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Isoxazole-type derivatives related to combretastatin A-4, synthesis and biological evaluation

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Abstract—Novel combretastatin analogues bearing various five-membered heterocycles with consecutive oxygen and nitrogen atoms, in place of the olefinic bridge of CA4, have been synthesized (isoxazole, isoxazoline, oxadiazole, etc). These compounds have been evaluated for cytotoxicity and their ability to inhibit the tubulin assembly. On the basis of the relative position of the aromatic A- and B-rings on the heterocyclic moiety, they could be split in two classes, the α,γ- or α,β-diaryl heterocyclic derivatives. In the first series, the 3,5-diaryloxadiazole **9a** displayed comparable antitubulin activity to that of CA4, but was devoid of cytotoxic effects. Among the α,β-diaryl heterocyclic derivatives, the 4,5-diarylisoxazole **35** exhibited greater antitubulin activity than that of CA4 (0.75 vs 1.2 μM), but modest antiproliferative activity. These data showed that minor alteration in the chemical structure of the heterocyclic ring and its relative orientation with regard to the two phenyl rings of CA4 could dramatically influence the tubulin binding properties.

1. Introduction

Combretastatin A-4 (CA4), a natural product isolated by Pettit et al. in 1989 from the South African willow tree *Combretum caffrum*,[1] strongly inhibits tubulin polymerization by binding to the colchicine site.[2] A disodium phosphate prodrug form (CA4P) was selected for further preclinical developments.[3] CA4, which displayed potent activity against a broad spectrum of human cancer cells, including multidrug-resistant cells,[4] has drawn significant attention due to its potent and selective effect on the established tumour vasculature.[5,6] CA4 has shown the ability to shut down tumour vasculature, whereas the blood flow to normal tissues was much less affected.[7] The most likely causes of the rapid vascular collapse are morphological and functional changes in endothelial cells,[8,9] associated with an increased permeability,[10,11] which resulted in the disruption of the tubulin cytoskeleton. In experimental tumours, the antivascular effects observed well below its maximum tolerated dose rapidly led to extensive haemorrhagic necrosis in areas that are often resistant to conventional anti-cancer treatments.[12,13] CA4P, in combination with cytotoxic chemotherapy and radiotherapy, is undergoing phase II trials, for the treatment of solid tumours.[14,15] Another CA4 derivative, AVE8062,[16,17] is currently under clinical evaluation as tumour vascular targeting agent.[18]

Given the encouraging antivascular/anticancer activity of CA4P, synthesis of numerous analogues has been reported in order to have a better understanding of the structure–activity relationships.[19] A key structural factor for tubulin affinity is the presence of the double bond, or of a suitable linker, forcing the two aromatic rings to stay within an appropriate distance.[2,20] To overcome the problem of the isomerization of the active *cis* double bond into an inactive form, heterocyclic rings were used in place of the ethene bridge.[19,21,22]

We report here, the synthesis and biological activities of a series of CA4 analogues with a five-membered heterocycle as linker of the two aromatic rings of CA4: isoxazole and other heterocyclic moieties with adjoined nitrogen and oxygen atoms (Fig. 1). We intended to

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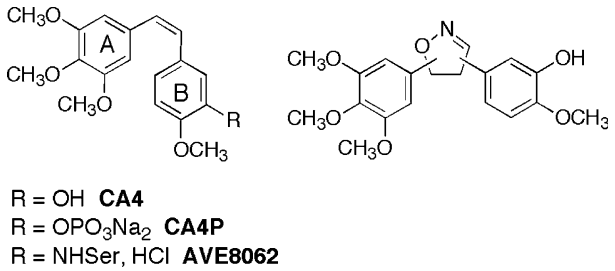


Figure 1.

investigate the influence of the position of the heteroatoms of the bridge ring, with regard to A- and B-aromatic rings, on the antitubulin and cytotoxicity activities of these analogues.

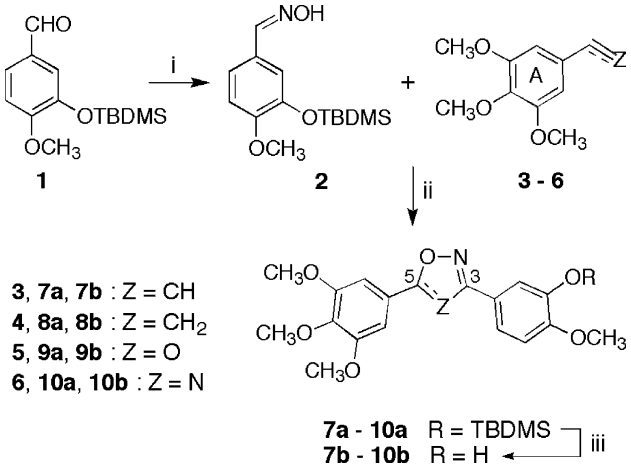
2. Results and discussion

2.1. Chemistry

We have previously communicated the synthesis of novel isoxazole-type derivatives related to CA4, using 1,3-dipolar cycloaddition reaction.[23] Recently, using the same approach, Simoni et al.[22] described the preparation of closely related heterocyclic analogues of CA4. These results prompted us to publish our additional result in this series.

As we have reported, the nitrile oxide generated in situ from aldoxime **2** following Lee procedure[24] underwent a [3 + 2] regioselective cycloaddition (Scheme 1) with 3,4,5-trimethoxyphenylacetylene **3**[25] to give 3,5-diarylisoxazole **7a** (38% yield). In the same manner, oxime **2** was allowed to react with the known olefine **4**,[26] the commercially available aldehyde **5** and nitrile **6** to give isoxazoline **8a**, dioxazole **9a** and oxadiazole **10a** in 62%, 10% and 6% yields, respectively.

In order to obtain more hydrophilic derivatives, coupling reactions have also been performed with olefines bearing a carboxylic or an amido group (Scheme 2). With 1,2-disubstituted alkenes as dipolarophiles[27] cycloaddition took place with reduced regioselectivity, leading to a mixture of the two possible regioisomeric δ^2-isoxazolines. Thus, *trans*-ethyl 3,4,5-trimethoxycinnamate **11** afforded the 3,5- and 3,4-diarylcycloadducts **13a** and **15a** in an 85:15 ratio. With the bulky cinnamide **12**, cycloadducts **14a** and **16a** were formed in almost equal amounts.[28]



Scheme 1. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, pyridine, EtOH, reflux, 1 h; (ii) aq NaOCl, Et_3N, CH_2Cl_2, 0 °C, then rt 24 h (additional reflux 24 h for **5** and **6**); (iii) TBAF, THF, rt, 2 h.

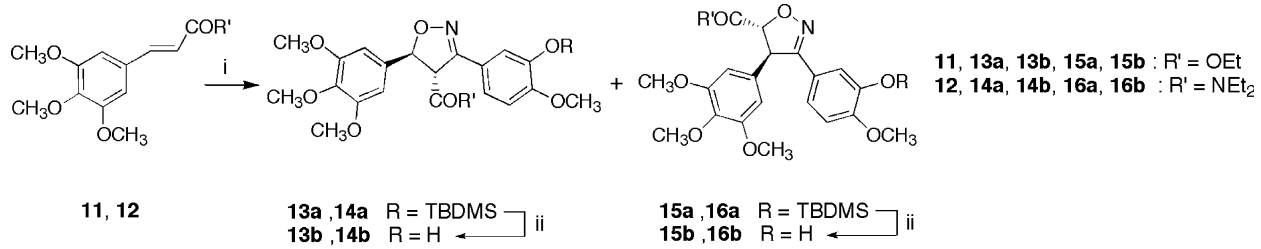
We next investigated the reversed cycloadditions, which were performed by reaction of the 3,4,5-trimethoxybenzaldehyde oxime[30] **17** with dipolarophiles providing the aromatic B-ring (Scheme 3). Reaction with the protected isovanillin **1** afforded the 3,5-diaryldioxazole **18a** (10% yield). With arylacetylene **19**, prepared[31,32] from aldehyde **1** by a Corey–Fuchs reaction, 3,5-diarylisoxazole **20a** was obtained in 53% yield. With cinnamide **23** (synthesized from the acid **21** by a carbodiimide coupling with Et_2NH) a mixture of two regioisomeric adducts was obtained in modest yield (25%) in a 4:6 ratio determinated by ^1H NMR, but only the major isomer, 3,4-diarylisoxazoline **24a**, could be isolated in a pure form.

