potential was lower than that of parent phenstatin or CA-4, which are known for their remarkable bioactivity to this end and are used as references. However, these derivatives could serve as lead compounds for designing novel anticancer agents that target microtubules.

Table 4. Inhibitory activities on tubulin polymerization.

Entry	Product	TPI [%][a,b]	IC_{50} [μM][b]
1	**8**	51	n.d.[c]
2	**27**	64	45.0
3	**33**	42	n.d.
4	**36**	21	n.d.
5	**39**	49	n.d.
6	**40**	13	n.d.
7	combretastatin A4[d] **3a**	88	12.7
8	phenstatin[d] **4a**	95	15.0

[a] Inhibition of tubulin polymerization at 50 μM concentration. [b] Values represent the mean of two experiments. [c] Not determined. [d] Concerning the tubulin polymerization inhibition measure, see ref. [21a] and the Supporting Information.

Conclusion

We have developed a rapid domino π-cationic arylation of aromatic carboxylic acids, mediated by Eaton's reagent, for the synthesis of lasi-red polymethoxylated-PAHs. This synthetic route is currently the most straightforward method for obtaining such methoxylated-PAHs. The domino process was generalized, the structures of the obtained red products were elucidated and the mechanism for their formation was investigated. Some of their photophysical properties were established, and the results of their biological evaluation on tubulin and cancer cells lines have been reported. These new derivatives showed significant activities against cellular proliferation and tubulin polymerization. Rubicene **39** proved to be the most potent compound synthesized, with cytotoxicity activity against HL-60(TB), K-562, SR, HCT-15, KM-12, SW-620, M14, MDA-MB-435, MALME-3M, NCI-ADR/RES, and SF-539 cells in the low nanomolar concentration range. Unexpectedly, the deprotected rubicene **40** showed decreased potency relative to chloroacetic precursor **39**. These results indicate that rubicene derivative **39** is an interesting starting point for further investigation as a new cytotoxic agent. This compound will be evaluated in the future against other biological targets to elucidate the mechanism of action and develop structural analogues with improved characteristics.

Experimental Section

General remarks

Starting materials are commercially available and were used without further purification. Melting points were measured with a MPA 100 OptiMelt apparatus and are uncorrected. NMR spectra were acquired at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR with a Varian 400 MHz Premium Shielded spectrometer. Chemical shifts (δ) are given in parts per million relative to $CDCl_3$ (7.26 ppm; 77.1 ppm). Splitting patterns are designed: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet, and sym m, symmetric multiplet. Coupling constants *J* are reported in hertz (Hz).

Table 3. GI_{50} values [nM] for rubicene **39** on different tumor cell lines (see the Supporting Information for reports of biological evaluation and all details of the measurements).

Cell type	**Leukemia**			**CNS cancer**			**Colon cancer**		
Cell line	HL-60(TB)	K-562	SR	SNB-75	SF-539	U251	SW-620	HCT-15	KM-12
39: GI_{50} (nM)	**<10**	**<10**	**<10**	20	**<10**	20	**<10**	**<10**	**<10**
Cell type	**Non-Small Cell Lung cancer**			**Melanoma**			**Ovarian cancer**		
Cell line	HOP-62	NCI-H460	NCI-H23	M14	MDA-MB-435	MALME-3M	OVCAR-3	NCI-ADR/RES	SK-OV-3
39: GI_{50} (nM)	40	30	40	**<10**	**<10**	**<10**	20	**<10**	10
Cell type	**Renal cancer**			**Prostate cancer**			**Breast cancer**		
Cell line	786-0	CAKI-1	UO-31	PC-3		DU-145	MCF-7	BT-549	HS 578T
39: GI_{50} (nM)	70	600	50	40		30	30	20	20

COSY and NOESY techniques were used in the assignment of ^{1}H–^{1}H relationships. HSQC and HMBC techniques were used throughout for the assignment of the ^{1}H–^{13}C relationships. Thin-layer chromatography was conducted on Macherey–Nagel silica gel plates with a fluorescent indicator and visualized with a UV-lamp at 254 and 366 nm. Column chromatography was performed with a CombiFlash R_f Companion (Teledyne-Isco System) using RediSep packed columns. IR spectra were recorded with a Varian 640-IR FTIR Spectrometer. UV/Vis spectra were recorded with a Cary UV 100 (Varian) spectrophotometer with cells of 1 cm path length, at 25 °C. A flow cell was used to allow pH adjustments. Elemental analyses (C, H, N) of new compounds were determined by the 'Pôle Chimie Moléculaire', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France.

General procedure A: Friedel–Crafts reaction in the presence of Eaton's reagent

Eaton's reagent was prepared from phosphorus pentoxide (P_2O_5) and methanesulfonic acid (CH_3SO_3H; weight ratio P_2O_5/CH_3SO_3H, 1:10). The mixture was heated at 40 °C under a nitrogen atmosphere until complete homogeneity. Carboxylic acid (1.15–1.5 equiv) and aromatic derivative (1.0 equiv) were then added and the mixture was heated at 50–60 °C under an inert atmosphere for 3–30 h. After cooling to RT, the reaction medium was diluted with dichloromethane and carefully poured into a separatory funnel containing aqueous sodium bicarbonate solution (50% $NaHCO_3$). The aqueous solution was extracted with dichloromethane and the combined organic layers were dried ($MgSO_4$). Solvent was removed under reduced pressure to produce a brownish oil. The crude product was purified by column chromatography on silica gel to afford pure benzophenones and red by-products.

