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New antitubulin derivatives in the combretastatin A4 series: synthesis and biological evaluation

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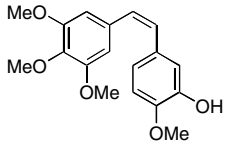
Abstract—Two series of combretastatin A4 derivatives (acrylamide = carboxamide and carbamate) were synthesized in order to improve the water solubility and stabilize the *cis*-configuration of the double bond. Their cytotoxic effects were evaluated against MCF-7, KB-3-1 and IGROV human cancer cell lines, as well as their inhibitory activity on tubulin polymerization. Results were compared to those of carboxamide **1**, chosen as reference. Potent inhibitions were observed on both tests in the carboxamide series, particularly for compound **4d** bearing a fluorine group in replacement of the 3-hydroxyl of **CA4**. In contrast, most of the carbamates were either inactive or displayed only moderate cytotoxicities. Interestingly, a submicromolar IC_{50} was measured on MCF-7 cells for **6g**, although this compound was totally devoid of antitubulin activity.

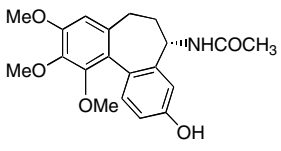
1. Introduction

Tubulin is a heterodimeric protein that polymerizes into microtubules and both forms coexist in a dynamic equilibrium. These filaments are involved in many kinetic processes in eukaryotic cells, for example, intracellular organelle transport, mitosis, cell motion or cell shape.[1] It constitutes a major target in the field of anticancer drugs research. Indeed, agents that inhibit the polymerization of tubulin and those that stabilize the resultant microtubules are widely used in current cancer chemotherapy, but have undesirable side effects and are subject to multidrug resistance.[2,3] Apart from these antimitotic properties, some antitubulin agents were recently shown to exhibit antivascular activities at dose levels far from the MTD (maximal tolerated dose).[4] These agents, like other vascular targeting agents (VTAs), disrupt the associated vasculature of tumour and lead to vascular shutdown and haemorrhagic necrosis, leaving normal vasculature intact. Consequences of blood supply arrest (i.e., hypoxia, lack of nutrients and waste products accumulation) lead to tumour cells death. Tubulin binding agents that display such an activity, target tubulin of the cytoskeleton on which they act as assembling inhibitors. Rapid endothelial cell shape changes are induced, leading to the aforementioned chain of fatal events for the tumour.[5,6]

In this context, two compounds, combretastatin A4 (**CA4**) and *N*-acetylcolchinol (**ZD 6126**), appear particularly promising due to potency and selectivity of their antivascular activity.[5–11] The former one is a natural stilbene extracted from the bark of *Combretum caffrum* Kuntze, whereas the last one is a semi-synthetic analogue of the natural antimitotic agent colchicine. Both corresponding **CA4** and **ZD 6126** phosphate prodrugs, are currently under clinical trials.[4,12]



combretastatin A4 (CA4)



N-acetylcolchinol

Keywords: Tubulin; Combretastatin A4; Carboxamides; Carbamates.
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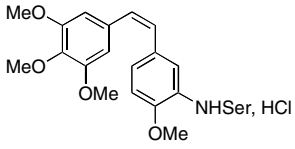
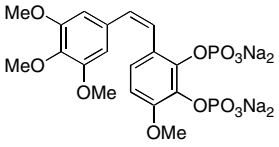
Nevertheless, the antitumour activity of these compounds, in terms of tumour mass reduction, is rather limited. Indeed, a rim of viable tumour cells survive at the periphery of the tumour, perfused by unaffected vessels in the surrounding normal tissues and it proliferates after drug removal. The search for compounds that display both antivascular and direct antimitotic activities is now well-established, particularly since **AVE 8062A** and **Oxi 4503**, two combretastatin analogues, were proved to possess such a dual activity correlated with increased antitumour effect.[13,14]



AVE 8062A



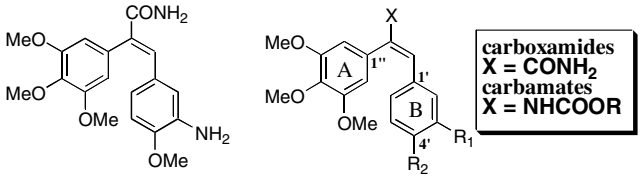
Oxi 4503 (CA1-P)

From the literature review, it appeared that numerous **CA4** analogues were previously prepared.[15,16] In the course of structure–activity relationship studies, many modifications, relative to chemical features of the molecule (i.e., double bond geometry, substitution pattern of both rings) were evaluated in order to make their influence on biological activity more precise. Following these works, different series of analogues where the double bond of **CA4** was replaced by a mimicking structure, were synthesized. Excellent results obtained with five-membered rings have to be underlined. In such restricted analogues, the essential *cis*-geometry between both A/B-rings is similar to that of **CA4**.

In contrast, only a few results concerned acyclic analogues where the double bond was maintained and stabilized by an electron-withdrawing group.[17] A series of (*Z*)-acrylonitriles was prepared by Ohsumi and co-workers.[18,19] The authors showed that the insertion of a nitrile group (CN) onto the olefin site adjacent to the A-ring, did not affect the activity of **CA4** and in terms of steric hindrance, CN was about the maximum tolerable size. Apart from these studies, a number of *N*-alkyl acrylamides were studied by Cushman et al. *N*-Alkylation in this position leads to a dramatic decrease of biological activities.[20]

These results have encouraged the preparation of **CA4** analogues with the goal of both a stabilized double bond and an increased hydrosolubility. Two series of compounds bearing a substituent, either a carboxamide or a carbamate group, on the 1 position of the olefin bridge adjacent to the A-ring were prepared.

To our knowledge, only one unsubstituted carboxamide was described.[18] Compound **1** was less cytotoxic than **CA4**, but displayed increased antitubulin activity. In order of studying the structure–activity relationships in this series, carboxamide **1** was chosen as a lead compound for further chemical modulations on the B-ring (general formula: X = $CONH_2$). The trimethoxy A-ring and the 4′-OCH_3 on the B-ring of **CA4**, which was reported necessary for potent activity were conserved and various substituents were introduced on the 3′-position (R_1 = NH_2, H, OH, F).

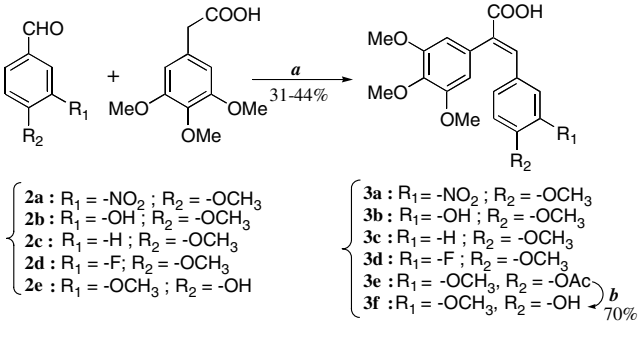


1 **General Formula of CA4 analogues**

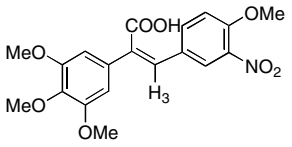
In order to complete this study, the synthesis of several isosteric carbamates (general formula: X = NHCOOR) was undertaken. Both series were prepared from common acrylic acid precursors (general formula: X = COOH). Biological evaluation of these intermediates is also presented.

2. Chemistry

Acrylic acids **3a**–**c** were synthesized by means of a Perkin reaction, as previously reported.[18,20,21] This method was also applied to the preparation of new acrylic acids **3d**–**f** (Scheme 1). Reaction of 4-hydroxy-3-methoxybenzaldehyde (**2e**) with 3,4,5-trimethoxyphenylacetic acid in the presence of acetic anhydride afforded the acetate of corresponding acrylic acid **3e**. In contrast, the regioisomeric isovanillin led to the phenol **3b** when treated under similar conditions.[21] Deprotection of **3e** in mild basic conditions afforded **3f**. Pure *cis*-(*E*) isomers were obtained in moderate yields (31–44%) after purification of the crude precipitate by fractional crystallization. For each compound, the *cis* configuration was unambiguously determined by ^{1}H NMR, on the basis of typical chemical shifts observed for the ethylenic proton H_3 signal.[18,21] As a general rule, the isomeric *trans*-(*Z*) isomers, known to be biologically inactive in the combretastatin series, were not separated from the crude reaction mixture.[20,22] Only *trans*-(*Z*) compound **3a** was isolated for spectroscopic comparison with the corresponding *cis*-(*E*) isomer. There is an upfield chemical shift displacement of 0.6 ppm in going from (*E*)-**3a** to (*Z*)-**3a**.



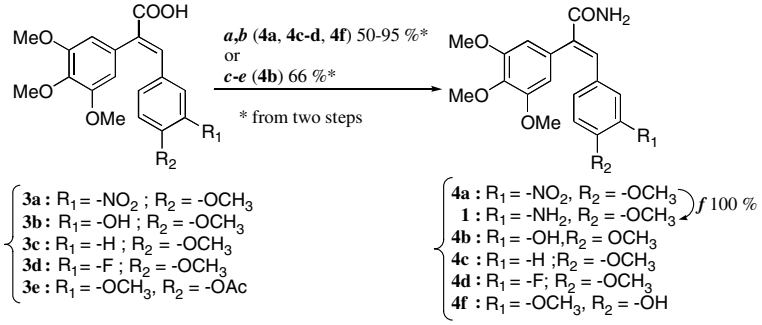
Scheme 1. Syntheses of acrylic acids **3a**–**f**. Reagents and conditions: (a) $(CH_3CO)_2O$, Et_3N, 140 °C; (b) K_2CO_3, MeOH/H_2O 3/1, rt.