Deprotection of the phenol function of the cycloadducts **7a**–**10a**, **13a**–**16a**, **18a**, **20a** and **24a**, carried out using TBAF (yields ranging from 80% to 95%), gave eleven new heterocyclic CA4 analogues (corresponding derivatives **b** in Schemes 1–3).[33]

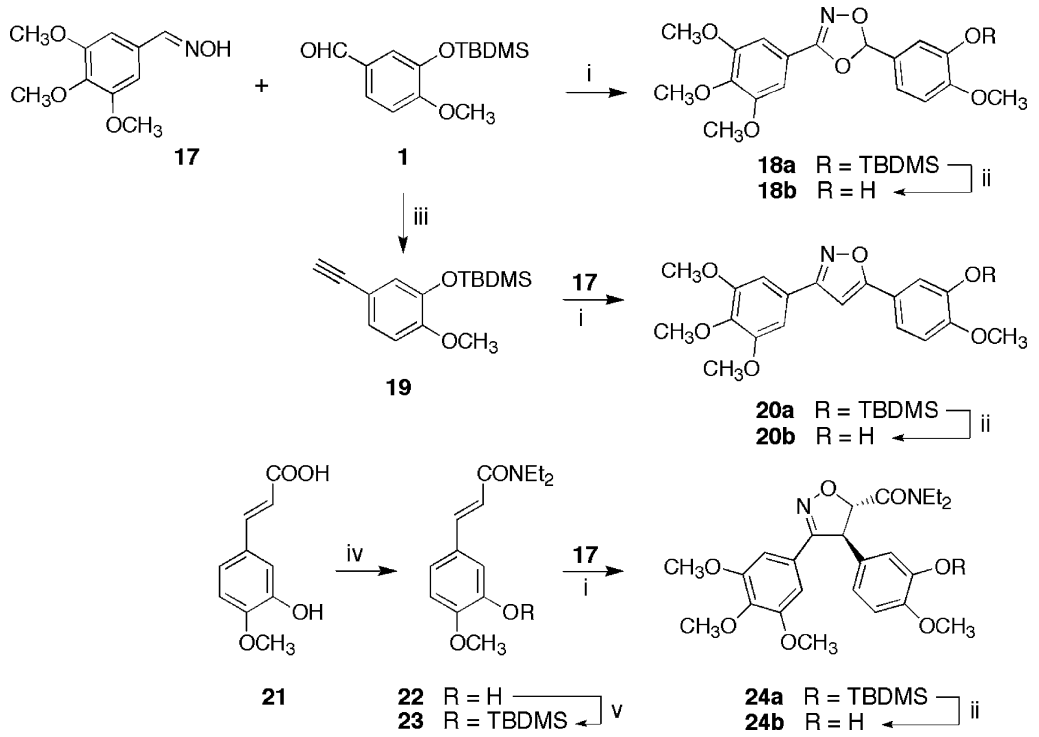
Next, we turned to the synthesis of 2,3-diarylisoxazolidines including a nitrogen at a bridgehead, which can be prepared through the 1,3-dipolar cycloaddition reactions of nitrones with ethylenic dipolarophiles.[34] So we have undertaken the reaction of methylacrylate with the *C*,*N*-diarylnitrone **26** (Scheme 4), which was easily obtained by one-pot preparation from nitroaryl **25** and isovanillin under zinc-mediated reductive conditions.[35] Condensation of methylacrylate with nitrone **26** was carried out in toluene under reflux conditions for 18 h. Along with nitrone which was mostly recovered unchanged, a less polar derivative was isolated, albeit in very low yield. On the basis of ^1H NMR its structure was assigned to be a *cis*-cycloadduct ($J_{3'4'} = 8.9$ Hz),[36,37] the isoxazolidine **27** bearing the ester group in position 4.

Our following aim was to obtain CA4 analogues having the olefinic bond incorporated into the heterocyclic ring (Fig. 2). We first attempted 1,3-dipolar cycloaddition with the known diarylacetylenic derivative **28** as dipolarophile (phenolic function protected by a TBDMS group).[25] However, compound **28** failed to react with nitrile oxide **A** generated, either from nitroalkane **29** under Mukaiyama conditions (phenylisocyanide and Et_3N),[38] or prepared in situ (Et_3N) from chloroaldoxime **30**,[39] and even in the ionic liquid [BMIM][BF_4].[40]

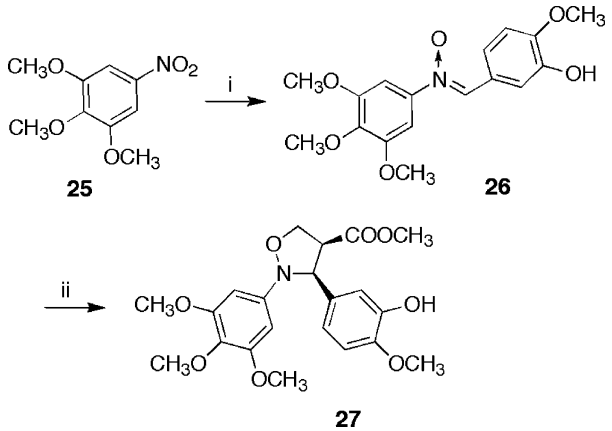
Thus, we investigated an alternative strategy based on the expected regioselective addition of hydroxylamine on a β-ketoaldehyde species **B**.[41] Such derivatives could



Scheme 2. Reagents and conditions: (i) aq NaOCl, Et_3N, CH_2Cl_2, 0 °C, then rt, 24 h; (ii) TBAF, THF, rt, 2 h.



Scheme 3. Reagents and conditions: (i) aq NaOCl, Et_3N, CH_2Cl_2, 0 °C, rt, 24 h (additional reflux 24 h for **1**; (ii) TBAF, THF, rt, 2 h; (iii) a—Ref. 31: CBr_4, PPh_3, CH_2Cl_2, 0 °C, 10 min; b—Ref. 32: *n*-BuLi (2 equiv.), THF, −78 °C to rt; (iv) EDCI, HOBT, Et_2NH, DMF, rt, 3 h; (v) TBDMSCl, *i*-Pr_2EtN, THF, rt, 24 h.



Scheme 4. Reagents and conditions: (i) isovanillin, Zn, AcOH, EtOH, 0 °C, 2 h then rt, 2 h; (ii) CH_2=CHCOOCH$_3$, toluene, reflux, 17 h.

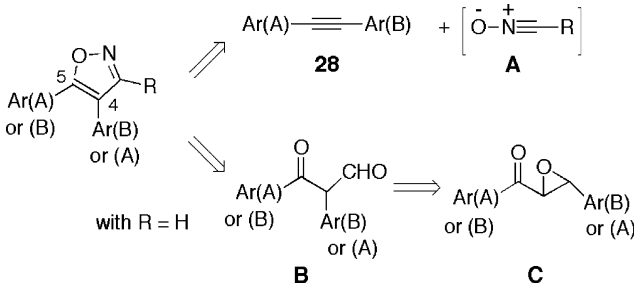
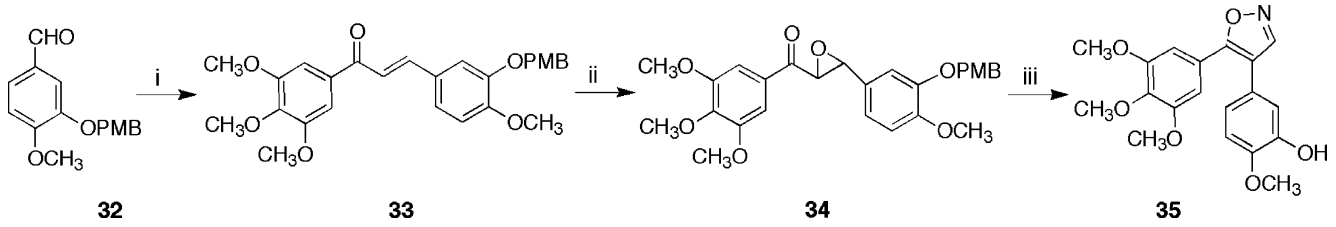