2-Methoxy-5-(3,4,5-trimethoxybenzoyl)phenyl chloroacetate (7; Scheme 1):[21a] General procedure A was followed by using **5** (11.42 g, 53.8 mmol), **6** (9.00 g, 44.9 mmol) and Eaton's reagent (4.09 g P_2O_5 in 27.6 mL CH_3SO_3H). The mixture was heated at 50 °C for 3 h. The final brown oil was purified by column chromatography on silica gel (EtOAc/*n*-heptane, 3:7) to give pure chloroacetate **7** (15.1 g, 85%; lit. (with PPA)[23a] 80%) as an off-white solid; m.p. 150–152 °C (EtOH/Et_2O); TLC: R_f=0.62 (EtOAc/*n*-heptane, 7:3); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=3.89 (s, 6H; 2OCH_3), 3.94 (s, 6H; 2OCH_3), 4.36 (s, 2H; $OCOCH_2Cl$), 7.03 (s, 2H; ArH), 7.07 (d, J=8.6 Hz, 1H; ArH), 7.62 (d, J=2.2 Hz, 1H; ArH), 7.79 ppm (dd, J=8.6, 2.2 Hz, 1H; ArH); elemental analysis calcd (%) for $C_{19}H_{19}O_7Cl$: C 57.80, H 4.85; found: C 57.74, H 4.78.

8-Hydroxy-3,5,6,7,10,11,12-heptamethoxybenzo[*a*]aceanthrylen-2-yl chloroacetate (8; Table 1): By-product from the synthesis of **7**. Red solid (2.3 g, 9%); m.p. 182–185 °C (EtOAc/*n*-heptane); R_f=0.74 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.84 (s, 3H; OCH_3), 4.00 (s, 3H; OCH_3), 4.06 (s, 6H; 2OCH_3), 4.15 (s, 3H; OCH_3), 4.22 (s, 3H; OCH_3), 4.34 (s, 3H; OCH_3), 4.45 (s, 2H; $OCOCH_2Cl$), 7.58 (s, 1H; ArH), 7.88 (s, 1H; ArH), 8.48 (s, 1H; ArH), 9.80 ppm (s, 1H; ArOH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=41.0 (CH_2), 55.9 (CH_3), 56.2 (CH_3), 61.4 (CH_3), 61.4 (CH_3), 61.6 (CH_3), 61.6 (CH_3), 62.3 (CH_3), 77.2 (CH), 98.0 (CH), 106.8 (C), 107.4 (CH), 119.1 (C), 119.2 (C), 120.4 (CH), 121.2 (C), 123.5 (C), 127.6 (C), 132.9 (C), 135.0 (C), 138.4 (C), 142.6 (C), 144.8 (C), 148.2 (C), 148.4 (C), 149.0 (C), 150.2 (C), 150.9 (C), 166.1 ppm (C); IR: $\tilde{\nu}$=847, 1069, 1112, 1254, 1291, 1312, 1453, 1606, 1674, 3341 cm^{-1}; LC/MS (APCI$^+$): *m/z*: 571.8 $[M+H]^+$; elemental analysis calcd (%) for $C_{29}H_{27}O_{10}Cl$: C 60.71, H 5.09; found: C 60.76, H 5.00.

5-(3,4-Dimethoxybenzoyl)-2-methoxyphenyl chloroacetate (17; Table 1): General procedure A was followed by using 3,4-dimethoxybenzoic acid **16** (5.0 g, 27.4 mmol), 2-methoxyphenyl chloroacetate **6** (3.67 g, 18.3 mmol), and Eaton's reagent (1.82 g P_2O_5 in 12.3 mL CH_3SO_3H). The mixture was heated at 50 °C for 8 h and the final product was recrystallized from absolute ethanol to give pure chloroacetate **17** (6.07 g, 91%) as a white solid; m.p. 112–114 °C (EtOH); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=3.93 (s, 3H; OCH_3), 3.94 (s, 3H; OCH_3), 3.97 (s, 3H; OCH_3), 4.36 (s, 2H; $OCOCH_2Cl$), 6.92 (d, J=8.2 Hz, 1H; ArH), 7.06 (d, J=8.6 Hz, 1H; ArH), 7.38 (dd, J=8.2, 1.9 Hz, 1H; ArH), 7.42 (d, J=2.0 Hz, 1H; ArH), 7.58 (d, J=1.9 Hz, 1H; ArH), 7.76 ppm (dd, J=8.6, 2.0 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=40.5 (CH_2), 56.0 (CH_3), 56.1 (CH_3), 56.2 (CH_3), 109.9 (CH), 111.6 (CH), 112.2 (CH), 124.6 (CH), 124.8 (CH), 130.1 (CH), 130.2 (C), 130.8 (C), 138.7 (C), 149.0 (C), 152.8 (C), 154.2 (C), 165.3 (C), 193.3 ppm (C); IR: $\tilde{\nu}$=770, 820, 865, 1021, 1104, 1130, 1226, 1262, 1424, 1509, 1597, 1635, 1789 cm^{-1}; elemental analysis calcd (%) for $C_{18}H_{15}O_6Cl$: C 59.27, H 4.70; found: C 59.32, H 4.49.