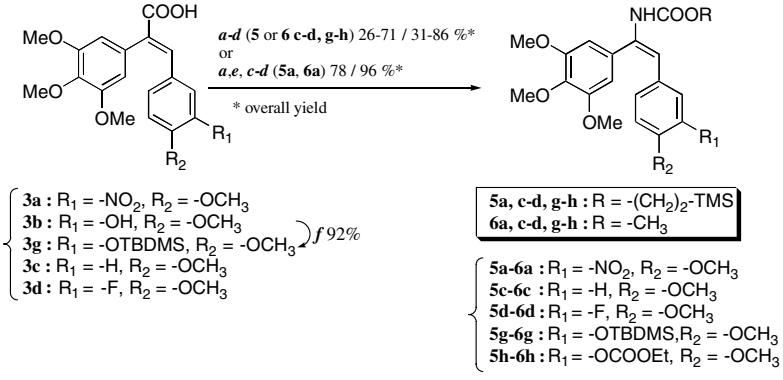
(*Z*)-**3a**

Reference compound **1** was prepared as previously described.[18] Treatment of acrylic acid **3a** with $SOCl_2$ followed by aminolysis of the corresponding acylchloride with aqueous NH_3 afforded the nitro compound **4a** (Scheme 2). Finally, zinc reduction in acetic acid afforded **1** in quantitative yield. Similarly, activation with $SOCl_2$ was applied to the synthesis of the new acrylamides **4c** and **4d** from acrylic acids **3c** and **3d**, respectively. When ester **3e** was used as starting material, phenol **4f** was directly obtained since deprotection occurred during aminolysis step. For the synthesis of the acrylamide analogous to **CA4** (**4b**), the protection of the phenol group and the activation of the carboxylic function were both ensured by treatment with ethylchloroformate. Thereby, reaction with ammonium chloride afforded an ethylcarbonate intermediate, which was directly deprotected by LiOH.

As outlined in Scheme 3, Curtius conditions gave an access to trimethylsilylethyl carbamates (**5a**,**c**,**d**,**g**,**h**) and methyl carbamates (**6a**,**c**,**d**,**g**,**h**) starting from the corresponding acrylic acids.[23] Thus, activation of the carboxylic function by treatment with ethylchloroformate led to a mixed anhydride, which reacted with sodium azide to afford a carbonyl azide derivative. Thermal rearrangement in toluene at 80 °C gave the corresponding isocyanate. Finally, alcoholysis by either trimethylsilylethan-2-ol or methyl alcohol afforded the corresponding carbamates in weak to good yield, depending on the starting acrylic acid and alcohol. Indeed, when the B-ring of acrylic acid bears either an electronegative or a mesomeric electron-withdrawing group such as –F or $–NO_2$, high yields were obtained (78–96%). In contrast, synthesis of carbamates bearing either a hydroxy or an amino group at R_1 failed. All other attempts toward the reduction of nitro carbamates **5a** and **6a** were unsuccessful, only leading to degradation products. Degradation was also observed when trying to deprotect the carbonate group of carbamates **5h** and **6h**. In order to circumvent the drawback of alkaline deprotection, *O*-(*tert*-butyldimethylsilanyloxy) acrylic acid **3g** was synthesized and subsequently converted into the corresponding carbamates **5g** and **6g**. Nevertheless, attempts to cleave the protecting group also failed in this case. The chemical stability of **5g** and **6g** strongly suggests that instability in the carbamate series mainly depends upon the presence of a proton donor group at R_1. Finally, carbonates **5h** and **6h** as well as silyl ethers **5g** and **6g** were submitted to biological tests.



Scheme 2. Syntheses of acrylamides **4a**–**d**,**f** and **1**. Reagents and conditions: (a) $SOCl_2$, Et_3N, CH_2Cl_2, rt; (b) NH_4OH, CH_2Cl_2, rt; (c) Et_3N, ClCOOEt, DMF, −10 °C; (d) NH_4Cl, Et_3N, DMF, −10 °C; (e) LiOH, MeOH/H_2O 6/1, rt; (f) Zn, AcOH, rt.



Scheme 3. Syntheses of carbamates **5a**,**c**,**d**,**g**,**h** and **6a**,**c**,**d**,**g**,**h**. Reagents and conditions: (a) Et_3N, ClCOOEt, anhyd acetone, −5 °C; (b) NaN_3, H_2O, −5 °C; (c) anhyd toluene, 80 °C; (d) trimethylsilylethan-2-ol (**5a**,**c**,**d**,**g**,**h**) or MeOH (**6a**,**c**,**d**,**g**,**h**), 50 °C; (e) NaN_3, THF, −5 °C; (f) TBDMSCl, imidazole, DMF, rt.

3. Biological activity

3.1. Inhibition of tubulin polymerization

Inhibition of tubulin polymerization (ITP) was determined for acrylic acids **3a**–**d**,**f** and **3g**; acrylamides **4a**–**d**,**f** and carbamates **5a/6a**, **5c**–**d/6c**–**d** and **5g**–**h/6g**–**h** in comparison with the parent compound **1** and colchicine used as standard. Results expressed in terms of the relative $IC_{50}/IC_{50colch}$ are summarized in Table 1. The potent activity of **1** was confirmed in our hands where this compound displayed an ITP value of 0.5, in good agreement with the literature data.[18]

ITP activity of acrylic acid **3c** had been previously determined by Cushman et al. and it was concluded that introduction of carboxylic group on the olefin site resulted in a lack of activity in comparison to that of **CA4**.[20] In good agreement with this statement, all the newly synthesized acrylic acids **3b**,**d**,**f** and **3g**, as well as **3a** were found inactive, except **3f** bearing a $-OCH_3$ group on the 3′-position of the B-ring, which displayed a weak activity with a ITP value of 21. Results obtained in carboxylic series dramatically contrasted with those observed in carboxamide series and an acidic function on this position of the olefin has likely a deleterious effect on the interaction between the inhibitor and tubulin. Indeed, with the exception of nitro derivative **4a** and **4f**, which derive from the aforementioned acrylic acid **3f**, all acrylamides **4b**–**d** displayed moderate to strong ITP activities. Surprisingly, compound **4b**, which bears the substitution pattern of **CA4**, showed about eightfold lower antitubulin activity than **1**. Only a slight decrease (threefold) is observed for **4c** (R_1 = H) and **4d** (R_1 = –F). Therefore, in the carboxamide series as well as in the **CA4** series, the nature of the substituent on the 3′-position of the B-ring dramatically influences ITP activity.[18,20,22]

Table 1. IC_{50} values of ITP[a]

Compound	$IC_{50}/IC_{50colch}$
Acrylic acids	
3a	Inactive
3b	Inactive
3c	Inactive
3d	Inactive
3f	21
3g	Inactive
Acrylamides	
4a	Inactive
4b	3.7
4c	1.7
4d	1.5
4f	Inactive
1	0.5[b]
Methylcarbamates	
6a	Inactive
6c	27
6d	15
6g	Inactive
6h	Inactive
Silylcarbamates	
5a	Inactive
5c	91
5d	71
5g	20
5h	Inactive
Reference	
Colchicine	1.0[c]

[a] Inhibition of tubulin polymerization. ITP was determined as described in Section 5.
[b] Lit. IC_{50} = 3 μM.[18]
[c] $IC_{50colch}$ varies from 1.8 to 6.7 μM for the different experiments according to the tubulin concentration.

All the newly synthesized carbamates exhibited weak ITP activities. Particularly, nitro compounds **5a** and **6a** ($R_1 = -NO_2$) and carbonates **5h** and **6h** (R_1 = –OCOOEt) were totally devoid of effect. Surprisingly, the *N*-methylcarbamate **5g** (R_1 = –OTBDMS) displayed a ITP value of 20, whereas, in the silylated series, **6g** was devoid of activity. In comparison to the corresponding acrylamides **4c** and **4d**, which possess potent inhibitory activities, unsubstituted carbamates **5c** and **6c** (R_1 = H) as well as those bearing a fluorine on the 3′-position of the B-ring (**5d** and **6d**) showed a 10- to 60-fold decreased antitubulin effects. The results also clearly underlined the influence of the bulkiness of the substituting group on the olefin site adjacent to the A-ring on ITP properties. Indeed, the *N*-methylcarbamates **6c** and **6d** were slightly more active than their bulky *N*-(trimethylsilyl)ethyl homologues **5c** and **5d**.

3.2. Cytotoxicity

Compounds were evaluated for their cytotoxic activities against three human cancer cell lines (KB-3-1, MCF-7 and IGROV) using an MTT assay. IC_{50} values are summarized in Tables 2,3 and represent the concentration inducing a 50% decrease of cell growth after 3 days incubation. Values superior to 10 μM are not reported.

All the new acrylic[†] acids were devoid of cytotoxic effects, even **3f**, which displayed a weak ITP activity. In the acrylamide series (Table 2), the cytotoxicity results were well-correlated with those of ITP tests. Indeed, ineffective compounds against tubulin polymerization assay (**4a** and **4f**) were also not cytotoxic, except a slight effect observed for the nitro acrylamide **4a** against MCF-7 cell line. In our hands, **1** displayed moderate cytotoxic activities against MCF-7 and KB-3-1 cells, and a strong one against IGROV cells. Interestingly, replacement of amino group by a hydroxy leads to a decreased cytotoxicity, except in the case of KB-3-1 cell lines. Thus, compared with **1**, acrylamide **4b** displayed 6-fold and 30-fold lower cytotoxic activity against IGROV and MCF-7 cells, respectively, but a 1.5-fold increased potency against KB-3-1 cells. In comparison with **CA4** values, introduction of a carboxamide group on the olefin site near the trimethoxyphenyl ring severely weakened the cell growth inhibition potency. This decrease

[†] According to Ref. 20, **3c** gave an IC_{50} of 12.8 μM on MCF-7 cells.

Table 2. IC_{50} values (μM, mean of 8 determinations ± SD) for cytotoxicity of carboxamides, colchicine and **CA4**

Compound	KB-3-1	IGROV	MCF-7
4a	>10	>10	8.00 ± 1.55
4b	0.15 ± 0.01	0.12 ± 0.02	1.13 ± 0.17
4c	0.051 ± 0.006	0.18 ± 0.02	0.46 ± 0.12
4d	0.016 ± 0.02	0.10 ± 0.03	0.14 ± 0.13
4f	>10	>10	>10
1[a]	0.20 ± 0.01	0.028 ± 0.012	0.44 ± 0.13
Colchicine	0.003 ± 0.0002	0.021 ± 0.002	0.032 ± 0.013
CA4[b]	0.005 ± 0.0006	0.014 ± 0.001	0.069 ± 0.013

[a] Lit. IC_{50} = 0.20 μM on Colon 26.[18]
[b] For an efficient synthesis of **CA4**, see Ref. 21.