Figure 2. Considered pathways of access to 4,5-diarylisoxazoles. Nitrile oxides A were generated from $O_2NCH_2CH_2CO_2Et$ **29** or HONCH(Cl)CO_2Et **30**.

result from the Lewis acid-promoted rearrangement (pinacol–pinacone type)[42,43] of ketoepoxides **C**, which could be easily obtained from chalcones. Thus, (Scheme 5) base-catalyzed Claisen–Schmidt condensation between 3,4,5-trimethoxyacetophenone **31** and the O-protected isovanillin **32**,[44] afforded the chalcone **33** in good yield. Its treatment with hydrogen peroxide in alkaline conditions at 0 °C, according to Jain and Krishnamurty[45] provided the ketoepoxide derivative **34** in



32 33 34 35

Scheme 5. Reagents and conditions: (i) **31**, 6 N NaOH, EtOH, 60 °C, 5 h; (ii) H_2O_2, 2 N NaOH, EtOH, 0 °C to rt, 4 h; (iii) a—$BF_3{\cdot}Et_2O$, THF, reflux, 45 min; b—$NH_2OH{\cdot}HCl$, pyridine, EtOH, reflux, 6 h.

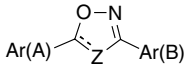
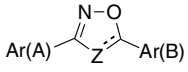
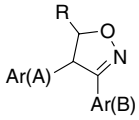
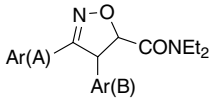
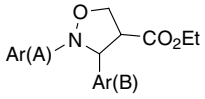
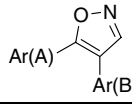
83% yield. Epoxide **34** was next stirred with an excess of $BF_3{\cdot}Et_2O$ in THF at 40 °C for 45 min and, after usual workup, the crude mixture was submitted to oximation with hydroxylamine hydrochloride.[42] The expected 4,5-diarylisoxazole **35**, with the phenolic group free, was isolated in 32% yield from a mixture of other uncharacterized products. The structure of **35** was assigned on the basis of the ^1H NMR chemical shift value (8.29 ppm) for the heterocyclic proton.[21b,46] The modest yield obtained for this two-step reaction could result from the lack of regioselectivity during the rearrangement process (hydride, aryl or acyl migration)[43] and the lack of chemoselectivity in the oximation step (aldehyde > ketone).[41]

2.2. Biological evaluation

Our main objective was to investigate the importance of (a) the nature and (b) the substitution pattern of the heterocyclic ring for the antitubulin and the antiproliferative activities of these new CA4 analogues (Table 1). These derivatives could be classified in two series, depending on the relative position of the aromatic A- and B-rings on the heterocyclic moiety (α,γ or α,β).

2.2.1. Inhibition of tubulin polymerization (ITP). In the first series, the 3,5-diaryldioxazole **9b** displayed an interesting inhibition of tubulin polymerization (IC_{50} = 5.0 μM), nevertheless 4-fold lower than that of

Table 1. Inhibition of tubulin polymerization (ITP) and cellular proliferation for heterocyclic analogues of CA4

	Compound	ITP[a] IC_{50} (μM)	HT29[b] IC_{50} (μM)	SVEC[b] IC_{50} (μM)
	**7b** Z = CH	>25	78 ± 20	23.5 ± 10
	8b Z = CH_2	>25	ND[c]	ND
	9b Z = O	5.0	>50	>50
	10b Z = N	>25	>50	>50
	13b Z = $CHCO_2Et$	>25	NE[d]	40
	14b Z = $CHCONEt_2$	>25	NE	NE
	**18b** Z = O	>25	>50	>50
	20b Z = CH	>25	>50	49
	**15b** R = CO_2Et	>25	NE	ND
	16b R = $CONEt_2$	>25	NE	NE
	**24b**	>25	7.1 ± 1.4	9.5 ± 0.5
	**27**	>25	NE	39
	**35**	0.75	3	8.5
	CA4	1.2	0.47 ± 0.03	3 ± 0.1

[a] Drug concentration needed to inhibit tubulin polymerization by 50%. The compounds were assayed at least twice and the reported IC_{50} values were the averages.

[b] Drug concentration needed to inhibit cell growth by 50% ± SD. Given values are the mean of two independent experiments in triplicate, with CA4 as positive control.

[c] ND: not determined.

[d] NE: no effect was shown at the highest concentration (50 μM) tested.

CA4 (IC_{50} = 1.2 μM). Switching the position of the two aryl groups (**18b** vs **9b**) resulted in a dramatic loss in inhibition potency. The other derivatives, with at the 4-position a methine (**7b**, **20b**), a methylene (**8b**), a nitrogen atom (**10b**) or an exocyclic carboxylate or amido group (**13b**, **14b**), were ineffective in the tubulin assay (IC_{50} values > 25 μM). From these data it appeared that in this series of α,γ-diaryl heterocyclic analogues, the substitution pattern on the heterocyclic moiety played an important role in the antitubulin activity: a CH group in the benzylic position of the 3,4,5-trimethoxyphenyl ring and a sp^2 carbon adjacent to the B-ring seemed necessary. Oxygen atom at the 4-position also had a great influence, since the isoxazoline analogue **8b**, with a CH_2 group replacing the heteroatom, did not interact with the tubulin; These three structural requirements (Fig. 3) were present in the two potent analogues developed at Abbott Laboratories, the oxadiazoline (A105972)[47] and the oxazoline (A289099).[48]

For the α,β-diaryl heterocyclic analogues, the 4,5-diarylisoxazole **35** exhibited a stronger activity (IC_{50} = 0.75 μM), almost twice more potent than that of CA4. The other analogues, the isoxazoline derivatives **15b**, **16b** and **24b** and the 2,3-diarylisoxazolidine **27**, were essentially inactive. Numerous CA4 analogues with an ethylene bridge as part of a five-membered heterocyclic ring have been reported in the literature.[19,21b] Among them, imidazole **36** and oxazole **37**[49] displayed, as isoxazole **35**, a higher antitubulin activity than that of CA4 (Fig. 4).

2.2.2. Antiproliferative activity. Compounds were evaluated for their antiproliferative activity against human colon adenocarcinoma cell line HT29 and transformed murine endothelial cell line SVEC 4–10, using an MTT assay.[50,51] The most potent tubulin inhibitor **35** showed some antiproliferative activities on HT29 and SVEC cells, nevertheless 6- and 3-fold lower than that of CA4, respectively. For its part, the other potent tubulin inhibitor, dioxazole **9b**, was devoid of cytotoxic effects. Proliferation of HT29 cells was not inhibited by any of the other derivatives, except the carboxamide group-bearing isoxazoline **24b**, which showed a weak cytotoxicity (7.1 μM, i.e., 15-fold lower than CA4). Moreover, against SVEC cells, **24b** was equipotent to the powerful antitubulin inhibitor **35** and, surprisingly, the isomeric isoxazoline **16b** was inactive. It is noteworthy that the four heterocyclic analogues, **7b**, **13b**, **20b** and **27**, which were ineffective in the tubulin assay and in the proliferation test on H29 cell line, displayed weak cytotoxicity (8- to 16-fold less than that of CA4) on the SVEC cell line. This suggests that these derivatives may have some specificity for the endothelial cells and another target than tubulin.

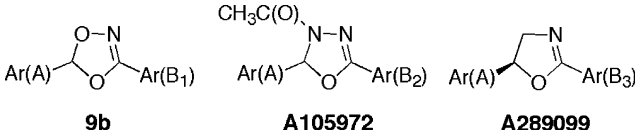


Figure 3. A = 3,4,5-trimethoxyphenyl; B_1 = 4-hydroxy-3-methoxyphenyl; B_2 = 4-amino-3-methylphenyl; B_3 = 5-(*N*-methyl)indolyl.