2-Methoxy-5-(2-methoxybenzoyl)phenyl chloroacetate (20; Table 1): General procedure A was followed by using 2-methoxybenzoic acid (**19**; 1.31 g, 8.59 mmol), 2-methoxyphenyl chloroacetate (**6**; 1.50 g, 7.48 mmol) and Eaton's reagent (0.48 g P_2O_5 in 3.21 mL CH_3SO_3H). The mixture was heated at 50 °C for 4 h, and the final product was recrystallized from absolute ethanol to give pure chloroacetate **20** (1.9 g, 76%) as an off-white solid; ^{1}H NMR ($CDCl_3$, 200 MHz): δ=3.74 (s, 3H; OCH_3), 3.90 (s, 3H; OCH_3), 4.33 (s, 2H; $OCOCH_2Cl$), 6.99 (d, J=8.6 Hz, 2H; ArH), 7.04 (td, J=7.6, 1.2 Hz, 1H; ArH), 7.33 (dd, J=7.4, 1.6 Hz, 1H; ArH), 7.46 (td, J=8.6, 1.6 Hz, 1H; ArH), 7.56 (d, J=2.0 Hz, 1H; ArH), 7.75 ppm (dd, J=8.6, 2.0 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=40.5 (CH_2), 55.6 (CH_3), 56.2 (CH_3), 111.4 (CH), 111.5 (CH), 111.6 (CH), 120.5 (CH), 124.3 (CH), 129.4 (C), 130.2 (CH), 131.0 (C), 131.8 (CH), 138.9 (C), 154.8 (C), 157.1 (C), 165.2 (C), 194.1 ppm (C); elemental analysis calcd (%) for $C_{17}H_{15}O_5Cl$: C 61.00, H 4.52; found: C 60.74, H 4.48.

5-{4-[(Ethoxycarbonyl)oxy]-3,5-dimethoxybenzoyl}-2-methoxyphenyl chloroacetate (23; Table 1):[21a] General procedure A was followed by using 4-[(ethoxycarbonyl)oxy]-3,5-dimethoxybenzoic acid (**22**; 10.10 g, 37.38 mmol), 2-methoxyphenyl chloroacetate (**6**; 5.00 g, 24.92 mmol), and Eaton's reagent (3.67 g P_2O_5 in 24.80 mL CH_3SO_3H). The mixture was heated at 50 °C for 3 h and the resulting brown oil was crystallized in 95% EtOH to give pure product **23** (8.70 g, 77%) as a white solid; m.p. 171–172 °C (EtOH); ^{1}H NMR ($CDCl_3$, 200 MHz): δ=1.41 (t, J=7.1 Hz, 3H; $OCO_2CH_2CH_3$), 3.87 (s, 6H; 2OCH_3), 3.94 (s, 3H; OCH_3), 4.35 (q, J=7.1 Hz, 2H; $OCOCH_2CH_3$), 4.37 (s, 2H; $OCOCH_2Cl$), 7.02 (s, 2H; ArH), 7.07 (d, J=8.5 Hz, 1H; ArH), 7.63 (d, J=2.2 Hz, 1H; ArH), 7.81 ppm (dd, J=8.5, 2.2 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 50 MHz): δ=13.9 (CH_3), 40.2 (CH_2), 56.0 (2CH_3), 56.2 (CH_3), 65.0 (CH_2), 106.5 (2CH), 111.5 (CH), 124.6 (CH), 129.7 (CH), 129.9 (C), 130.3 (C), 135.4 (C), 138.5 (C), 151.9 (2C), 152.3 (C), 154.4 (C), 165.2 (C), 193.2 ppm (C); IR: $\tilde{\nu}$=760, 1105, 1127, 1209, 1253, 1336, 1409, 1597, 1651, 1770, 1792 cm^{-1}; elemental analysis calcd (%) for $C_{21}H_{21}O_9Cl$: C 55.70, H 4.67; found: C 55.64, H 4.79.