Table 3. IC_{50} values (μM, mean of 8 determinations ± SD) for cytotoxicity of carbamates, colchicine and **CA4**

Compound	KB-3-1	IGROV	MCF-7
Methylcarbamates			
6a	>10	>10	>10
6c	1.21 ± 0.12	2.34 ± 0.22	2.02 ± 0.43
6d	0.91 ± 0.30	1.27 ± 0.08	1.47 ± 0.14
6g	2.87 ± 0.27	6.96 ± 1.60	0.84 ± 0.40
6h	1.41 ± 0.22	>10	2.83 ± 1.15
Silylcarbamates			
5a	>10	>10	>10
5c	>10	>10	>10
5d	0.80 ± 0.33	>10	>10
5h	2.43 ± 0.60	>10	>10
5g	>10	>10	>10
References			
Colchicine	0.003 ± 0.0002	0.021 ± 0.002	0.032 ± 0.013
CA4	0.005 ± 0.0006	0.014 ± 0.001	0.069 ± 0.013

is correlated with a ITP decrease observed for compound **4b**. Compared to **1**, **4c** (R_1 = –H) and **4d** (R_1 = –F) were significantly more potent in terms of cytotoxicity against KB-3-1 cell line (i.e., respectively, 4-fold and 13-fold) and also, but to a lower extent, against MCF-7 (respectively, by a 1.2- and 4-fold). In contrast, both compounds were less potent than **1** against the IGROV cell line, but the decrease was limited to a 3.6-factor for fluoro compound **4d**. Results obtained for **4d** are remarkable since cytotoxicity against KB-3-1 was comparable to that of **CA4**. This observation[‡] did not comply with Hsieh group's hypothesis involving that presence of an available donor group in the B-ring was important for cytotoxic activity in **CA4** analogues.[25]

As previously noted for ITP activity, carbamates displayed on the whole, significantly lower potency than the corresponding isosteric carboxamide (Table 3). Bulky silylcarbamates **5a**,**c** and **5g** were considered inactive. In contrast, compounds **5d** and **5h**, showed, respectively, submicromolar and micromolar IC_{50}, but only against one cancer cell line (KB-3-1). According to their cytotoxic effects, three categories of *N*-methylcarbamates might be considered. Firstly, compound **6a** was devoid of any activity for both ITP and cytotoxicity tests. Secondly, **6c** (R_1 = –H) and **6d** (R_1 = –F), which displayed moderate ITP activities, were shown to exhibit micromolar IC_{50} against the three cell lines. Thirdly, **6h** (R_1 = –OCOOEt) and **6g** (R_1 = –OTBDMS) were significantly cytotoxic, particularly **6g**, which displayed a IC_{50} of 0.84 μM against MCF-7, but neither **6h** nor **6g** exhibited antitubulin properties. Another mechanism might be evoked in order to explain observed cytotoxicities of these *N*-methylcarbamates.

4. Conclusion

In conclusion, two series of analogues of **CA4** were synthesized and evaluated for their ITP and cytotoxic activities. Results for carbamates were rather disappointing likely due to the bulkiness of the alkoxy groups. Furthermore, the instability of the synthesis intermediates could not allow the access to the expected molecules bearing a hydroxy or an amino group on the 3′-position of the B-ring. In contrast, acrylamide derivatives displayed promising antitumour and cytotoxic properties. As far as SAR are concerned, introduction of polar carboxamide group on olefin site near the A-ring seemed to induce deleterious effects on both cytotoxic and ITP activities. Substitution on the 3′-position of the B-ring with a polar amino group increased ITP effect but reduced cytotoxicity.[18] A more hydrophobic fluorine group could restore cytotoxic activity against several cancer cell lines and in this case slightly weakened ITP properties. These results could be advantageously exploited for the development of new drugs with potential antivascular and correlated direct cytotoxicity. Fluorine analogue **4d** whose biological activity is within the same range as those of reference compound **1** is currently selected for further biological studies.

5. Experimental

Most reagents were commercially available reagent-grade chemicals and used without further purification. Reactions were monitored by thin-layer chromatography using silica gel plates (Merck Si gel $60F_{254}$). Where appropriate, crude products were separated by column chromatography, silica gel (Merck, particle size 0.035–0.070 mm or 0.025–0.045 mm) with an overpressure of 300 mbars. All melting points were determined on a Leica micromelting point apparatus and are uncorrected. NMR spectra were recorded at 300 or 400 MHz (^{1}H NMR) and at 75 or 100 MHz (^{13}C NMR) using a Bruker AC 300 or a AVANCE 400 spectrophotometer; *J* values are given in hertz (Hz) and δ in ppm, relative to solvent peaks as internal standards (δ: $CDCl_3$: 7.27 ppm (^{1}H), 77 ppm (^{13}C); Me_2CO-d_6: 2.10 ppm (^{1}H), 29.8 ppm (^{13}C); Me_2SO-d_6: 2.50 ppm (^{1}H), 40.6 ppm (^{13}C)); ^{1}H and ^{13}C signals were unambiguously attributed by 2D NMR experiments. Mass spectra

[‡] A synthesis of 3-fluoro-3-deoxy **CA4** was recently published.[24] Cytotoxicity against K562 human chronic myelogenous leukaemia cell line was reported and was similar to that of **CA4**. ITP activity was not evaluated.

(MS) were measured on a Nermag R10-10C (DIC) or a ZQ 2000 Waters (ES) spectrophotometer. The IR spectra were obtained using a Nicolet 510 FT-IR spectrophotometer. The UV spectra were recorded on a Beckman DU 640 apparatus. Elemental analysis was determined by the microanalyses service of Pierre et Marie Curie University.

5.1. General procedure for the preparation of acrylic acids 3b,d–e

A mixture of 3,4,5-trimethoxyphenylacetic acid (2 g, 8.84 mmol), substituted benzaldehyde (4.4 mmol) and triethylamine (2 mL) in Ac_2O (4 mL) was heated at 140 °C until the benzaldehyde had disappeared. After cooling, the reaction mixture was acidified with 35% aqueous HCl (6 mL) and kept at room temperature overnight. The precipitate was collected by filtration and recrystallized from absolute EtOH to give pure acids.

5.1.1. (*E*)-3-(3′-Hydroxy-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (3b).[20] The general procedure was performed with isovanilline **2b** (670 mg, 4.4 mmol). The reaction mixture was heated for 3 h and the precipitate was recrystallized from ethanol to give 500 mg (31%) of **3b** as pale yellow crystals. Mp 184–186 °C; ^{1}H NMR (DMSO-d_6): δ 8.95 (s, 1H, OH), 7.56 (s, 1H, H3), 6.80 (d, 1H, J = 8.5 Hz, H5′), 6.59 (dd, 1H, J = 2, 8.5 Hz, H6′), 6.52 (d, 1H, J = 2 Hz, H2′), 6.43 (s, 2H, H2″, H6″), 3.72 (s, 3H, 4′-OCH_3), 3.70 (s, 3H, 4″-OCH_3), 3.68 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR (DMSO-d_6): 169.2 (C1), 153.6 (C3″,C5″), 149.5 (C4′), 146.4 (C3′), 139.7 (C3), 137.5 (C4″), 132.7 (C1″), 130.9 (C2), 127.6 (C1′), 123.5 (C6′), 117.8 (C2′), 112.1 (C5′), 107.3 (C2″,C6″), 60.7 (4″-OCH_3), 56.5 (3″,5″-OCH_3), 56.0 (4′-OCH_3); MS (DIC/NH_3) m/z = 378 $[M+NH_4]^+$, 361 $[M+H]^+$; UV (MeOH) λ (nm) 203 ($\log\varepsilon$ = 4.56), 284 ($\log\varepsilon$ = 3.98); IR (KBr) ν' (cm^{-1}) 3328, 2912, 1665, 1583, 1502, 1443, 1266, 1124, 1017. Anal. Calcd ($C_{19}H_{20}O_7$) C = 63.33%, H = 5.59%. Found C = 63.30%, H = 5.61%.

5.1.2. (*E*)-3-(3′-Fluoro-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (3d). The general procedure was performed with 3-fluoro-4-methoxybenzaldehyde **2d** (494 mg, 3.2 mmol). The reaction mixture was heated for 18 h and the precipitate was recrystallized from ethanol to give 484 mg (42%) of **3d** as white crystals. Mp 203–205 °C; ^{1}H NMR (DMSO-d_6): δ 7.64 (s, 1H, H3), 7.05 (t, 1H, J = 9 Hz, H5′), 6.98 (dd, 1H, J = 1.5, 9 Hz, H6′), 6.81 (dd, 1H, J = 13, 1.5 Hz, H2′), 6.45 (s, 2H, H2″,H6″), 3.79 (s, 3H, 4′-OCH_3), 3.70 (s, 3H, 4″-OCH_3), 3.68 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR (DMSO-d_6): δ 169.3 (C1), 154.4 (C3″,C5″), 151.82 (J = 244 Hz, C3′), 149.1 (J = 11 Hz, C4′), 138.7 (C3), 138.3 (C4″), 133.1 (C1″), 132.9 (C2), 128.9 (C6′), 128.3 (C1′), 118.03 (J = 19 Hz, C2′), 114.6 (C5′), 107.7 (C2″,C6″), 61.3 (4″-OCH_3), 57.1 (4′,3″,5″-OCH_3); MS(DIC/NH_3) m/z = 363 $[M+H]^+$; UV (MeOH) λ (nm) 205 ($\log\varepsilon$ = 4.60), 282 ($\log\varepsilon$ = 4.17), 297 ($\log\varepsilon$ = 4.16); IR (KBr) ν' (cm^{-1}) 3448, 2998, 2942, 2626, 1667, 1616, 1516, 1506, 1414, 1278, 1257, 1129, 1024, 1003, 924, 818, 772. Anal. Calcd ($C_{19}H_{19}FO_6$) C = 62.98%, H = 5.29%. Found C = 60.33%, H = 5.36%.