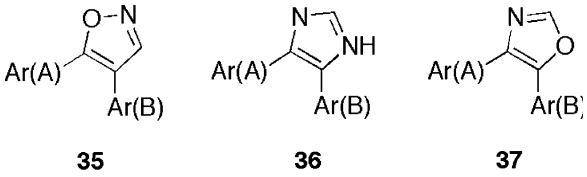


Figure 4. A = 3,4,5-trimethoxyphenyl; B = 4-hydroxy-3-methoxyphenyl.

3. Conclusion

By using 1,3-dipolar cycloaddition reaction a series of twelve CA4 analogues had been synthesized (6–62% yields). The olefinic bond was replaced by five-membered heterocyclic rings: isoxazole or other moieties with adjoined nitrogen and oxygen atoms. On the other hand, the isoxazole-based CA4 derivative **35** had been obtained by a concise synthesis starting from an easily accessible chalcone. All these compounds were evaluated for their antitubulin and antiproliferative activities. Among the eight derivatives with A- and B-rings attached to an heterocyclic moiety in an α,γ-relationship, only the 3,5-diaryloxadiazole **9b** showed an antitubulin activity comparable to that of CA4, but was nevertheless devoid of cytotoxicity. Of the five α,β-diaryl heterocyclic derivatives, the isoxazole **35** displayed higher antitubulin activity than that of CA4. With this constrained derivative, the liability of CA4 to isomerize to an inactive form is prevented. So, it seems appropriate to undertake the synthesis of a series of such isoxazole derivatives in which the natural B-ring would be replaced by other rings, for example heteroaromatic moieties, to increase the solubility.

These results demonstrated that minor alteration in the chemical structure of the heterocyclic ring and its relative orientation with regard to the two phenyl rings of CA4 could dramatically influence the tubulin binding properties.

4. Experimental

4.1. General methods

NMR spectra were recorded on a Bruker AM-300 spectrometer at 300 MHz (^1H) and at 75 MHz (^{13}C) using $CDCl_3$ as the solvent. The residual proton-solvent peak is used as the internal standard [(δ 7.25 (^1H) and 77.0 ppm (^{13}C)] and *J* values are given in hertz. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublet; br, broad; and m, multiplet. Chemical ionization (CI) mass spectra were recorded on a Nermag R10-10-C spectrometer. High-resolution mass spectra (HRMS) were obtained on a Jeol-700 spectrometer. Melting points were determined on an Electrothermal 9200 apparatus and are uncorrected. Elemental analyses were performed by the 'Service de Microanalyses du CNRS' (Vernaison-Lyon, France). The thin-layer chromatographic analyses were performed using pre-coated silica gel (Merck, $60F_{254}$) plates and the spots were examined with UV

light and phosphomolybdic acid spray. Preparative column chromatographies were carried out on Merck silica gel (230–240 mesh). THF was distilled from sodium benzophenone ketyl prior to use. Derivatives **1**,[52] **3**,[25] **4**,[26] **17**,[30] **19**,[31,32] and **20b**,[22] were prepared according to published procedures.

Numbering for ^{1}H NMR assignments is usual for the A-ring, ′ for B-ring and ″ for the heterocyclic ring.

4.2. 3-(*tert*-Butyldimethylsilanyloxy)-4-methoxybenzaldehyde oxime (2)

To a solution of silylated isovanillin **1** (16.7 g, 62.9 mmol) in methanol (300 mL) were added, under argon, hydroxylamine hydrochloride (4.4 g, 63.3 mmol) and then pyridine (5.6 mL, 69.1 mmol). After refluxing for 1 h, the reaction mixture was concentrated to 50 mL, diluted with water (300 mL) and extracted with EtOAc. Combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residual yellow oil was washed with pentane to give oxime **2** (15.54 g, 88%) as a thick oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (s, 1H, CH), 7.14 (d, 1H, J = 2.0 Hz, H-2), 7.08 (dd, 1H, J = 8.3 Hz, J = 2.0 Hz, H-6), 6.83 (d, 1H, J = 8.3 Hz, H-5), 3.83 (s, 3H, OCH_3), 0.99 [s, 9H, $C(CH_3)_3$], 0.15 [s, 6H, $Si(CH_3)_2$].

4.3. *N*,*N*-Diethyl-3-(3-hydroxy-4-methoxyphenyl)-acrylamide (22)

To a solution of 3-hydroxy-4-methoxycinnamic acid **21** (2.5 g, 12.9 mmol) in dry DMF (25 mL) were added diethylamine (1.4 mL, 13.4 mmol), hydroxybenzotriazole (2.1 g, 15.5 mmol) and EDCI (3.0 g, 15.6 mmol). After stirring at room temperature for 3 h, the yellow mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc, the combined organic layers were washed with brine, dried ($MgSO_4$), filtered, concentrated under reduced pressure and chromatographed (CH_2Cl_2/MeOH 97:3) to give amide **22** as a white solid, which was recrystallized from EtOAc (2.10 g, 65%). Mp 112–114 °C; ^{1}H NMR(300 MHz, $CDCl_3$): δ 7.63 (d, 1H, J = 15.3 Hz, ArCH), 7.19 (d, 1H, J = 2.0 Hz, H-2), 6.99 (dd, 1H, J = 8.3 Hz, J = 2.0 Hz, H-6), 6.81 (d, 1H, J = 8.3 Hz, H-5), 6.67 (d, 1H, J = 15.3 Hz, CHCO), 6.31 (br s, 1H, OH), 3.89 (s, 3H, OCH_3), 3.45 (m, 4H, 2× CH_2), 1.23 (t, 3H, J = 7.1 Hz, CH_3), 1.17 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0 (CO), 148.1 (C), 145.9 (C), 142.3 (CH), 128.9 (C), 121.3 (CH), 115.6 (CH), 112.9 (CH), 110.6 (CH), 55.9 (CH_3), 42.3 (CH_2), 41.1 (CH_2), 15.0 (CH_3), 13.2 (CH_3). Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.68; N, 5.62. Found C: 67.13; H, 7.89; N, 5.51.

4.4. 3-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-*N*,*N*-diethyl-acrylamide (23)

Diisopropylethylamine (1.2 mL, 6.87 mmol) was added, under argon, to a stirred solution of phenol **22** (914 mg, 3.67 mmol) in dry THF followed by *tert*-butyldimethylsilyl chloride (773 mg, 5.13 mmol). After stirring at room temperature for 24 h, water was added (100mL). The aqueous layer was extracted with EtOAc, the combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Following flash chromatography (CH_2Cl_2/MeOH 97:3), silylated compound **23** was isolated as a white crystalline solid (2.14 g, 65%). Mp 90–92 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 1H, J = 15.3 Hz, ArCH), 7.10 (dd, 1H, J = 8.3 Hz, J = 2.1 Hz, H-6), 7.02 (d, 1H, J = 2.1 Hz, H-2), 6.82 (d, 1H, J = 8.3 Hz, H-5), 6.65 (d, 1H, J = 15.3 Hz, CHCO), 3.83 (s, 3H, OCH_3), 3.45 (m, 4H, 2× CH_2), 1.25 (t, 3H, J = 7.1 Hz, CH_3), 1.18 (t, 3H, J = 7.1 Hz, CH_3), 0.99 [s, 9H, $C(CH_3)_3$], 0.15 [s, 6H, $Si(CH_3)_2$].

4.5. General procedure for the cycloaddition reactions

To dipolarophile **3**, **4**, **5**, **6**, **11**, **12**, **1**, **19** or **23** (1 equiv) and triethylamine (0.1 equiv) in dichloromethane were added, under argon atmosphere, a 13% aqueous solution of NaOCl (1.6 equiv) and dropwise (over a period of 1 h) at 0 °C, the appropriate oxime **2** or **17** (1 equiv) in dichloromethane. After being stirred at room temperature for 24 h (an additional reflux period was necessary for less reactive dipolarophiles **5**, **6** and **1**), water was added to the reaction mixture and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was chromatographed as indicated.