5-(3,5-Dimethoxy-4-methylbenzoyl)-2-methoxyphenyl chloroacetate (26; Table 1): General procedure A was followed by using 3,5-dimethoxy-4-methyllbenzoic acid (**25**; 3.80 g, 19.5 mmol), 2-methoxyphenyl chloroacetate (**6**; 3.60 g, 17.7 mmol), and Eaton's reagent (1.82 g P_2O_5 in 12.3 mL CH_3SO_3H). The mixture was heated at 60 °C for 4 h and the final brown oil was purified by column chromatography on silica gel (EtOAc/*n*-heptane, 15:85→3:7) and the resulting solid was recrystallized from diethyl ether to give pure chloroacetate **26** (2.58 g, 38%) as white crystals; m.p. 125–130 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=2.17 (s, 3H; $ArCH_3$), 2.85 (s, 6H; 2OCH_3), 3.94 (s, 3H; OCH_3), 4.36 (s, 2H; $OCOCH_2Cl$), 6.95 (s, 2H; 2ArH), 7.06 (d, J=8.6 Hz, 1H; ArH), 7.63 (d, J=1.9 Hz, 1H;

ArH), 7.81 ppm (dd, $J=8.6$, 1.9 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=8.7$ (CH_3), 40.5 (CH_2), 55.9 (2CH_3), 56.2 (CH_3), 105.3 (2CH), 111.7 (CH), 119.8 (C), 124.9 (CH), 130.3 (CH), 130.6 (C), 135.8 (C), 138.7 (C), 154.4 (C), 158.0 (2C), 165.4 (C), 194.3 ppm (C); IR: $\tilde{\nu}=$ 760, 821, 1015, 1135, 1234, 1268, 1406, 1606, 1639, 1788 cm^{-1}; elemental analysis calcd (%) for $C_{19}H_{19}O_6Cl$: C 60.24, H 5.06; found: C 60.55, H 4.98.

8-Hydroxy-3,5,7,10,12-pentamethoxy-6,11-dimethylbenzo[*a*]-aceanthrylen-2-yl chloroacetate (27; Table 1): By-product from the synthesis of compound **26**. Red solid (0.95 g, 10% yield); m.p. 175–180 °C (EtOAc/*n*-heptane); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=2.45$ (s, 3H; $ArCH_3$), 2.53 (s, 3H; $ArCH_3$), 3.68 (s, 3H; OCH_3), 4.00 (s, 3H; OCH_3), 4.03 (s, 3H; OCH_3), 4.09 (s, 3H; OCH_3), 4.10 (s, 3H; OCH_3), 4.46 (s, 2H; $OCOCH_2Cl$), 7.54 (s, 1H; ArH), 7.85 (s, 1H; ArH), 8.50 (s, 1H; ArH), 9.77 ppm (s, 1H; ArOH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=$ 9.7 (CH_3), 9.8 (CH_3), 41.0 (CH_2), 55.6 (CH_3), 56.2 (CH_3), 60.9 (CH_3), 61.1 (CH_3), 62.2 (CH_3), 96.2 (CH), 107.3 (CH), 107.5 (CH), 119.3 (C), 120.5 (CH), 121.4 (C), 121.7 (C), 121.9 (C), 122.2 (C), 124.2 (C), 130.2 (C), 133.3 (C), 134.9 (C), 138.5 (C), 148.3 (C), 149.7 (C), 154.2 (C), 154.8 (C), 155.2 (C), 155.3 (C), 166.1 ppm (C); IR: $\tilde{\nu}=837$, 979, 1090, 1120, 1141, 1210, 1292, 1462, 1612, 1780, 3345 cm^{-1}; elemental analysis calcd (%) for $C_{29}H_{27}O_8Cl$: C 64.63, H 5.05; found: C 65.01, H 5.17.

2,6-Dimethoxy-3-(3,4,5-trimethoxybenzoyl)phenyl chloroacetate (29; Table 1):[21a] General procedure A was followed by using 3,4,5-trimethoxybenzoic acid (**5**; 1.25 g, 5.9 mmol), 2,6-dimethoxyphenyl chloroacetate (**28**; 0.90 g, 3.9 mmol), and Eaton's reagent (0.57 g P_2O_5 in 3.83 mL CH_3SO_3H). The mixture was heated at 60 °C for 5 h and the final brown oil was purified on silica gel (EtOAc/*n*-heptane, 4:6) to give pure chloroacetate **29** (1.35 g, 80%) as an off-white solid; m.p. 118–120 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=3.68$ (s, 3H; OCH_3), 3.86 (s, 6H; 2OCH_3), 3.91 (s, 3H; OCH_3), 3.93 (s, 3H; OCH_3), 4.38 (s, 2H; $OCOCH_2Cl$), 6.81 (d, $J=8.6$ Hz, 1H; ArH), 7.10 (s, 2H; ArH), 7.33 ppm (d, $J=8.6$ Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=40.4$ (CH_2), 56.2 (2CH_3), 56.3 (CH_3), 60.9 (CH_3), 62.6 (CH_3), 106.9 (CH), 107.4 (2CH), 125.7 (C), 128.3 (CH), 132.3 (C), 132.5 (C), 142.6 (C), 151.8 (C), 152.9 (2C), 154.3 (C), 165.0 (C), 193.5 ppm (C). IR: $\tilde{\nu}=522$, 758, 814, 1093, 1120, 1293, 1290, 1414, 1489, 1667, 1754, 1781 cm^{-1}; elemental analysis calcd (%) for $C_{20}H_{21}O_8Cl$: C 56.54, H 4.98; found: C 56.37, H 4.81.