5.1.3. (*E*)-3-(4′-Acetoxy-3′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (3e). The general procedure was performed with vanilline **2e** (670 mg, 4.4 mmol). The reaction mixture was heated for 3 h 30 min and the precipitate was crystallized from ethanol to give 479 mg (30%) of **3e** as pale yellow crystals. Mp 205–207 °C; ^{1}H NMR (DMSO-d_6): δ 7.70 (s, 1H, H3), 6.99 (d, 1H, J = 8 Hz, H5′), 6.86 (dd, 1H, J = 2, 8 Hz, H5′), 6.70 (d, 1H, J = 2 Hz, H2′), 6.45 (s, 2H, H2″,H6″), 3.68 (s, 6H, 3″,5″-OCH_3), 3.66 (s, 3H, 4″-OCH_3), 3.40 (s, 3H, 4′-OCH_3), 2.20 (s, 3H, –COCH_3); ^{13}C NMR (DMSO-d_6): δ 169.7 (–COCH_3), 169.5 (C1), 154.5 (C3″,C5″), 151.4 (C3′), 141.1 (C4′), 139.4 (C3), 138.3 (C4″), 134.6 (C2), 134.4 (C1′), 133.1 (C1″), 125.1 (C6′), 124.0 (C5′), 114.7 (C2′), 108.0 (C2″,C6″), 61.3 (4″-OCH_3), 57.3 (3″,5″-OCH_3), 56.3 (3′-OCH_3), 21.5 (–COCH_3); MS(ES) m/z = 425 $[M+Na]^+$; UV (MeOH) λ (nm) 205 ($\log\varepsilon$ = 4.69), 271 ($\log\varepsilon$ = 4.15), 301 ($\log\varepsilon$ = 4.05); IR (KBr) ν' (cm^{-1}) 3068, 2942, 1762, 1676, 1584, 1510, 1411, 1257, 1238, 1222, 1126, 1035, 1009, 913, 854, 837, 772, 714. Anal. Calcd ($C_{21}H_{22}O_8$) C = 62.68%, H = 5.51%. Found C = 62.31%, H = 5.61%.

5.2. General procedure for the preparation of acrylic acids 3a and 3c

A mixture of 3,4,5-trimethoxyphenylacetic acid (1 g, 4.4 mmol), substituted benzaldehyde (4.4 mmol) and triethylamine (1 mL) in Ac_2O (10 mL) was heated at 140 °C. After cooling, the mixture was acidified with 35% aqueous HCl dropwise added (6 mL). The mixture was left overnight and then the precipitated product was filtered. The precipitate was recrystallized from absolute EtOH to give pure products.

5.2.1. (*E*)-3-(4′-Methoxy-3′-nitrophenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (*E*-3a) and (*Z*)-3-(4′-methoxy-3′-nitrophenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (*Z*-3a).[18] The general procedure was performed with 4-methoxy-3-nitrobenzaldehyde **2a** (850 mg, 4.7 mmol). The reaction mixture was stirred for 20 h. After acidification, *E*-**3a** precipitated and was collected by filtration. The aqueous filtrate was allowed to stand overnight at rt leading to precipitation of *Z*-**3a**, which was collected by filtration as an amorphous yellow powder (50 mg, <3%). The former precipitate was recrystallized from ethanol to give 800 mg (44%) of pure *E*-**3a** as yellow crystals. Compound *E*-**3a**: mp 223 °C; ^{1}H NMR (DMSO-d_6): δ 7.70 (s, 1H, H3), 7.53 (d, 1H, J = 1.5 Hz, H2′), 7.40 (dd, 1H, J = 1.5, 9 Hz, H6′), 7.26 (d, 1H, J = 9 Hz, H5′), 6.48 (s, 2H, H2″,H6″), 3.88 (s, 3H, 4′-OCH_3), 3.70 (s, 3H, 4″-OCH_3), 3.68 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR (DMSO-d_6): δ 169.0 (C1), 154.5 (C3″,C5″), 153.3 (C4′), 139.8 (C3′), 138.6 (C4″), 137.3 (C3), 137.2 (C6′), 134.5 (C2), 132.3 (C1″), 127.9 (C1'), 127.5 (C2′), 115.3 (C5′), 107.7 (C2″,C6″), 61.2 (4″-OCH_3), 57.9 (4′-OCH_3), 57.1 (3″,5″-OCH_3); MS (ES) m/z = 412 $[M+Na]^+$; UV (MeOH) λ (nm) 204 ($\log\varepsilon$ = 4.63), 297 ($\log\varepsilon$ = 3.95); IR (KBr) ν' (cm^{-1}) 3448, 2990, 2941, 2626, 1666, 1612, 1583, 1536, 1413,

1291, 1266, 1128, 1088, 1015, 996, 930, 830; Anal. Calcd ($C_{19}H_{19}NO_8$) C = 58.61%, H = 4.92%, N = 3.60%. Found C = 58.63%, H = 4.88%, N = 3.76%.Compound *Z*-**3a**: ^{1}H NMR (DMSO-d_6): δ 8.03 (d, 1H, J = 2Hz, H2′), 7.78 (dd, 1H, J = 9, 2 Hz, H6′), 7.42 (d, 1H, J = 9 Hz, H5′), 7.10 (s, 1H, H3), 6.78 (s, 2H, H2″,H6″), 3.95 (s, 3H, 4″-OCH_3), 3.81 (s, 6H, 3″,5″-OCH_3), 3.69 (s, 3H, 4′-OCH_3); ^{13}C NMR (DMSO-d_6): δ 171.4 (C1), 154.0 (C3″,C5″), 152.7 (C4′), 140.0 (C3′), 139.1 (C4″), 137.0 (C2), 134.8 (C6′), 132.8 (C1″), 129.2 (C1'), 125.9 (C3), 125.6 (C2′), 115.7 (C5′), 104.7 (C2″,C6″), 61.2 (4″-OCH_3), 57.9 (4′-OCH_3), 57.1 (3″,5″-OCH_3); MS (DIC/NH_3) m/z = 407 $[M+NH_4]^+$, 390 $[M+H]^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.55), 207 ($\log\varepsilon$ = 4.50), 237 ($\log\varepsilon$ = 4.23), 306 ($\log\varepsilon$ = 4.42); IR (KBr) ν' (cm^{-1}) 3440, 2941, 1691, 1618, 1582, 1529, 1508, 1465, 1413, 1340, 1283, 1270, 1242, 1128, 1009, 815. Anal. Calcd ($C_{19}H_{19}NO_8$) C = 58.61%, H = 4.92%, N = 3.60%. Found C = 58.20%, H = 4.92%, N = 3.92%.

5.2.2. (*E*)-3-(4′-Methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (3c).[21] The general procedure was performed with 4-methoxybenzaldehyde **2c** (536 mL, 4.4 mmol). The reaction mixture was heated during 20 h and the precipitate was recrystallized from ethanol to give 617 mg (41%) of **3c** as yellow crystals. mp 212 °C; ^{1}H NMR (DMSO-d_6): δ 7.67 (s, 1H, H3), 7.05 (d, 2H, J = 8.5 Hz, H2′,H6′), 6.80 (d, 2H, J = 8.5 Hz, H3′,H5′), 6.44 (s, 2H, H2″,H6″), 3.70 (s, 6H, 4′,4″-OCH_3), 3.67 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR (DMSO-d_6): δ 169.1 (C1), 160.6 (C4′), 153.8 (C3″,C5″), 139.3 (C3), 137.5 (C4″), 132.8 (C1″), 132.6 (C2′,C6′), 131.1 (C2), 127.3 (C1'), 114.4 (C3′,C5′), 107.1 (C2″,C6″), 60.7 (4″-OCH_3), 56.5 (3″,5″-OCH_3), 55.7 (4′-OCH_3); MS (DIC/NH_3) m/z = 362 $[M+NH_4]^+$, 345 $[M+H]^+$; UV (EtOH) λ (nm) 301 ($\log\varepsilon$ = 4.17); IR (KBr) ν' (cm^{-1}) 2994, 2942, 2630, 1664, 1604, 1582, 1508, 1464, 1414, 1253, 1242, 1180, 1127, 1029, 1008, 830. Anal. Calcd ($C_{19}H_{20}O_6$) C = 66.27%, H = 5.85%. Found C = 65.76%, H = 5.85%.

5.2.3. (*E*)-3-(4′-Hydroxy-3′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (3f). A solution of K_2CO_3 (21 mg, 0.15 mmol) in water (0.5 mL) was added to a solution of **3e** (20 mg, 0.05 mmol) in methanol (1.5 mL). The reaction mixture was stirred overnight at rt and then neutralized with 1 N aqueous HCl. After concentration, the resulting solution was extracted with AcOEt. The combined organic layers were washed with water, brine and dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. Purification of the residue by flash chromatography (eluent: cyclohexane/AcOEt, 60/40 (v/v), 1% AcOH) afforded 12 mg (70%) of **3f** as an amorphous white powder. ^{1}H NMR (DMSO-d_6): δ 9.50 (s, 1H, –OH), 7.61 (s, 1H, H3), 6.75 (dd, 1H, J = 1.5, 8 Hz, H6′), 6.66 (d, 1H, J = 8 Hz, H5′), 6.48 (d, 1H, J = 1.5 Hz, H2′), 6.47 (s, 2H, H2″,H6″), 3.70 (s, 6H, 3″,5″-OCH_3), 3.67 (s, 3H, 4″-OCH_3), 3.35 (s, 3H, 3′-OCH_3); ^{13}C NMR (DMSO-d_6): δ 169.6 (C1), 154.4 (C3″,C5″), 149.3 (C4′), 148.0 (C3′), 140.6 (C3), 137.9 (C4″), 134.6 (C1″), 130.6 (C2), 126.7 (C1′), 126.6 (C6′), 116.3 (C5′), 113.9 (C2′), 107.9 (C2″,C6″), 61.0 (4″-OCH_3), 57.1 (3″,5″-OCH_3), 55.7 (3′-OCH_3); MS(ES) m/z = 383 $[M+Na]^+$; UV (MeOH) λ (nm) 207 ($\log\varepsilon$ = 4.59), 292 ($\log\varepsilon$ = 4.06), 319 ($\log\varepsilon$ = 4.16); IR (NaCl film) ν' (cm^{-1}) 3316, 2937, 1767, 1672, 1588, 1503, 1460, 1413, 1245, 1169, 1126, 1025, 991. Anal. Calcd ($C_{21}H_{22}O_8$) C = 63.33%, H = 5.59%. Found C = 62.75%, H = 5.60%.