4.5.1. 3-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)isoxazole (7a). From alkyne **3** (271 mg, 1.41 mmol) and oxime **2** (398 mg, 1.42 mmol), isoxazole **7a** was obtained as a yellow oil (251 mg, 38% yield) after a flash chromatography (cyclohexane/EtOAc 9:1–8:2). ^{1}H NMR data are in agreement with those described by Simoni et al.[22]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9 (C-Het), 162.7 (C-Het), 153.6 (2× C), 152.6 (C), 145.3 (C), 139.8 (C), 123.0 (C), 121.8 (C), 120.6 (CH), 119.3 (CH), 111.9 (CH), 103.1 (2× CH), 97.0 (CH-Het), 61.0 (CH_3), 56.3 (2× CH_3), 55.4 (CH_3), 25.7 (3× CH_3), 18.5 (C), −4.6 (2× CH_3).

4.5.2. 3-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (8a). From alkene **4** (1.56 g, 8.0 mmol) and oxime **2** (2.24 g, 8.0 mmol), dihydroisoxazole **8a** was obtained as a yellow oil (2.34 g, 62% yield) after a flash chromatography (cyclohexane/EtOAc 8:2). ^{1}H NMR data are in agreement with those described by Simoni et al.[22]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.8 (C-Het), 153.5 (2× C), 152.8 (C), 145.1 (C), 137.7 (C), 136.7 (C), 122.1 (C), 120.9 (CH), 119.0 (CH), 111.5 (CH), 102.6 (2× CH), 82.4 (CH-Het), 60.8 (CH_3), 56.1 (2× CH_3), 55.4 (CH_3), 43.6 (CH_2-Het), 25.6 (3× CH_3), 18.4 (C), −4.6 (2× CH_3).

4.5.3. 3-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)-1,2,4-dioxazole (9a). From trimethoxybenzaldehyde **5** (985 mg, 5.02 mmol) and oxime **2** (1.40 g, 4.98 mmol), dioxazole **9a** was obtained as a colourless oil (235 mg, 10% yield) after a flash chromatography (cyclohexane/EtOAc 9:1–8:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, H-6′),

7.33 (d, 1H, J = 2.1 Hz, H-2′), 6.88 (d, 1H, J = 8.4 Hz, H-5′), 6.82 (s, 2H, H-2, H-6), 6.73 (s, 1H, H-5″), 3.89 (s, 6H, 2× OCH_3), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 0.99 [s, 9H, $C(CH_3)_3$], 0.16 [s, 6H, $Si(CH_3)_2$].

4.5.4. 3-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (10a). From trimethoxybenzonitrile **6** (970 mg, 5.02 mmol) and oxime **2** (1.59 g, 5.04 mmol), oxadiazole **10a** was obtained as an oil (142 mg, 6% yield) after a flash chromatography (cyclohexane/EtOAc 9:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (dd, 1H, J = 8.5 Hz, J = 2.1 Hz, H-6′), 7.63 (d, 1H, J = 2.1 Hz, H-2′), 7,44 (s, 2H, H-2, H-6), 6.95 (d, 1H, J = 8.5 Hz, H-5′), 4.00 (s, 6H, 2× OCH_3), 3.94 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 1.03 [s, 9H, $C(CH_3)_3$], 0.20 [s, 6H, $(SiCH_3)_3$].

4.5.5. *trans*-Ethyl-3-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-4-carboxylate (13a). *trans*-Ethyl-3-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-4-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxylate (15a). From ethyl-3,4,5-trimethoxycinnamate **11** (2.08 g, 7.80 mmol) and oxime **2** (2.11 g, 7.50 mmol) a mixture of dihydroisoxazoles **13a** and **15a** (2.57 g, 63% yield, ratio 85:15 determined by ^{1}H NMR) was obtained after a flash chromatography (cyclohexane/EtOAc 8:2). They could be separated after another tedious chromatography (CH_2Cl_2/acetone 99:1) to give **13a** (1.99 g, 49%) and **15a** (139 mg, 3%) as white foams. Cycloadduct **13a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (d, 1H, J = 2.1 Hz, H-2′), 7.23 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, H-6′), 6.83 (d, 1H, J = 8.4 Hz, H-5′), 6.58 (s, 2H, H-2, H-6), 5.86 (d, 1H, J = 6.7 Hz, H-5″), 4.37 (d, 1H, J = 6.7 Hz, H-4″), 4.23 (q, 2H, J = 7.1 Hz, CH_2), 3.86 (s, 6H, 2× OCH_3), 3.83 (s, 6H, 2× OCH_3), 1.22 (t, 3H, J = 7.1 Hz, CH_3), 0.99 [s, 9H, $C(CH_3)_3$], 0.16 [s, 6H, $Si(CH_3)_2$]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.3 (CO), 153.6 (2× C), 155.3 (C-Het), 152.9 (C), 145.1 (C), 138.1 (C), 135.3 (C), 121.1 (CH), 121.0 (C), 119.3 (CH), 111.5 (CH), 102.2 (2× CH), 86.7 (CH-Het), 62.3 (CH-Het), 62.1 (CH_2), 60.8 (CH_3), 56.1 (2× CH_3), 55.3 (CH_3), 25.6 (3× CH_3), 18.4 (C), 14.0 (CH_3), −4.7 (2× CH_3). Cycloadduct **15a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (dd, 1H, J = 8.5 Hz, J = 2.1 Hz, H-6′), 7.07 (d, 1H, J = 2.1 Hz, H-2′), 6.76 (d, 1H, J = 8.5 Hz, H-5′), 6.44 (s, 2H, H-2, H-6), 4.89 (m, 2H, H-4″, H-5″), 4.29 (m, 2H, CH_2), 3.80 (s, 9H, 3× OCH_3), 3.78 (s, 3H, OCH_3), 1.33 (t, 3H, J = 7.1 Hz, CH_3), 0.92 [s, 9H, $C(CH_3)_3$], 0.06 (s, 3H, $SiCH_3$), 0.02 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1 (CO), 157.3 (C-Het), 153.9 (2× C), 152.8 (C), 144.7 (C), 137.6 (C), 133.7 (C), 121.7 (CH), 120.4 (C), 120.0 (CH), 111.6 (CH), 104.2 (2× CH), 86.2 (CH-Het), 62.1 (CH_2), 60.8 (CH_3), 58.7 (CH-Het), 56.1 (2× CH_3), 55.4 (CH_3), 25.6 (3× CH_3), 18.4 (C), 14.1 (CH_3), −4.8 (2× CH_3).

4.5.6. *trans*-*N*,*N*-Diethyl-3-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-4-carboxamide (14a). *trans*-*N*,*N*-Diethyl-3-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-4-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide (16a). From *N*,*N*-diethylcinnamide **12** (587 mg, 2.0 mmol) and oxime **2** (562 mg, 2.0 mmol) a mixture of dihydroisoxazoles **14a** and **16a** (660 mg, 58% yield, ratio 45:55 determined by ^{1}H NMR) was obtained after a flash chromatography (cyclohexane/EtOAc 6:4–4:6). They could be separated after another two tedious chromatographies (cyclohexane/EtOAc 7:3) to give **14a** (312 mg, 27%) and **16a** (251 mg, 22%) as white foams. Cycloadduct **14a**: TLC (cyclohexane/ EtOAc 3:7) R_f 0.57; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 (d, 1H, J = 2.1 Hz, H-2′), 7.13 dd, 1H, J = 8.3 Hz, J = 2.1 Hz, H-6′), 6.81 (d, 1H, J = 8.3 Hz, H-5′), 6.62 (s, 2H, H-2, H-6), 5.65 (d, 1H, J = 9.9 Hz, H-5″), 4.59 (d, 1H, J = 9.9 Hz, H-4″), 3.84 (s, 9H, 3× OCH_3), 3.81 (s, 3H, OCH_3), 3.41 (q, 2H, J = 7.1 Hz, CH_2), 3.19 (m, 2H, CH_2), 1.25 (t, 3H, J = 7.1 Hz, CH_3), 1.15 (t, 3H, J = 7.1 Hz, CH_3), 0.99 [s, 9H, $C(CH_3)_3$], 0.14 [s, 6H, $Si(CH_3)_2$]. Cycloadduct **16a**: TLC (cyclohexane/EtOAc 3:7) R_f 0.51; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (dd, 1H, J = 8.5 Hz, J = 2.1 Hz, H-6′), 7.07 (d, 1H, J = 2.1 Hz, H-2′), 6.75 (d, 1H, J = 8.5 Hz, H-5′), 6.46 (s, 2H, H-2, H-6), 5.54 (d, 1H, J = 5.7 Hz, H-4″), 5.05 (d, 1H, J = 5.7 Hz, H-5″), 3.79 (s, 9H, 3× OCH_3), 3.77 (s, 3H, OCH_3), 3.45 (m, 4H, 2× CH_2), 1.27 (t, 3H, J = 7.0 Hz, CH_3), 1.25 (t, 3H, J = 7.0 Hz, CH_3); 0.92 [s, 9H, $C(CH_3)_3$], 0.05 (s, 3H, $SiCH_3$), 0.02 (s, 6H, $SiCH_3$).