6-(3,4,5-Trimethoxybenzoyl)-1,3-benzoxazol-2(3*H*)-one (32; Table 1):[21a] General procedure A was followed by using 1,3-benzoxazol-2(3*H*)-one (**31**; 4.00 g, 29.6 mmol) and Eaton's reagent (4.00 g P_2O_5 in 27.00 mL CH_3SO_3H). 3,4,5-Trimethoxybenzoic acid (**5**; 10.99 g, 51.8 mmol) was added to the reaction mixture in small portions (1.0 g every 30 min) and the resulting viscous solution was heated at 60 °C for 30 h. The final brown oil was purified by column chromatography on silica gel (EtOAc/*n*-heptane, 1:9) to give pure compound **32** (2.24 g, 23%) as a beige solid; m.p. 220–221 °C (EtOAc/*n*-heptane). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=3.89$ (s, 6H; 2OCH_3), 3.95 (s, 3H; OCH_3), 7.02 (s, 2H; ArH), 7.16 (d, $J=$ 8.6 Hz, 1H; ArH), 7.71 (dd, $J=8.6$, 1.7 Hz, 1H; ArH), 7.73 (d, $J=$ 1.7 Hz, 1H; ArH), 8.32 ppm (large s, 1H; NH); ^{13}C NMR ($CDCl_3$ + [D_6]DMSO, 100 MHz): $\delta=56.9$ (2CH_3), 61.4 (CH_3), 108.1 (2CH), 109.8 (CH), 111.5 (CH), 127.8 (CH), 132.3 (C), 133.5 (C), 135.4 (C), 142.3 (C), 144.3 (C), 153.5 (2C), 155.7 (C), 194.7 ppm (C); IR: $\tilde{\nu}=583$, 704, 815, 922, 1104, 1119, 1290, 1413, 1493, 1574, 1643, 1773, 3021 cm^{-1}; elemental analysis calcd (%) for $C_{17}H_{15}O_6N$: C 62.00, H 4.59, N 4.25; found: C 61.89, H 4.40, N 4.19.

5-Hydroxy-1,2,3,6,7,8-hexamethoxybenzo[1,2]fluorantheno[8,9-*d*][1,3]oxazol-11(10*H*)-one (33; Table 1): By-product from the synthesis of compound **32**. Red solid (1.65 g, 11% yield); m.p. > 250 °C (EtOAc/*n*-heptane); ^{1}H NMR ([D_6]DMSO, 400 MHz): $\delta=3.77$ (s, 3H; OCH_3), 3.99 (s, 3H; OCH_3), 4.02 (s, 3H; OCH_3), 4.04 (s, 3H; OCH_3), 4.18 (s, 3H; OCH_3), 4.25 (s, 3H; OCH_3), 7.62 (s, 1H; ArH), 7.81 (s, 1H; ArH), 8.49 (s, 1H; ArH), 10.06 (s, 1H; ArOH), 11.62 ppm (s, 1H; ArNH); ^{13}C NMR ($CDCl_3$ + [D_6]DMSO, 100 MHz): $\delta=55.7$ (CH_3), 61.1 (CH_3), 61.2 (CH_3), 61.4 (2CH_3), 62.2 (CH_3), 98.2 (CH), 104.7 (C), 106.5 (C), 110.4 (C), 119.0 (C), 119.2 (C), 120.6 (C), 123.2 (C), 127.0 (C), 127.5 (CH), 131.8 (C), 134.2 (CH), 142.5 (C), 143.6 (C), 144.7 (C), 147.9 (C), 148.5 (C), 150.3 (C), 150.4 (C), 150.6 (C), 155.9 ppm (C); IR: $\tilde{\nu}=850$, 938, 1112, 1129, 1298, 1425, 1503, 1599, 1638, 1793, 3019, 3339 cm^{-1}; LC/MS (APCI$^+$): *m/z*: 506.1 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{27}H_{23}O_9N$: C 64.16, H 4.59, N 2.77; found: C 64.52, H 4.63, N 2.89.

3-Methyl-6-(3,4,5-trimethoxybenzoyl)-1,3-benzoxazol-2(3*H*)-one (35; Table 1): General procedure A was followed by using 3,4,5-trimethoxybenzoic acid (**5**; 1.64 g, 7.73 mmol), 3-methyl-1,3-benzoxazol-2(3*H*)-one (**34**; 1.0 g, 6.7 mmol), and Eaton's reagent (0.60 g P_2O_5 in 4.0 mL CH_3SO_3H). The mixture was heated at 60 °C for 20 h and the final brown oil was purified by column chromatography on silica gel (EtOAc/*n*-heptane, 4:6) to give pure **35** (1.38 g, 60%) as a white solid; m.p. 155–156 °C (EtOAc/*n*-heptane); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=3.49$ (s, 3H; NCH_3), 3.89 (s, 6H; 2OCH_3), 3.95 (s, 3H; OCH_3), 7.02 (s, 2H; ArH), 7.06 (d, $J=8.1$ Hz, 1H; ArH), 7.72 (dd, $J=3.6$, 1.6 Hz, 1H; ArH), 7.74 ppm (dd, $J=10.9$, 1.5 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=24.4$ (CH_3), 56.3 (2CH_3), 61.0 (CH_3), 107.3 (CH), 107.6 (2CH), 111.6 (CH), 127.2 (CH), 132.5 (C), 132.6 (C), 135.4 (C), 142.1 (C), 142.3 (C), 152.9 (2C), 154.6 (C), 194.1 ppm (C); elemental analysis calcd (%) for $C_{18}H_{17}O_6N$: C 62.97, H 4.99, N 4.08; found: C 62.87, H 4.91, N 4.29.