5.2.4. (*E*)-3-(4′-Methoxy-3′-[(*tert*-butyldimethylsilyl)-oxy]phenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylic acid (3g). TBDMSCl (440 mg, 2.8 mmol) was added to a solution of **3b** (200 mg, 0.56 mmol) and imidazole (161 mg, 2.44 mmol) in DMF (2 mL). The mixture was stirred at rt for 3 h, then diluted with water (5 mL) and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude residue was dissolved in a mixture of THF (2 mL) and 1 mL of a saturated aqueous $NaHCO_3$ solution. The solution was stirred for 2 h at rt, then water was added and the reaction mixture was extracted with AcOEt. Combined organic layers were washed with water, brine and dried over anhydrous $MgSO_4$ and evaporated to dryness to give 245 mg (92%) of **3g** as a white powder. Mp (CH_2Cl_2/*n*-hexane) 170 °C; ^{1}H NMR ($CDCl_3$): δ 7.84 (s, 1H, H3), 6.85 (dd, 1H, J = 2, 8.5 Hz, H6′), 6.73 (d, 1H, J = 8.5 Hz, H5′), 6.62 (d, 1H, J = 2 Hz, H2′), 6.46 (s, 2H, H2″, H6″), 3.89 (s, 3H, 4″-OCH_3), 3.79 (s, 9H, 3″,5″,4′-OCH_3), 0.90 (s, 6H, C–CH_3 in TBDMS), 0.01 (s, 9H, Si–CH_3); ^{13}C NMR ($CDCl_3$): δ 170.1 (C1), 153.8 (C3″,C5″), 152.9 (C4′), 144.6 (C3′), 142.5 (C3), 137.7 (C4″), 131.3 (C1″), 128.8 (C2), 127.1 (C1′), 126.8 (C6′), 122.5 (C2′), 111.3 (C5′), 106.5 (C2″,C6″), 61.0 (4″-OCH_3), 56.1 (3″,5″-OCH_3), 55.5 (4′-OCH_3), 25.6 (C–CH_3 in TBDMS), 18.4 (Si–C), −4.8 (Si–CH_3); MS(ES) m/z = 497 $[M+Na]^+$, 433, 301; UV (EtOH) λ (nm) 206 ($\log\varepsilon$ = 4.64), 285 ($\log\varepsilon$ = 4.15), 313 ($\log\varepsilon$ = 4.17); IR (KBr) ν' (cm^{-1}) 3001, 2929, 1665, 1598, 1583, 1506, 1272, 1127, 1028, 992, 862, 836, 778. Anal. Calcd ($C_{25}H_{34}O_7Si$) C = 63.27%, H = 7.22%. Found C = 62.36%, H = 7.27%.

5.3. General procedure for the preparation of amides (4a,c–d and 4f)

Step 1: To a solution of carboxylic acid (0.28 mmol) in freshly distilled CH_2Cl_2 (1 mL), triethylamine (167 μL, 1.21 mmol) and thionyl chloride (38 μL, 0.52 mmol) were added dropwise. The reaction mixture was stirred at rt for 2 h and concentrated to dryness under reduce pressure. **Step 2**: To 4 mL of an aqueous 28% NH_4OH solution a solution of the crude residue dissolved in CH_2Cl_2 (4 mL) was added. The reaction mixture was vigorously stirred at room temperature overnight, then diluted with water and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated to dryness under reduce pressure. The residue was purified by flash chromatography.

5.3.1. (*E*)-3-(4′-Methoxy-3′-nitrophenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylamide (4a).[18] The general procedure was performed with *E*-**3a** (108 mg, 0.28 mmol)

and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH 99/1 (v/v)) to give 82 mg (81%) of **4a** as a pale yellow powder. Mp (CH_2Cl_2/*n*-hexane) 187–189 °C; ^{1}H NMR ($CDCl_3$): δ 7.57 (s, 1H, H3), 7.52 (d, 1H, J = 2 Hz, H2′), 7.27 (d, 1H, J = 9 Hz, H5′), 6.92 (dd, 1H, J = 2, 9 Hz, H6′), 6.49 (s, 2H, H2″,H6″), 5.62 (s, 2H, –$CONH_2$), 3.93 (s, 6H, 4′,4″-OCH_3), 3.82 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 168.3 (C1), 154.7 (C3″,C5″), 153.0 (C4′), 139.3 (C3′), 138.8 (C4″), 136.1 (C6′), 134.9 (C3), 134.2 (C2), 130.7 (C1″), 127.3 (C1′,C2′), 113.2 (C5′), 106.2 (C2″,C6″), 61.2 (4″-OCH_3), 56.6 (4′-OCH_3), 56.4 (3″,5″-OCH_3); MS(ES) m/z = 411 $[M+Na]^+$; UV (EtOH) λ (nm) 209 ($\log\varepsilon$ = 4.7), 287 ($\log\varepsilon$ = 4.3); IR (NaCl film) ν' (cm^{-1}) 3418, 3173, 2912, 1679, 1528, 1351, 1279, 1126. Anal. Calcd ($C_{19}H_{20}N_2O_7$) C = 58.76%, H = 5.19%, N = 7.21%. Found C = 58.70%, H = 5.14%, N = 7.30%.

5.3.2. (*E*)-3-(3′-Amino-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylamide (1).[18] To a solution of **4a** (100 mg, 0.26 mmol) in AcOH (5 mL) zinc powder (1.0 g) was added at rt. The suspension was vigorously stirred for 1 h and then, was filtered over Celite. The filtrate was evaporated to dryness to give **1** (90 mg, quantitative yield) as a yellow powder in a pure form (**1** was analyzed without further purification). Mp (CH_2Cl_2/*n*-hexane) 189 °C; ^{1}H NMR ($CDCl_3$): δ 7.73 (s, 1H, H3), 6.62 (d, 1H, J = 8.5 Hz, H5′), 6.54 (dd, 1H, J = 2, 8.5 Hz, H6′), 6.51 (s, 2H, H2″,H6″), 6.40 (d, 1H, J = 2 Hz, H2′), 5.86 (s, 1H, –CONH), 5.55 (bs, 1H, –CONH), 3.92 (s, 3H, 4″-OCH_3), 3.81 (s, 9H, 4′,3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 169.3 (C1), 154.2 (C3″,C5″), 148.3 (C4′), 138.5 (C3), 138.1 (C4″), 135.7 (C3′), 132.3 (C1″), 130.5 (C2), 127.4 (C1′), 122.5 (C6′), 116.6 (C2′), 109.9 (C5′), 106.6 (C2″,C6″), 61.1 (4″-OCH_3), 56.3 (3″,5″-OCH_3), 55.4 (4′-OCH_3); MS(ES) m/z = 381 $[M+Na]^+$; UV (MeOH) λ (nm) 204 ($\log\varepsilon$ = 4.6), 251 ($\log\varepsilon$ = 4.2), 297 ($\log\varepsilon$ = 4.0), 336 ($\log\varepsilon$ = 3.9); IR (NaCl film) ν' (cm^{-1}) 3421, 2900, 1653, 1581, 1236, 1124.

5.3.3. (*E*)-3-(4′-Methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylamide (4c). The general procedure was performed with **3c** (96 mg, 0.28 mmol) and the residue was purified by flash column chromatography using an eluting gradient of AcOEt in cyclohexane to give 90 mg (94%) of **4c** as a brown powder. Mp (CH_2Cl_2/*n*-hexane)172–174 °C, ^{1}H NMR ($CDCl_3$): δ 7.79 (s, 1H, H3), 7.02 (d, 2H, J = 8 Hz, H2′,H6′), 6.72 (d, 2H, J = 8 Hz, H3′,H5′), 6.50 (s, 2H, H2″,H6″), 5.75 (bs, 1H, –CONH), 5.57 (bs, 1H, –CONH), 3.93 (s, 3H, 4″-OCH_3), 3.81 (s, 6H, 3″,5″-OCH_3), 3.77 (s, 3H, 4′-OCH_3); ^{13}C NMR ($CDCl_3$): δ 169.3 (C1), 160.3 (C4′), 154.4 (C3″,C5″), 138.1 (C4″), 137.8 (C3), 132.4 (C2′,C6′), 132.3 (C1″), 131.0 (C2), 127.2 (C1′), 113.9 (C3′,C5′), 106.4 (C2″,C6″), 61.2 (4″-OCH_3), 56.3 (3″,5″-OCH_3), 55.3 (4′-OCH_3); MS(ES) m/z = 366 $[M+Na]^+$; UV (MeOH) λ (nm) 205 ($\log\varepsilon$ = 4.57), 228 ($\log\varepsilon$ = 4.31), 282.5 ($\log\varepsilon$ = 4.16); IR (KBr) ν' (cm^{-1}) 3429, 3162, 2937, 1677, 1582, 1508, 1236, 1128, 1029, 995, 823, 691. Anal. Calcd ($C_{19}H_{21}NO_5$) C = 66.46%, H = 6.16%, N = 4.08%. Found C = 65.66%, H = 6.19%, N = 4.16%.

5.3.4. (*E*)-3-(3′-Fluoro-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylamide (4d). The general procedure was performed with **3d** (100 mg, 0.28 mmol) and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH 99/1 (v/v)) to give 65 mg (64%) of **4d** as a brown powder. Mp (CH_2Cl_2/*n*-hexane) 165 °C; ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 1H, H3), 6.89 (dd, 1H, J = 2, 8.5 Hz, H6′), 6.79 (dd, 1H, J = 9, 8.5 Hz, H5′), 6.71 (dd, 1H, J = 13, 2 Hz, H2′), 6.49 (s, 2H, H2″,H6″), 5.76 (bs, 1H, –CONH), 5.58 (bs, 1H, –CONH), 3.90 (s, 3H, 4″-OCH_3), 3.85 (s, 3H, 4′-OCH_3), 3.80 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 168.9 (C1), 154.4 (C3″,C5″), 151.6 (C3′, J = 244 Hz), 148.3 (C4′, J = 11 Hz), 138.3 (C4″), 136.4 (C3), 134.4 (C2), 131.4 (C1″), 127.6 (C1′,C6′), 117.3 (C2′, J = 19 Hz), 112.7 (C5′), 106.2 (C2″,C6″), 61.0 (4″-OCH_3), 56.2 (3″,5″-OCH_3), 56.0 (4′-OCH_3); MS (DIC/NH_3) m/z = 362 $[M+H]^+$; UV (MeOH) λ (nm) 207 ($\log\varepsilon$ = 4.56), 282.5 ($\log\varepsilon$ = 4.12), 303.5 ($\log\varepsilon$ = 4.16); IR (KBr) ν' (cm^{-1}) 3421–3157, 2912, 1681, 1585, 1508, 1278, 1237, 1129. Anal. Calcd ($C_{19}H_{20}FNO_5$) C = 63.15%, H = 5.19%, N = 7.21%. Found C = 63.63%, H = 5.47%, N = 4.27%.