4.5.7. 5-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-3-(3,4,5-trimethoxyphenyl)-1,2,4-dioxazole (18a). From silylated isovanillin **1** (2.66 g, 10.00 mmol) and oxime **17** (2.11 g, 10.0 mmol), dioxazole **18a** was obtained as a white foam (472 mg, 10% yield) after a flash chromatography (cyclohexane/EtOAc 85:15). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.16 (dd, 1H, J = 8.3 Hz, J = 2.1 Hz, H-6′), 7.08 (d, 1H, J = 2.1 Hz, H-2′), 7.07 (s, 2H, H-2, H-6), 6.90 (d, 1H, J = 8.3 Hz, H-5′), 6.74 (s, 1H, H-5″), 3.89 (s, 3H, OCH_3), 3.88 (s, 6H, 2× OCH_3), 3.84 (s, 3H, OCH_3), 1.00 [s, 9H, $C(CH_3)_3$], 0.16 [s, 6H, $Si(CH_3)_2$]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4 (C-Het), 153.3 (2× C), 152.3 (C), 145.3 (C), 140.8 (C), 126.8 (C), 120.8 (C), 119.4 (CH), 118.0 (C), 111.7 (CH), 108.9 (CH-Het), 104.1 (2× CH), 61.0 (CH_3), 56.3 (2× CH_3), 55.5 (CH_3), 25.7 (3× CH_3), −4.6 (2× CH_3).

4.5.8. 5-[3-(*tert*-Butyldimethylsilanyloxy)-4-methoxyphenyl]-3-(3,4,5-trimethoxyphenyl) isoxazole (20a). From alkyne **19** (346 mg, 1.32 mmol) and oxime **17** (217 mg, 1.00 mmol), isoxazole **20a** was obtained as a yellow oil (258 mg, 53% yield) after a flash chromatography (cyclohexane/EtOAc 8:2). Data are in agreement with those described by Simoni et al.[22]

4.5.9. *trans*-*N*,*N*-Diethyl-4-[3-(*tert*-butyldimethylsilanyloxy)-4-methoxyphenyl]-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide (24a). From *N*,*N*-diethylcinnamide **23** (1.52 g, 4.20 mmol) and oxime **17** (0.88 g, 4.20 mmol) and after three tedious chromatographies (cyclohexane/EtOAc 8:2), the cycloadduct **24a** could be isolated (143 mg, 6% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.86 dd, 1H, J = 8.3 Hz, J = 2.1 Hz, H-6′), 6.83 (s, 2H, H-2, H-6), 6.79 (d, 1H, J = 8.3 Hz, H-5′), 6.74 (d, 1H, J = 2.1 Hz, H-2′), 5.52 (d, 1H, J = 6.2 Hz, H-4″), 5.07 (d, 1H, J = 6.2 Hz, H-

5″), 3.80 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.72 (s, 3H, 2× OCH_3) 3.44 (m, 4H, 2× CH_2), 1.22 (t, 3H, J = 7.1 Hz, CH_3), 1.16 (t, 3H, J = 7.1 Hz, CH_3), 0.92 [s, 9H, $C(CH_3)_3$], 0.04 (s, 3H, $SiCH_3$), 0.03 (s, 6H, $SiCH_3$).

4.6. General procedure for removal of the TBDMS-protecting group

A solution of the appropriate silyl ether **7a**-**10a**, **13a**-**16a**, **18a**, **20a** or **24a** in dry THF (1 equiv) was treated with a 1 M solution of tetrabutylammonium fluoride in THF (1 equiv). The mixture was stirred at room temperature during 2 h, then diluted with water and extracted with EtOAc. The organic layers were washed with water, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was chromatographed as indicated.

4.6.1. 3-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)isoxazole (7b). Isoxazole **7b** was obtained from protected compound **7a** (453 mg, 0.96 mmol) after a flash chromatography (cyclohexane/EtOAc 6:4–3:7) as a white crystalline solid (302 mg, 88%). ^{1}H NMR data are in agreement with those described by Simoni et al.[22]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9 (C-Het), 162.6 (C-Het), 153.6 (2× C), 148.0 (C), 145.9 (C), 139.7 (C), 122.9 (C), 122.3 (C), 119.0 (CH), 113.0 (CH), 110.7 (CH), 103.1 (2× CH), 97.1 (CH-Het), 61.0 (CH_3), 56.3 (2× CH_3), 56.0 (CH_3); MS (DCI/NH_3) m/z 358 $(M+H)^+$; Anal. Calcd for $C_{19}H_{19}NO_6 \cdot 0.25\ H_2O$: C, 63.06; H, 5.43; N, 3.87. Found C: 63.32; H, 5.31; N, 3.97.

4.6.2. 3-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (8b). Dihydroisoxazole **8b** was obtained from protected compound **8a** (2.08 g, 4.39 mmol) after a flash chromatography (cyclohexane/EtOAc 5:5–3:7) as a white crystalline solid (1.40 g, 89%). ^{1}H NMR data are in agreement with those described by Simoni et al.[22]; ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9 (C-Het), 153.5 (2× C), 148.2 (C), 145.7 (C), 137.6 (C), 136.6 (C), 122.7 (C), 119.3 (CH), 112.8 (CH), 110.5 (CH), 102.6 (2× C), 82.4 (CH-Het), 60.8 (CH_3), 56.1 (2× CH_3), 56.0 (CH_3), 43.5 (CH_2-Het); MS (DCI/NH_3) m/z 360 $(M+H)^+$; Anal. Calcd for $C_{19}H_{21}NO_6 \cdot 0.5\ H_2O$: C, 61.95; H, 6.02; N, 3.80. Found C: 61.98; H, 5.91; N, 3.72.

4.6.3. 3-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-dioxazole (9b). Dioxazole **9b** was obtained from protected compound **9a** (310 mg, 0.65 mmol) after a flash chromatography (cyclohexane/EtOAc 6:4) as a white crystalline solid (195 mg, 83%). Mp 118–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (s, 1H, H-2′), 7.39 (d, 1H, J = 8.5 Hz, H-6′), 6.90 d, 1H, J = 8.5 Hz, H-5′), 6.82 (s, 2H, H-2, H-6), 5.76 (s, 1H, H-5″), 5.68 (s, 1H, OH), 3.95 (s, 3H, OCH_3), 3.89 (s, 6H, 2× OCH_3), 3.87 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2 (C-Het), 153.4 (2× C), 149.4 (C), 145.7 (C), 139.6 (C), 130.1 (C), 119.9 (CH), 115.7 (C), 113.1 (CH), 110.4 (CH), 108.4 (CH-Het), 103.7 (2× CH), 60.8 (CH_3), 56.2 (2× CH_3), 56.0 (CH_3); MS (DCI/NH_3) m/z 362 $(M+H)^+$; Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88. Found C: 59.99; H, 5.33; N, 3.88.