5-Hydroxy-1,2,3,6,7,8-hexamethoxy-10-methylbenzo-[1,2]fluorantheno[8,9-*d*]-[1,3]oxazol-11(10*H*)-one (36; Table 1): By-product from the synthesis of **35**. Red solid (0.24 g, 7% yield); m.p. > 250 °C (EtOAc/*n*-heptane); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=$ 3.53 (s, 3H; NCH_3), 3.85 (s, 3H; OCH_3), 4.07 (s, 6H; 2OCH_3), 4.18 (s, 3H; OCH_3), 4.26 (s, 3H; OCH_3), 4.34 (s, 3H; OCH_3), 7.61 (s, 1H; ArH), 7.80 (s, 1H; ArH), 8.72 (s, 1H; ArH), 9.86 ppm (s, 1H; ArOH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=28.3$ (CH_3), 55.9 (CH_3), 61.3 (CH_3), 61.4 (CH_3), 61.6 (CH_3), 61.6 (CH_3), 62.3 (CH_3), 98.1 (CH), 102.9 (CH), 106.6 (C), 108.6 (CH), 119.2 (C), 119.3 (C), 120.8 (C), 123.7 (C), 127.2 (C), 128.9 (C), 132.1 (C), 135.1 (C), 142.4 (C), 142.6 (C), 145.1 (C), 148.1 (C), 148.8 (C), 150.5 (C), 150.6 (C), 150.9 (C), 155.6 ppm (C); LC/MS (APCI$^+$): *m/z*: 520.2 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{28}H_{25}O_9N$: C 64.74, H 4.85, N 2.70; found: C 64.36, H 5.03, N 3.06.

5-{4-[(Chloroacetyl)oxy]-3,5-dimethoxybenzoyl}-2-methoxyphenyl chloro-acetate (9), 5-{3,5-dimethoxy-4-[(methylsulfonyl)oxy]-benzoyl}-2-methoxy-phenyl chloroacetate (10), 2,6-dimethoxy-4-{4-methoxy-3-[(methylsulfonyl)-oxy]benzoyl}phenyl methanesulfonate (11), and 5-(chloroacetyl)-2-methoxy-phenyl chloroacetate (12; Scheme 2): Eaton's reagent was prepared from phosphorus pentoxide (P_2O_5) and methanesulfonic acid (CH_3SO_3H; weight ratio P_2O_5/CH_3SO_3H, 1:10). The mixture was heated at 40 °C under a nitrogen atmosphere until complete homogeneity. Chloroacetate (**7**; 5 g, 12.7 mmol) was then added to Eaton's reagent and the mixture was heated at 60 °C under an inert atmosphere for 18 h. After cooling to RT, the reaction medium was diluted with dichloromethane and carefully poured into a separatory funnel containing sodium bicarbonate aqueous solution (50% $NaHCO_3$). The aqueous solution was extracted with dichloromethane and the combined organic layers were dried ($MgSO_4$). Solvent was removed under reduced pressure to give a brownish oil. No red coloration of the media was observed, but a multitude of products were formed, from which four compounds were isolated and characterized. The crude product was purified by column chromatography on silica gel (EtOAc/*n*-heptane, 1:9) to give pure compounds **9**

(1.33 g, 23%), **10** (0.58 g, 10%), **11** (0.70 g, 12%), and **12** (0.17 g, 5%).

Compound 9: White solid; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.85$ (s, 6H; $2OCH_3$), 3.94 (s, 3H; OCH_3), 4.36 (s, 2H; $OCOCH_2Cl$), 4.41 (s, 2H; $OCOCH_2Cl$), 7.02 (s, 2H; ArH), 7.07 (d, $J = 8.8$ Hz, 1H; ArH), 7.63 (d, $J = 2.4$ Hz, 1H; ArH), 7.81 ppm (dd, $J = 8.8$, 2.4 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 40.4$ (CH_2), 40.5 (CH_2), 56.3 (CH_3), 56.4 ($2CH_3$), 106.6 (2CH), 111.8 (CH), 125.0 (CH), 129.9 (C), 130.4 (CH), 131.4 (C), 136.1 (C), 138.8 (C), 151.9 (2C), 154.7 (C), 164.8 (C), 165.4 (C), 193.3 ppm (C); elemental analysis calcd (%) for $C_{20}H_{18}O_8Cl_2$: C 52.53, H 3.97; found: C 52.56, H 3.97.