5.3.5. (*E*)-3-(4′-Hydroxy-3′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylamide (4f). The general procedure was performed with **3e** (112 mg, 0.28 mmol) and the residue was purified by flash column chromatography (cyclohexane/AcOEt 20/80 (v/v)) to give 75 mg (77%) of **4g** as an amorphous brown powder. ^{1}H NMR ($CDCl_3$): δ 7.77 (s, 1H, H3), 6.79 (s, 2H, H5′,H6′), 6.54 (s, 2H, H2″,H6″), 6.46 (bs, 1H, H2′), 5.86 (bs, 1H, OH), 5.71 (bs, 1H, –CONH), 5.57 (bs, 1H, –CONH), 3.89 (s, 3H, 4″-OCH_3), 3.83 (s, 6H, 3″,5″-OCH_3), 3.53 (s, 3H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$): δ 169.0 (C1), 154.5 (C3″,C5″), 146.9 (C4′), 146.0 (C3′), 138.1 (C3, C4″), 132.4 (C1″), 130.7 (C2), 126.9 (C1′), 126.1 (C5′), 114.3 (C6′), 111.8 (C2′), 106.5 (C2″,C6″), 60.9 (4″-OCH_3), 56.3 (3″,5″-OCH_3), 55.3 (3′-OCH_3); MS(ES) m/z = 382 $[M+Na]^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.64), 206 ($\log\varepsilon$ = 4.64), 234 ($\log\varepsilon$ = 4.24), 281 ($\log\varepsilon$ = 4.02), 321 ($\log\varepsilon$ = 4.16); IR (NaCl film) ν' (cm^{-1}) 3471, 3347, 3179, 2935, 1663, 1582, 1514, 1463, 1412, 1280, 1237, 1126, 1030, 1104, 895, 818, 734. Anal. Calcd ($C_{19}H_{21}NO_6$) C = 63.50%, H = 5.89%, N = 3.90%. Found C = 63.21%, H = 6.39%, N = 3.77%.

5.3.6. (*E*)-3-(3′-Hydroxy-4′-methoxyphenyl)-2-(3″,4″,5″-trimethoxyphenyl)acrylamide (4b). A solution of **3b** (0.055 mmol) in DMF (1 mL) was cooled at −10 °C and stirred under argon. Triethylamine (31 μL, 0.22 mmol) and ethylchloroformate (16 μL, 0.17 mmol) were added dropwise. After 30 min, a solution of NH_4Cl (5 mg, 0.11 mmol) and triethylamine (15 μL, 0.11 mmol) in DMF (1 mL) was added. The reaction mixture was stirred for 1 h and evaporated to dryness under reduced pressure. The residue was taken up with AcOEt and washed with water and brine. The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. To a solution of the residue in methanol (3 mL), a solution (0.5 mL) of LiOH (5 mg, 0.11 mmol) in water was added. After 5 h, the reaction mixture was neutralized with 1 N aqueous HCl and evaporated to dryness under vacuum. The residue was

treated with AcOEt. The resulting solution was washed successively with water and brine, dried over anhydrous $MgSO_4$, filtered and then evaporated to dryness. The crude residue was purified by recrystallization in CH_2Cl_2/*n*-hexane to give 13 mg (66%) of **4b** as yellow crystals. Mp 163–165 °C; ^{1}H NMR ($CDCl_3$): δ 7.75 (s, 1H, H3), 6.69 (d, 1H, J = 8 Hz, H5′), 6.65 (dd, 1H, J = 2, 8 Hz, H6′), 6.64 (bs, 1H, H2′), 6.51 (s, 2H, H2″,H6″), 5.54 (bs, 2H, –$CONH_2$), 5.50 (s, 1H, OH), 3.93 (s, 3H, 4″-OCH_3), 3.86 (s, 3H, 4′-OCH_3), 3.82 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 169.1 (C1), 154.3 (C3″,C5″), 145.2 (C4′), 144.7 (C3), 137.8 (C4″,C3′), 131.9 (C1″), 131.5 (C2), 129.0 (C1′), 123.8 (C6′), 116.3 (C2′), 110.2 (C5′), 106.5 (C2″,C6″), 61.1 (4″-OCH_3), 56.3 (3″,5″-OCH_3), 55.9 (4′-OCH_3); MS(ES) m/z = 382 $[M+Na]^+$; UV (MeOH) λ (nm) 205 ($\log\varepsilon$ = 4.53), 207 ($\log\varepsilon$ = 4.53), 242 ($\log\varepsilon$ = 4.10), 296 ($\log\varepsilon$ = 3.94), 320 ($\log\varepsilon$ = 3.99); IR (NaCl film) ν' (cm^{-1}) 3460, 3346, 3186, 2934, 1661, 1581, 1508, 1459, 1410, 1275, 1237, 1125, 1024. Anal. Calcd ($C_{19}H_{21}NO_6$) C = 63.50%, H = 5.89%, N = 3.90%. Found C = 56.28%, H = 5.70%, N = 3.45%.

5.4. General procedure for the preparation of carbamates 5a and 6a

Step 1: To a solution of *E*-**3a** (0.14 mmol) in anhydrous acetone (3 mL), cooled at −5 °C and stirred under argon, triethylamine (39 μL, 0.28 mmol) and ethylchloroformate (19 μL, 0.18 mmol) were added dropwise. After 30 min, ice (3 mL) was added and the solution was extracted with CH_2Cl_2 (4 mL). The combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. **Step 2**: The residue was taken up in 3 mL of THF and the solution was cooled at 0 °C. A solution of NaN_3 (19 mg, 0.28 mmol) in water (1 mL) was added. The solution was extracted with ether (4 mL). The combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered and evaporated to dryness under reduced pressure. **Step 3**: The residue was taken up with 3 mL of anhydrous toluene and heated under argon at 80 °C for 1 h. **Step 4**: The reaction mixture was cooled at 50 °C and 2-trimethylsilylethanol or methanol (1.4 mmol) was added. The solution was stirred under argon at 50 °C, then allowed to cool at rt and evaporated to dryness. The crude residue was purified by flash column chromatography.

5.4.1. (*E*)-[2-(Trimethylsilyl)ethyl]-*N*-[2-(4′-methoxy-3′-nitrophenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl] carbamate (5a). The general procedure was performed with *E*-**3a** (56 mg, 0.14 mmol). The reaction mixture was heated for 18 h and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 75/25 (v/v)) to give 55 mg (78%) of **5a** as yellow powder. Mp (CH_2Cl_2/*n*-hexane) = 141–142 °C; ^{1}H NMR ($CDCl_3$): δ 7.42 (d, 1H, J = 2Hz, H2′), 7.08 (dd, 2H, J = 2, 8.5 Hz, H2, H6′), 6.71 (d, 1H, J = 8.5 Hz, H5′), 6.50 (s, 2H, H2″,H6″), 6.11 (bs, 1H, NH), 4.27 (t, 2H, OCH_2), 3.88 (s, 6H, 4′,4″-OCH_3), 3.72 (s, 3H, 3″,5″-OCH_3), 1.50 (t, 2H, –CH_2–TMS), 0.7 (s, 9H, Si–CH_3); ^{13}C NMR ($CDCl_3$): δ 153.9 (C3″,C5″), 153.6 (–NH*C*OO), 150.7 (C4′), 139.4 (C3′), 139.3 (C4″), 136.0 (C1″), 134.2 (C6′), 131.3 (C1), 129.7 (C1′), 125.5 (C2′), 112.9 (C5′), 111.2 (C2), 106.4 (C2″,C6″), 63.8 (-CH_2O), 61.0 (4″-OCH_3), 56.5 (3″,5″-OCH_3), 56.4 (4′-OCH_3), 17.7 (–*C*H_2–TMS), −1.4 (Si–CH_3); MS(ES) m/z = 527 $[M+Na]^+$; UV (MeOH) λ (nm) 204 ($\log\varepsilon$ = 4.60), 240 ($\log\varepsilon$ = 4.23), 304 ($\log\varepsilon$ = 4.13); IR (KBr) ν' (cm^{-1}) 3332, 2920, 1588, 1456, 1406, 1126, 1045, 839. Anal. Calcd ($C_{24}H_{32}N_2O_5Si$) C = 57.13%, H = 6.39%, N = 5.55%. Found C = 57.35%, H = 6.50%, N = 5.52%.

5.4.2. (*E*-Methyl-*N*-[2-(4′-methoxy-3′-nitrophenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]carbamate (6a). The general procedure was performed with *E*-**3a** (56 mg, 0.14 mmol). The reaction mixture was heated for 4 h 30 min and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 70/30 (v/v)) to give 52 mg (96%) of **6a** as an amorphous yellow powder. ^{1}H NMR ($CDCl_3$): δ 7.44 (d, 1H, J = 2 Hz, H2′), 7.08 (m, 2H, H2, H6′), 6.82 (d, 1H, J = 9 Hz, H5′), 6.51 (s, 2H, H2″,H6″), 6.16 (bs, 1H, NH), 3.89 (s, 6H, 4′,4″-OCH_3), 3.79 (s, 3H, –$COOCH_3$), 3.75 (s, 3H, 3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 153.9 (–NH-*C*$OOCH_3$, C3″,C5″), 150.8 (C4′), 139.3 (C3′), 139.2 (C4″), 135.9 (C1), 134.2 (C6′), 131.2 (C1″), 129.6 (C1′), 125.6 (C2′), 113.0 (C5′), 111.5 (C2), 106.3 (C2″,C6″), 61.1 (4″-OCH_3), 56.4 (4′-OCH_3), 56.3 (3″,5″-OCH_3), 52.5 (–$COOCH_3$); MS(ES) m/z = 441 $[M+Na]^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.59), 203 ($\log\varepsilon$ = 4.57), 220 ($\log\varepsilon$ = 4.44), 245 ($\log\varepsilon$ = 4.10), 282 ($\log\varepsilon$ = 4.04); IR (KBr) ν' (cm^{-1}) 3300, 2943, 1585, 1518, 1460, 1414, 1282, 1127, 1021, 1006, 764. Anal. Calcd ($C_{20}H_{22}N_2O_8$) C = 57.41%, H = 5.30%, N = 6.70%. Found C = 56.36%, H = 5.57%, N = 6.18%.

5.5. General procedure for the preparation of carbamates (5c,d,g,h, 6c,d,g–h)

Step 1: A solution of acrylic acid (0.14 mmol) in freshly distilled anhydrous acetone (3 mL) was cooled at −5 °C and stirred under argon. Triethylamine (39 μL, 0.28 mmol) and ethyl chloroformate (19 μL, 0.18 mmol) were added dropwise. **Step 2**: After 30 min, a solution of NaN_3 (19 mg, 0.28 mmol) in water (1 mL) was added and the reaction was quenched after 1 h by addition of ice (3 mL). Further steps are identical to those previously described for the preparation of carbamates **5a** and **6a**.