4.6.4. 3-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (10b). Oxadiazole **10b** was obtained from protected compound **10a** (123 mg, 0.26 mmol) after a flash chromatography (cyclohexane/EtOAc 62:38) as a white crystalline solid (65 mg, 70%). Mp 154–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, 1H, J = 2.0 Hz, H-2′), 7.7 (dd, 1H, J = 8.3 Hz, J = 2.0 Hz, H-6′), 7.43 (s, 2H, H-2, H-6), 6.95 d, 1H, J = 8.3 Hz, H-5′), 5.76 (br s, 1H, OH), 3.94 (s, 3H, OCH_3), 3.96 (s, 6H, 2× OCH_3), 3.94 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.2 (C-Het), 168.6 (C-Het), 153.6 (2× C), 149.0 (C), 145.8 (C), 141.9 (C), 120.1 (C, CH), 119.4 (C), 113.6 (CH), 110.5 (CH), 105.3 (2× C), 61.0 (CH_3), 56.4 (2× CH_3), 56.0 (CH_3); HRMS (DCI/NH_3) m/z 359.1240 [$(M + H)^+$ calcd for $C_{19}H_{20}NO_6$: 359.1243].

4.6.5. *trans*-Ethyl-3-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-4-carboxylate (13b). Ester **13b** was obtained from protected compound **13a** (624 mg, 1.10 mmol) after a flash chromatography (cyclohexane/EtOAc 1:1) as a yellow crystalline solid (356 mg, 75%). Mp 111–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (d, 1H, J = 2.1 Hz, H-2′), 7.19 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, H-6′), 6.83 (d, 1H, J = 8.4 Hz, H-5′), 6.57 (s, 2H, H-2, H-6), 5.86 (d, 1H, J = 6.6 Hz, H-5″), 5.78 (br s, 1H, OH), 4.38 (d, 1H, J = 6.6 Hz, H-4″), 4.23 (m, 2H, CH_2CH_3), 3.92 (s, 3H, OCH_3), 3.84 (s, 6H, 2× OCH_3), 3.81 (s, 3H, OCH_3), 1.22 (t, 1H, J = 7.0 Hz, CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2 (CO), 153.6 (2× CH), 153.4 (CH-Het), 148.4 (C), 145.7 (C), 138.2 (C), 135.2 (C), 121.6 (C), 119.6 (CH), 113.0 (CH), 110.4 (CH), 102.2 (2× CH), 86.7 (CH-Het), 62.1 (CH-Het, CH_2), 60.7 (CH_3), 56.1 (2× CH_3), 55.9 (CH_3), 13.9 (CH_3); MS (DCI/NH_3) m/z 432 $(M+H)^+$; Anal. Calcd for $C_{22}H_{25}NO_8$: C, 61.25; H, 5.84; N, 3.25. Found C: 60.89; H, 5.91; N, 3.03.

4.6.6. *trans*-Ethyl-3-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxylate (15b). Ester **15b** was obtained from protected compound **15a** (139 mg, 0.25 mmol) after a flash chromatography (cyclohexane/EtOAc 1:1) as a white crystalline solid (113 mg, 95%). Mp 125–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (1H, J = 2.1 Hz, H-2′), 7.12 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, H-6′), 6.76 (d, 1H, J = 8.4 Hz, H-5′), 6.45 (s, 2H, H-2, H-6), 5.57 (s, 1H, OH), 4.90 (m, 2H, H-5″, H-4″), 4.29 (m, 2H, CH_2CH_3), 3.88 (s, 3H, OCH_3), 3.82 (s, 9H, 3× OCH_3), 1.33 (t, 3H, J = 7.1 Hz, CH_2CH_3), ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1 (CO), 153.9 (2× C), 157.5 (C-Het), 148.3 (C), 145.5 (C), 137.7 (C), 133.5 (C), 121.2 (C), 120.3 (CH), 113.5 (CH), 110.4 (CH), 104.2 (2× CH), 86.3 (CH-Het), 62.1 (CH_2), 60.8 (CH_3), 58.5 (CH-Het), 56.2 (2× CH_3), 55.9 (CH_3), 14.1 (CH_3); MS (DCI/NH_3) m/z 432 $(M+H)^+$; Anal. Calcd for $C_{22}H_{25}NO_8$: C, 61.25; H, 5.84; N, 3.25. Found C: 61.27; H, 5.84; N, 3.24.

4.6.7. *trans*-*N*,*N*-Diethyl-3-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-4-carboxamide (14b). Amide **14b** was obtained from protected compound **14a** (248 mg, 0.43 mmol) after a flash chromatography (CH_2Cl_2/MeOH 96:4) as a white crys-

talline solid (187 mg, 95%). Mp 144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 (d, 1H, J = 2.1 Hz, H-2′), 7.13 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, H-6′), 6.83 (d, 1H, J = 8.4 Hz, H-5′), 6.62 (s, 2H, H-2, H-6), 5.67 (d, 1H, J = 9.8 Hz, H-5″), 5.61 (s, 1H, OH), 4.62 (d, 1H, J = 9.8 Hz, H-4″), 3.91 (s, 3H, OCH_3), 3.85 (s, 9H, 3× OCH_3), 3.50–3.25 (m, 4H, 2× CH_2), 1.15 (t, 3H, J = 7.1 Hz, CH_3), 1.00 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.1 (CO), 155.6 (C-Het), 153.7 (2× C), 148.2 (C), 145.7 (C), 138.2 (C), 134.5 (C), 122.1 (C), 119.2 (CH), 112.8 (CH), 110.5 (CH), 103.0 (2× CH), 88.8 (CH-Het), 60.9 (CH_3), 60.3 (CH-Het), 56.1 (2× CH_3), 55.9 (CH_3), 42.4 (CH_2), 41.0 (CH_2), 14.6 (CH_3), 12.7 (CH_3); MS (DCI/NH_3) m/z 459 $(M+H)^+$; Anal. Calcd for $C_{24}H_{30}N_2O_7 \cdot 0.25\ H_2O$: C, 62.26; H, 6.64; N, 6.05. Found C: 62.30; H, 6.59; N, 6.13.

4.6.8. *trans*-*N*,*N*-Diethyl-3-(3-hydroxy-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide (16b). Amide **16b** was obtained from protected compound **16a** (320 mg, 0.56 mmol) after a flash chromatography (CH_2Cl_2/MeOH 97:3) as a white crystalline solid (218 mg, 85%). Mp 193–195 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, 1H, J = 2.1 Hz, H-2′), 7.09 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, H-6′), 6.74 (d, 1H, J = 8.4 Hz, H-5′), 6.46 (s, 2H, H-2, H-6), 5.57 (s, 1H, OH), 5.54 (d, 1H, J = 5.3 Hz, H-4″), 5.05 (d, 1H, J = 5.3 Hz, H-5″), 3.87 (s, 3H, OCH_3), 3.80 (s, 9H, 3× OCH_3), 3.48 (q, 2H, J = 7.1 Hz, CH_2), 3.42 (q, 2H, J = 7.2 Hz, CH_2), 1.25 (t, 3H, J = 7.1Hz, CH_3), 1.16 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.7 (CO), 158.6 (C-Het), 153.7 (2× C), 148.1 (C), 145.4 (C), 137.2 (C), 134.4 (C), 121.5 (C), 120.3 (CH), 113.4 (CH), 110.3 (CH), 104.6 (2× CH), 87.0 (CH-Het), 60.8 (CH_3), 56.3 (CH-Het), 56.1 (2× CH_3,), 55.8 (CH_3), 42.0 (CH_2), 40.7 (CH_2), 14.3 (CH_3), 12.6 (CH_3); MS (DCI/NH_3) m/z 459 $(M+H)^+$; Anal. Calcd for $C_{24}H_{30}N_2O_7$: C, 62.87; H, 6.59; N, 6.11. Found C: 63.04; H, 6.64; N, 6.13.