Compound 10: Colorless solid; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.36$ (s, 3H; OSO_2CH_3), 3.92 (s, 6H; $2OCH_3$), 3.94 (s, 3H; OCH_3), 4.37 (s, 2H; $OCOCH_2Cl$), 7.02 (s, 2H; ArH), 7.07 (d, $J = 8.8$ Hz, 1H; ArH), 7.62 (d, $J = 2.0$ Hz, 1H; ArH), 7.80 ppm (dd, $J = 8.8$, 2.0 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 40.2$ (CH_3), 40.5 (CH_2), 56.3 (CH_3), 56.6 ($2CH_3$), 106.7 (2CH), 111.9 (CH), 124.9 (CH), 129.7 (C), 130.4 (CH), 131.0 (C), 136.6 (C), 138.7 (C), 153.1 (2C), 154.9 (C), 165.4 (C), 193.1 ppm (C); elemental analysis calcd (%) for $C_{19}H_{19}ClO_9S$: C 49.73, H 4.17, S 6.99; found: C 49.68, H 4.02, S 7.41.

Compound 11: Colorless solid; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.25$ (s, 3H; OSO_2CH_3), 3.35 (s, 3H; OSO_2CH_3), 3.93 (s, 6H; $2OCH_3$), 4.01 (s, 3H; OCH_3), 7.06 (s, 2H; ArH), 7.13 (d, $J = 8.8$ Hz, 1H; ArH), 7.81 (d, $J = 2.0$ Hz, 1H; ArH), 7.88 ppm (dd, $J = 8.8$, 2.0 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 38.8$ (CH_3), 40.2 (CH_3), 56.4 (CH_3), 56.6 ($2CH_3$), 106.9 (2CH), 112.6 (CH), 127.0 (CH), 130.0 (C), 130.9 (CH), 131.2 (C), 136.1 (C), 137.4 (C), 153.2 (2C), 155.4 (C), 192.6 ppm (C); elemental analysis calcd (%) for $C_{18}H_{20}O_{10}S_2$: C 46.95, H 4.38, S 13.93; found: C 46.81, H 4.41, S 13.79.

Compound 12: Beige solid; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.93$ (s, 3H; OCH_3), 4.36 (s, 2H; $OCOCH_2Cl$), 4.63 (s, 2H; $OCOCH_2Cl$), 7.05 (d, $J = 8.6$ Hz, 1H; ArH), 7.73 (d, $J = 2.1$ Hz, 1H; ArH), 7.91 ppm (dd, $J = 8.6$, 2.1 Hz, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 40.4$ (CH_2), 45.4 (CH_2), 56.3 (CH_3), 112.0 (CH), 123.2 (CH), 127.4 (C), 129.0 (CH), 139.4 (C), 155.6 (C), 165.1 (C), 188.9 ppm (C); elemental analysis calcd (%) for $C_{11}H_{10}O_4Cl_2$: C 47.68, H 3.64; found: C 48.00, H 3.99.

13-[(Chloroacetyl)oxy]-1,2,3,5,8,9,10,12-octamethoxyrubicen-6-yl chloroacetate (39; Scheme 6): Eaton's reagent was prepared from phosphorus pentoxide (P_2O_5) and methanesulfonic acid (CH_3SO_3H; weight ratio P_2O_5/CH_3SO_3H, 1:10). The mixture was heated at 40 °C under a nitrogen atmosphere until complete homogeneity. Chloroacetate **7** (0.3 g, 0.76 mmol) and benzoic acid **37** (0.093 g, 0.76 mmol) were then added and the mixture was heated at 50 °C under an inert atmosphere for 18 h. The mixture became red very quickly. After cooling to RT, the reaction medium was diluted with dichloromethane and carefully poured into a separatory funnel containing aqueous sodium bicarbonate solution (50% $NaHCO_3$). The aqueous solution was extracted with dichloromethane, and the combined organic layers were dried ($MgSO_4$). Solvent was removed under reduced pressure to give a red dark oil. The crude product was purified by column chromatography on silica gel (EtOAc/*n*-heptane, 2:8→6:4) to obtain rubicene derivative **39** (106 mg, 37%) as a red solid; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 4.02$ (s, 6H; $2OCH_3$), 4.04 (s, 6H; $2OCH_3$), 4.18 (s, 6H; $2OCH_3$), 4.26 (s, 6H; $2OCH_3$), 4.45 (s, 4H; $2OCOCH_2Cl$), 7.89 (s, 2H; 2ArH), 8.58 ppm (s, 2H; 2ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 40.9$ ($2CH_2$), 56.2 ($2CH_3$), 61.3 ($2CH_3$), 61.6 ($2CH_3$), 61.9 ($2CH_3$), 107.5 (2CH), 119.5 (2C), 121.0 (2CH), 123.8 (2C), 130.3 (2C), 130.9 (2C), 131.2 (2C), 137.2 (2C), 138.2 (2C), 147.5 (2C), 150.2 (2C), 150.9 (2C), 151.1 (2C), 166.0 ppm (2C); elemental analysis calcd (%) for $C_{38}H_{32}Cl_2O_{12}$: C 60.73, H 4.29; found: C 61.01, H 4.40.