5.5.1. (*E*)-[2-(Trimethylsilyl)ethyl]-*N*-[2-(4′-methoxyphenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]carbamate (5c). The general procedure was performed with **3c** (48 mg, 0.14 mmol). The reaction mixture was heated for 5 h and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 90/10 (v/v)) to give 20 mg (31%) of **5c** as an amorphous white powder. ^{1}H NMR ($CDCl_3$): δ 7.00 (s, 1H, H2), 6.91 (bd, 2H, J = 8.8 Hz, H2′,H6′), 6.67 (bd, 2H, J = 8.8 Hz, H3′,H5′), 6.54 (s, 2H, H2″,H6″), 6.02 (bs, 1H, –CONH), 4.24 (t, 2H, –OCH_2), 3.87 (s, 3H, 4″-OCH_3), 3.74 (s, 3H, 4′-OCH_3), 3.71 (s, 6H, 3″,5″-OCH_3), 1.03 (t, 2H,

–CH_2–TMS), 0.06 (9H, s, Si–CH_3); ^{13}C NMR ($CDCl_3$): δ 157.9 (C4′), 154.2 (–NH*C*O), 153.5 (C3″, C5″), 138.5 (C4″), 133.3 (C1), 132.4 (C1″), 130.1 (C2′,C6′), 129.2 (C1′), 115.5 (C2), 113.5 (C3′,C5′), 106.4 (C2″,C6″), 63.6 (–OCH_2), 61.1 (4″-OCH_3), 56.2 (3″,5″-OCH_3), 55.3 (4′-OCH_3), 17.8 (–*C*H_2–TMS), −1.4 (Si–CH_3); MS(ES) *m*/*z* = 482 $[M+Na]^+$; UV (MeOH) λ (nm) 206 ($\log\varepsilon$ = 4.63), 244 ($\log\varepsilon$ = 4.26), 290 ($\log\varepsilon$ = 4.17); IR (NaCl film) ν' (cm^{-1}) 3379, 2954, 1705, 1584, 1510, 1421, 1249, 1129, 1037, 836. Anal. Calcd ($C_{24}H_{33}NO_6Si$) C = 62.72%, H = 7.24%, N = 3.05%. Found C = 62.16%, H = 7.12%, N = 3.27%.

5.5.2. (*E*)-[2-(Trimethylsilyl)ethyl]-*N*-[2-(3′-fluoro-4′-methoxyphenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]-carbamate (5d). The general procedure was monitored with **3d** (50 mg, 0.14 mmol). The reaction mixture was heated for 5 h and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 80/20 (v/v)) to give 48 mg (71%) of **5d** as a white powder. Mp (CH_2Cl_2/*n*-hexane) 126 °C; ^{1}H NMR (DMSO-d_6): δ 8.88 (bs, 1H, –CONH), 6.94 (t, 1H, *J* = 9 Hz, H5′), 6.70 (s, 1H, H2), 6.69 (dd, 1H, *J* = 2, 9 Hz, H6′), 6.60 (dd, 1H, *J* = 13, 2 Hz, H2′), 6.49 (s, 2H, H2″,H6″), 4.10 (t, 2H, –OCH_2), 3.74 (s, 3H, 4′-OCH_3), 3.66 (s, 3H, 4″-OCH_3), 3.62 (s, 6H, 3″,5″-OCH_3), 0.94 (t, 2H, –CH_2–TMS), 0.03 (9H, s, Si–CH_3); ^{13}C NMR (DMSO-d_6): δ 155.0 (–NH*C*O), 153.8 (C3″,C5″), 151.83 (C3′, *J* = 252 Hz), 145.5 (C4′), 138.8 (C4″), 136.8 (C1), 132.5 (C1″), 130.95 (C1′, *J* = 8 Hz), 125.9 (C6′), 116.35 (C2′, *J* = 17 Hz), 114.8 (C2), 114.4 (C5′), 107.8 (C2″,C6″), 63.0 (–OCH_2), 61.2 (4″-OCH_3), 56.9 (4′,3″,5″-OCH_3), 18.3 (–*C*H_2–TMS), −0.4 (Si–CH_3); MS(ES) : *m*/*z* = 500 $[M+Na]^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.60), 204 ($\log\varepsilon$ = 4.57), 243 ($\log\varepsilon$ = 4.31), 290 ($\log\varepsilon$ = 4.17); IR (KBr) ν' (cm^{-1}) 3371, 2928, 1724, 1635, 1518, 1437, 1127, 1052, 862. Anal. Calcd ($C_{24}H_{32}FNO_6Si$) C = 60.36%, H = 6.75%, N = 2.93%. Found C = 58.78%, H = 6.72%, N = 2.77%.

5.5.3. (*E*)-[2-(Trimethylsilyl)ethyl]-*N*-[2-(4′-methoxy-3′-[(*tert*-butyldimethylsilyl)oxy]phenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]carbamate (5g). The general procedure was performed with **3g** (63 mg, 0.14 mmol). The reaction mixture was heated for 4 h 30 min and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 90/10 (v/v)) to give 31 mg (26%) of **5g** as an amorphous white powder. ^{1}H NMR ($CDCl_3$): δ 6.98 (s, 1H, H2), 6.65 (d, 1H, *J* = 8.5 Hz, H5′), 6.61 (dd, 1H, *J* = 2, 8.5 Hz, H6′), 6.54 (s, 2H, H2″,H6″), 6.48 (d, 1H, *J* = 2 Hz, H2′), 5.96 (bs, 1H, NH), 4.24 (t, 2H, –OCH_2), 3.85 (s, 3H, 4″-OCH_3), 3.73 (s, 3H, 4′-OCH_3), 3.72 (s, 3H, 3″,5″-OCH_3), 1.02 (t, 2H, –CH_2–TMS), 0.89 (s, 6H, C–CH_3 in TBDMS), 0.05 (s, 9H, Si–CH_3), 0.00 (s, 9H, Si–CH_3); ^{13}C NMR ($CDCl_3$): δ 153.9 (–NH*C*O), 153.5 (C3″,C5″), 149.5 (C4′), 144.4 (C3′), 138.3 (C4″), 133.3 (C1), 132.6 (C1″), 129.6 (C1′), 122.9 (C6′), 121.2 (C2′), 115.2 (C2), 111.7 (C5′), 106.5 (C2″,C6″), 63.5 (CH_2O), 60.9 (4″-OCH_3), 56.1 (3″,5″-OCH_3), 55.5 (4′-OCH_3), 25.6 (C–*C*H_3 in TBDMS), 18.2 (Si–C in TBDMS), 17.7 (–*C*H_2-TMS), −1.5 (Si–CH_3), −5.0 (Si–CH_3 in TBDMS); MS(DIC/NH_3) *m*/*z* = 590 $[M+H]^+$, 562, 446, 196, 90, 73; UV (MeOH) λ (nm) 201 ($\log\varepsilon$ = 4.66), 204 ($\log\varepsilon$ = 4.66), 242 ($\log\varepsilon$ = 4.24), 290 ($\log\varepsilon$ = 4.13); IR (KBr) ν' (cm^{-1}) 3305, 2951, 2899, 2858, 1710, 1585, 1544, 1508, 1288, 1244, 1228, 1127, 866, 838. Anal. Calcd ($C_{30}H_{47}NO_7Si_2$) C = 61.09%, H = 8.03%, N = 2.37%. Found C = 60.69%, H = 8.11%, N = 1.98%.

5.5.4. (*E*)-Ethyl [5′-{2-[2-(trimethylsilyl)ethylcarbamoyl]-2-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl}]-2′-methoxyphenyl carbonate (5h). The general procedure was performed with **3b** (50 mg, 0.14 mmol). The reaction mixture was heated for 4 h 30 min and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 80/20 (v/v)) to give 38 mg (50%) of **3b** as an amorphous white powder. ^{1}H NMR ($CDCl_3$): δ 6.98 (s, 1H, H1), 6.83 (dd, 1H, *J* = 1.5, 8.5 Hz, H4′), 6.73 (d, 1H, *J* = 8.5 Hz, H3′), 6.71 (d, 1H, *J* = 1.5 Hz, H6′), 6.52 (s, 2H, H2″,H6″), 6.07 (bs, 1H, –CONH), 4.25 (4H, m, 2-OCH_2), 3.85 (s, 3H, 4″-OCH_3), 3.75 (s, 3H, 2′-OCH_3), 3.72 (s, 6H, 3″,5″-OCH_3), 1.33 (t, 3H, –CH_2CH_3), 1.03 (t, 2H, –CH_2–TMS), 0.02 (s, 9H, Si–CH_3); ^{13}C NMR ($CDCl_3$): δ 153.9 (–NH*C*O), 153.5 (C3″,C5″), 153.1 (–*C*OOEt), 149.4 (C2′), 139.6 (C1′), 138.5 (C4″), 134.3 (C2), 131.8 (C1″), 129.7 (C5′), 127.6 (C4′), 122.6 (C6′), 113.9 (C1′), 112.0 (C3′), 106.4 (C2″,C6″), 64.7 (–O*C*H_2–CH_3), 63.6 (–O*C*H_2–CH_2), 60.9 (4″-OCH_3), 56.2 (3″,5″-OCH_3), 55.9 (2′-OCH_3), 17.7 (–*C*H_2–TMS), 14.1 (–OCH_2*C*H_3), −1.4 (Si–CH_3); MS(ES) *m*/*z* = 570 $[M+Na]^+$; UV (EtOH) λ (nm) 201 ($\log\varepsilon$ = 4.69), 203 ($\log\varepsilon$ = 4.66), 244 ($\log\varepsilon$ = 4.24), 289 ($\log\varepsilon$ = 4.14); IR (KBr) ν'(cm^{-1}) 3329, 2955, 1762, 1732, 1588, 1544, 1504, 1268, 1234, 1125, 1050, 862, 834. Anal. Calcd ($C_{27}H_{37}NO_9Si$) C = 59.21%, H = 6.81%, N = 2.56%. Found C = 57.91%, H = 6.98%, N = 2.60%.