General procedure B: synthesis of phenols from chloroacetic esters

(Mono or di)chloroacetic ester (1.0 equiv) and sodium acetate ($AcONa\cdot 3H_2O$; 4.5 equiv for monochloroacetic ester and 9.0 equiv for dichloroacetic ester) were dissolved in MeOH. The solution was heated to refluxed for 5 h. After cooling to RT, the mixture was concentrated under reduced pressure and the residue was taken into distilled water. The resulting precipitate was filtered, washed with water several times to remove remaining sodium acetate, and recrystallized from absolute EtOH or purified by flash chromatography to obtain pure phenols.

1,2,3,5,8,9,10,12-Octamethoxyrubicene-6,13-diol (40; Scheme 7): General procedure B was followed by using dichloroacetate **39** (0.08 g, 0.106 mmol) and sodium acetate ($AcONa\cdot 3H_2O$; 0.13 g, 0.958 mmol) in MeOH (5 mL). The resulting solid was collected by filtration and recrystallized from absolute EtOH to obtain diphenol **40** (52 mg, 82%) as a dark-red solid. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta =$ 4.05 (s, 6H; $2OCH_3$), 4.08 (s, 6H; $2OCH_3$), 4.16 (s, 6H; $2OCH_3$), 4.23 (s, 6H; $2OCH_3$), 5.70 (s, 2H; 2ArOH), 7.76 (s, 2H; 2ArH), 8.50 ppm (s, 2H; 2ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 56.2$ ($2CH_3$), 61.2 ($2CH_3$), 61.5 ($2CH_3$), 61.7 ($2CH_3$), 106.7 (2CH), 113.4 (2CH), 119.7 (2C), 123.1 (2C), 130.2 (2C), 130.8 (2C), 131.6 (2C), 132.4 (2C), 145.0 (2C), 146.0 (2C), 147.6 (2C), 149.5 (2C), 149.8 ppm (C); LC/MS ($APCI^+$): *m/z*: 599.2 $[M+H]^+$.

5-Hydroxy-2,3,6,8,9,10-hexamethoxyacenaphtho[1,2,3,4-*klmn*]xanthen-11(11c*H*)-one (42; Scheme 7): General procedure B was followed by using chloroacetate **8** (0.2 g, 0.35 mmol) and sodium acetate ($AcONa\cdot 3H_2O$; 0.21 g, 1.54 mmol) in MeOH (5 mL). After evaporation of the methanol in vacuo, the residue was poured in distilled water. The residue was purified by column chromatography (EtOAc/*n*-heptane, 15:85) and recrystallized from EtOH to obtain heterocycle **42** (52 mg, 31%) as a red solid. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.48$ (s, 3H; OCH_3), 3.61 (s, 3H; OCH_3), 3.80 (s, 3H; OCH_3), 3.91 (s, 3H; OCH_3), 3.95 (s, 3H; OCH_3), 4.16 (s, 3H; OCH_3), 4.76 (s, 1H; ArH), 6.86 (s, 1H; ArH), 7.35 ppm (s, 1H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 54.9$ (CH_3), 56.1 (CH_3), 60.6 (CH_3), 60.7 (CH_3), 61.0 (CH_3), 61.5 (CH_3), 62.0 (CH_3), 97.6 (CH), 107.4 (CH), 115.5 (CH), 117.2 (C), 118.7 (C), 121.6 (C), 129.1 (C), 132.3 (C), 132.5 (C), 143.4 (C), 145.3 (C), 145.5 (C), 149.9 (C), 154.0 (C), 154.2 (C), 155.1 (C), 156.8 (C), 178.3 (C), 195.9 ppm (C); LC/MS ($APCI^+$): *m/z*: 479.1 $[M+H]^+$.

Cell proliferation assay:[36] The compounds were tested against a panel of 60 human cancer cell lines at the National Cancer Institute, Rockville, MD. The cytotoxicity studies were conducted using a 48 h exposure protocol using the sulforhodamine B assay.

Tubulin studies: All the studied compounds and the reference compounds (phenstatin and CA-4) were tested under identical operating conditions. Turbidimetric assays of microtubules were performed as described,[37] except when 50–150 μL quartz microcuvettes were utilized instead of 96-well plates. Lyophilized bovine brain tubulin (97%) was purchased from Cytoskeleton Inc. and was reconstituted to 5 mg mL^{-1} with buffer (80 mM PIPES, pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 1.0 mM GTP, 5% glycerol). To this solution (63 μL) in an ultramicro quartz cuvette at 0 °C was added test compound in dimethyl sulfoxide (7 μL). The increase in absorbance was monitored at 340 nm and 37 °C with a HeklOS Gamma&Delta. The samples were prepared in duplicate. The IC_{50} value was defined as the compound molar concentration that inhibited the extent of assembly by 50% after 30 min incubation.

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