5.5.5. (*E*)-Methyl-*N*-[-2-(4′-methoxyphenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]carbamate (6c). The general procedure was performed with **3c** (48 mg, 0.14 mmol). The reaction mixture was heated for 4 h 10 min and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 80/20 (v/v)) to give 20 mg (38%) of **6c** as an amorphous pale yellow powder. ^{1}H NMR ($CDCl_3$): δ 7.00 (s, 1H, H2), 6.91 (d, 2H, *J* = 8.5 Hz, H2′,H6′), 6.67 (d, 2H, *J* = 8.5 Hz, H3′,H5′), 6.53 (s, 2H, H2″,H6″), 6.11 (bs, 1H, –CONH), 3.86 (s, 3H, 4″-OCH_3), 3.76 (s, 3H, 4′-OCH_3), 3.74 (s, 3H, –$COOCH_3$), 3.71 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 158.0 (–NH*C*$OOCH_3$), 154.5 (C4′), 153.5 (C3″, C5″), 138.3 (C4″), 133.1 (C1), 132.3 (C1″), 130.2 (C2′,C6′), 129.1 (C1′), 115.6 (C2), 113.5 (C3′,C5′), 106.5 (C2″,C6″), 61.1 (4″-OCH_3), 56.2 (3″,5″-OCH_3), 55.3 (–$COOCH_3$), 52.4 (4′-OCH_3); MS (DIC/NH_3) *m*/*z* = 374 $[M+H]^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.50), 205 ($\log\varepsilon$ = 4.48), 242 ($\log\varepsilon$ = 4.16), 292 ($\log\varepsilon$ = 4.08); IR (NaCl film) ν' (cm^{-1}) 3328, 3002, 2940, 1730, 1583, 1509, 1245, 1127, 1031, 1004, 826. Anal. Calcd ($C_{20}H_{23}NO_6$) C = 64.33%, H = 6.21%, N = 3.75%. Found C = 62.98%, H = 6.26%, N = 3.48%.

5.5.6. (*E*)-Methyl-*N*-[2-(3′-fluoro-4′-methoxyphenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]carbamate (6d). The general procedure was performed with **3d** (50 mg,

0.14 mmol). The reaction mixture was heated for 4 h and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 70/30 (v/v)) to give 48 mg (86%) of **6d** as an amorphous white powder. ^{1}H NMR ($CDCl_3$): δ 7.00 (s, 1H, H2), 6.72 (dd, 1H, J = 8, 9 Hz, H5′), 6.70 (dd, 1H, J = 1.5, 8 Hz, H6′), 6.68 (dd, 1H, J = 12, 1.5 Hz, H2′), 6.52 (s, 2H, H2″,H6″), 6.08 (bs, 1H, –CONH), 3.87 (s, 3H, 4″-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.77 (s, 3H, –$COOCH_3$), 3.74 (s, 6H, 3″,5″-OCH_3); ^{13}C NMR ($CDCl_3$): δ 154.1 (–NH*C*OOCH$_3$), 153.6 (C3″,C5″), 151.8 (C3′, J = 243 Hz), 145.77 (C4′, J = 12 Hz), 138.5 (C4″), 134.3 (C1), 131.7 (C1″), 129.95 (C1′, J = 7.5 Hz), 124.9 (C6′), 116.173 (C2′, J = 18 Hz), 113.8 (C2), 112.9 (C5′), 106.4 (C2″,C6″), 61.0 (4″-OCH_3), 56.4 (4′,3″,5″-OCH_3), 52.4 (–COO*C*H$_3$); MS(ES) m/z = 414 [M+Na]$^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.63), 204 ($\log\varepsilon$ = 4.58), 242 ($\log\varepsilon$ = 4.20), 274 ($\log\varepsilon$ = 3.95), 281 ($\log\varepsilon$ = 3.94), 295 ($\log\varepsilon$ = 3.84); IR (NaCl film) ν' (cm^{-1}) 3336, 2936, 1584, 1518, 1441, 1416, 1281, 1129, 1023. Anal. Calcd ($C_{20}H_{22}FNO_6$) C = 61.38%, H = 5.67%, N = 3.58%. Found C = 61.35%, H = 5.80%, N = 3.59%.

5.5.7. (*E*)-Methyl-*N*-[2-(4′-methoxy-3′-[(*tert*-butyldimethylsilyl)oxy]phenyl)-1-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]carbamate (6g). The general procedure was performed with **3g** (63 mg, 0.14 mmol). The reaction mixture was heated for 4 h 30 min and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 80/20 (v/v)) to give 22 mg (31%) of **6g** as an amorphous white powder. ^{1}H NMR ($CDCl_3$): δ 6.98 (s, 1H, H2), 6.66 (d, 1H, J = 8.5 Hz, H5′), 6.62 (dd, 1H, J = 1, 8.5 Hz, H6′), 6.54 (s, 2H, H2″,H6″), 6.48 (d, 1H, J = 2 Hz, H2′), 6.03 (bs, 1H, –NH), 3.86 (s, 3H, 4″-OCH_3), 3.76 (s, 3H, $COOCH_3$), 3.74 (s, 3H, 4′-OCH_3), 3.73 (s, 3H, 3″,5″-OCH_3), 0.90 (s, 9H, C–CH_3), 0.00 (s, 6H, Si–CH_3); ^{13}C NMR ($CDCl_3$): δ 154.2 (–NH*C*OOCH$_3$), 153.5 (C3″,C5″), 149.6 (C4′), 144.4 (C3′), 138.3 (C4″), 133.1 (C1), 132.4 (C1″), 129.4 (C1′), 122.9 (C6′), 121.2 (C2′), 115.4 (C2), 111.6 (C5′), 106.4 (C2″,C6″), 60.9 (4″-OCH_3), 56.1 (3″,5″-OCH_3), 55.5 (4′-OCH_3), 52.3 (–COO*C*H$_3$), 25.6 (C–*C*H$_3$ in TBDMS), 18.3 (Si–C), −0.7 (Si–CH_3); MS(ES) m/z = 526 [M+Na]$^+$; UV (MeOH) λ (nm) 205 ($\log\varepsilon$ = 4.70), 207 ($\log\varepsilon$ = 4.69), 242 ($\log\varepsilon$ = 4.17), 288 ($\log\varepsilon$ = 4.12), 314 ($\log\varepsilon$ = 4.07); IR (NaCl film) ν' (cm^{-1}) 3312, 2939, 1773, 1676, 1587, 1499, 1463, 1416, 1339, 1250, 1174, 1127, 1028, 1002, 836, 775. Anal. Calcd ($C_{26}H_{37}NO_7Si$) C = 62.00%, H = 7.40%, N = 2.78%. Found C = 61.77%, H = 7.62%, N = 2.75%.

5.5.8. (*E*)-Ethyl{5′-[2-methylcarbamoyl-2-(3″,4″,5″-trimethoxyphenyl)ethen-1-yl]-2′-methoxyphenyl}carbonate (6h). The general procedure was performed with **3b** (50 mg, 0.14 mmol). The reaction mixture was heated for 4 h 30 min and the residue was purified by flash column chromatography (eluent: cyclohexane/AcOEt 70/30 (v/v)) to give 23 mg (36%) of **6b** as a white powder. Mp (CH_2Cl_2/*n*-hexane) 180–185 °C; ^{1}H NMR ($CDCl_3$): δ 7.00 (s, 1H, H1), 6.84 (dd, 1H, J = 2, 8.5 Hz, H4′), 6.74 (d, 1H, J = 8.5 Hz, H3′), 6.72 (d, 1H, J = 2 Hz, H6′), 6.52 (s, 2H, H2″,H6″), 6.10 (bs, 1H, –CONH), 4.25 (q, 2H, –OCH_2), 3.86 (s, 3H, 4″-OCH_3), 3.79 (s, 3H, 2′-OCH_3), 3.76 (s, 3H, –NHCOO*CH*$_3$), 3.73 (s, 6H, 3″,5″-OCH_3), 1.35 (t, 3H, –CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 154.3 (–NH*C*OOCH$_3$), 153.6 (C3″, C5″), 153.3 (–*C*OOEt), 149.5 (C2′), 139.6 (C1′), 138.5 (C4″), 134.5 (C2), 131.8 (C1″), 129.6 (C5′), 127.7 (C4′), 122.7 (C6′), 114.1 (C1), 112.0 (C3′), 106.3 (C2″,C6″), 64.9 (–OCH_2), 61.0 (4″-OCH_3), 56.2 (3″,5″-OCH_3), 56.0 (2′-OCH_3), 52.5 (–$COOCH_3$), 14.2 (–CH_2–*C*H$_3$); MS(ES) m/z = 484 [M+Na]$^+$; UV (MeOH) λ (nm) 202 ($\log\varepsilon$ = 4.46), 216 ($\log\varepsilon$ = 4.33), 243 ($\log\varepsilon$ = 4.06), 293 ($\log\varepsilon$ = 4.04); IR (NaCl film) ν' (cm^{-1}) 3313, 2940, 1763, 1732, 1584, 1505, 1236, 1127, 1055, 1003, 917, 770, 731. Anal. Calcd ($C_{23}H_{27}NO_9$) C = 59.86%, H = 5.90%, N = 3.04%. Found C = 59.42%, H = 6.01%, N = 3.05%.

5.6. Tubulin binding assay

Calf brain tubulin was purified according to the method of Shelanski, by three cycles of assembly–disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 2 mM EGTA and 1 mM GTP pH = 6.6 (the concentration of tubulin was about 2–3 mg/mL).[26] Tubulin assembly was monitored and recorded continuously by turbidimetry at 400 nm in a UV spectrophotometer, equipped with a thermostated cell at 37 °C.[27] The IC_{50}, defined as the concentration value of inhibitor, which decreased by 50% the maximum assembly rate of tubulin without drug, was determined. The IC_{50} for all compounds were compared to the IC_{50} of colchicine, measured the same day under the same conditions.

5.7. Cell growth inhibition assay

MTT colorimetric assay was employed according to the established procedure.[28] The human cell lines MCF-7 (breast adenocarcinoma), KB-3-1 (epidermoid carcinoma) and IGROV (ovary adenocarcinoma) were maintained in DMEM medium (Eurobio) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 UI/mL penicillin and 50 UI/mL streptomycin. Cells were grown at 37 °C and 5% CO_2 in humidified atmosphere. 10,000 cells/well were seeded in 200 μL of growth medium in 96-well microtitre plates (ATGC Biotechnologies, France) and incubated for 24 h prior to addition of experimental drugs (final dimethylsulfoxide concentration, 0.1%). The cells were treated with eleven concentrations of test compounds in a CO_2 incubator for 72 h. At the end of this period, 20 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was added to each well and the plates were incubated for 3 h at 37 °C. The medium was aspirated and the formazan solubilized by 100 L of DMSO. The UV absorbance of the solubilized formazan crystals was measured spectrophotometrically (Metermech Σ960 Microplate Reader, Bioblock Scientific, France). The IC_{50} (concentration reducing by 50% the absorbance at 570 nm) was calculated by a

linear regression performed on the linear zone of the dose–response curve.

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