4.6.9. 5-(3-Hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1,2,4-dioxazole (18b). Dioxazole **18b** was obtained from protected compound **18a** (607 mg, 1.28 mmol) after a flash chromatography (cyclohexane/EtOAc 6:4) as a white crystalline solid (410 mg, 89%). Mp 121–123 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12 (d, 1H, J = 2.0 Hz, H-2′), 7.10 (dd, 1H, J = 8.3 Hz, J = 2.0 Hz, H-6′), 7.06 (s, 2H, H-2, H-6), 6.90 (d, 1H, J = 8.3 Hz, H-5′), 6.77 (s, 1H, H-5″), 5.74 (sl, 1H, OH), 3.91 (s, 3H, C-4′OCH_3), 3.89 (s, 9H, C-3OCH_3, C-5OCH_3, C-4OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2 (C-Het), 153.3 (2× C), 148.4 (C), 145.5 (C), 140.8 (C), 127.7 (C), 119.2 (CH), 117.9 (C), 112.9 (CH), 110.4 (CH), 108.8 (CH-Het), 104.1 (2× CH), 60.9 (CH_3), 56.2 (2× CH_3), 56.0 (CH_3); HRMS (DCI/NH_3) m/z 361.1157 [M^+ calcd for $C_{18}H_{19}NO_7$: 361.1162].

4.6.10. *trans*-*N*,*N*-Diethyl-4-(3-hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-5-carboxamide (24b). Amide **24b** was obtained from protected compound **24a** (86 mg, 0.15 mmol) after a flash chromatography (CH_2Cl_2/MeOH 97:3) as a white foam (64 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.86 (s, 3H, H-Ar), 6.79 (s, 2H, H-Ar), 5.70 (s, 1H, OH), 5.50 (d, 1H, J = 5.5 Hz, H-4″), 5.09 (d, 1H, J = 5.5 Hz, H-5″), 3.85 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.73 (s, 6H, 2× OCH_3), 3.46 (q, 2H, J = 7.1 Hz, CH_2), 3.40 (q, 2H, J = 7.2 Hz, CH_2), 1.23 (t, 3H, J = 7.1 Hz, CH_3), 1.15 (t, 3H, J = 7.1 Hz, CH_3) ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.6 (CO), 158.9 (C-Het), 152.9 (2× C), 146.2 (2× C), 139.4 (C), 132.0 (C), 123.6 (C), 119.6 (CH), 113.9 (CH), 111.0 (CH), 104.9 (2× CH), 87.1 (CH-Het), 60.8 (CH_3), 60.0 (2× CH_3), 55.9 (CH_3), 55.7 (CH-Het), 42.0 (CH_2), 40.7 (CH_2), 14.4 (CH_3), 12.7 (CH_3); HRMS (DCI/NH_3) m/z 459.2128 [$(M + H)^+$ calcd for $C_{24}H_{31}N_2O_7$: 459.2131].

4.7. *C*-(3-Hydroxy-4-methoxyphenyl)-*N*-(3,4,5-trimethoxyphenyl)nitrone (26)

To a stirred solution of isovanillin (1.52 g, 10 mmol) and 3,4,5-trimethoxynitrophenyl **25** (3.2 g, 15 mmol) in EtOH (200 mL) was added zinc powder (1.46 g) at 0 °C under argon. Glacial acetic acid (2.6 mL) was then added dropwise, and the mixture was stirred for 2 h at 0 °C and then raised to room temperature for 2 h. After evaporation under reduced pressure, the crude material was chromatographed (CH_2Cl_2/MeOH 97:3) to give 2.7 g of nitrone **26**, which was recrystallized from EtOH (2.06 g, 62%). Mp 186–189 °C; ^{1}H NMR ($CDCl_3$): δ 8.28 (d, 1H, J = 1.9 Hz, H-2′), 7.84 (dd, 1H, J = 8.5 Hz, J = 1.9 Hz, H-6′), 7.77 (s, 1H, CH=N), 7.02 (s, 2H, H-2, H-6), 6.93 (d, 1H, J = 8.5 Hz, H-5′), 3.95 (s, 3H, OCH_3), 3.92 (s, 6H, 2× OCH_3), 3.88 (s, 3H, OCH_3); MS (DCI/NH_3) m/z 334 $(M + H)^+$, 318 (imine + H)$^+$.

4.8. Methyl-3-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-isoxazolidine-4-carboxylate (27)

To a suspension of nitrone **26** (1.0 g, 3.0 mmol) in toluene (15 mL) was added methylacrylate (1.1 mL, 12.2 mmol) under argon. After stirring under reflux for 18 h, the resulting mixture was evaporated to dryness. The residue was chromatographed (cyclohexane/EtOAc 5:5 then 4:6) to give isoxazolidine **27** (75mg, 6%). ^{1}H NMR ($CDCl_3$): δ , 7.02 (d, 1H, J = 2.1 Hz, H-2′), 6.97 (dd, 1H, J = 8.3 Hz, J = 2.1 Hz, H-6′), 6.81 (d, 1H, J = 8.3 Hz, H-5′), 6.26 (s, 2H, H-2, H-6), 5.60 (br, 1H, OH), 4.83 (d, 1H, J = 8.9 Hz, H-3″), 4.50 (t, 1H, J = 8.1 Hz, H-5″a),), 4.33 (t, 1H, J = 8.3 Hz, H-5″b) 3.87 (s, 3H, OCH_3), 3.78 (s, 9H, 3× OCH_3), 3.76 (m, 1H, H-4″), 3.39 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 169.6 (CO), 153.2 (2× C), 146.6 (C), 146.2 (C), 145.4 (C), 133.3 (C), 130.5 (C), 119.2 (CH), 114.0 (CH), 110.3 (CH), 93.1 (2× CH), 71.5 (CH-Het), 67.4 (CH_2), 60.8 (CH_3), 55.8 (2× CH_3), 55.7 (2× CH_3), 52.8 (CH-Het), 51.7 (CH_3). HRMS (DCI/NH_3) m/z 420.1654 [$(M + H)^+$ calcd for $C_{21}H_{26}NO_8$: 420.1658].

4.9. 1-[(3-*p*-Methoxybenzyloxy)-4-methoxyphenyl]-3-(3,4,5-trimethoxyphenyl)-propenone (33)

A mixture of 3,4,5-trimethoxyacetophenone (2.10 g, 10 mmol), aldehyde **32** (2.72 g, 10 mmol) and aqueous NaOH (6N, 0.4 mL) in EtOH (25 mL) was stirred at 60 °C for 5 h. After standing at room temperature, the

chalcone crystallized from the solution (4.26 g, 91%). Mp 86–87 °C; 1H NMR ($CDCl_3$): δ 7.71 (d, 1H, J = 15.6 Hz, CH), 7.38 (d, 2H, J = 8.4 Hz, 2× Ar-H), 7.26–7.18 (5H, m, H-2, H-6, H-2′, H-6′, CH), 6.91 (3H, m, H-5′, 2× Ar-H), 5.12 (s, 2H, CH_2), 3.94 (s, 6H, 2× OCH_3), 3.82 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3).

4.10. (*E*)-2,3-Epoxy-3-[3-(*p*-methoxybenzyloxy)-4-methoxyphenyl]-1-(3,4,5-trimethoxyphenyl)propenone (34)

H_2O_2 (35%, 1.0 mL) was added dropwise to a stirred suspension of chalcone **33** (2.32 g, 5 mmol) in EtOH (30 mL) and aqueous NaOH (2N, 1.25 mL) at 0 °C. After complete addition, the ice bath was removed and the mixture was stirred for 4 h at room temperature. The precipitated white crystals were filtered out, washed with cold EtOH and dried *in vacuo* (1.99 g, 83%). Mp 122–123 °C; ^{1}H NMR ($CDCl_3$): δ 7.36 (d, 2H, J = 8.5 Hz, 2× Ar-H), 7.26 (s, 2H, H-2, H-6), 6.94–6.88 (m, 5H, H-2′, H-5′, H-6′, 2× Ar-H), 5.08 (m, 2H, CH_2), 4.11 (d, 1H, J = 1.7 Hz, CH), 3.99 (d, 1H, J = 1.7 Hz, CH), 3.93 (s, 3H, OCH_3), 3.87 (s, 9H, 2× OCH_3), 3.79 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 192.0 (CO), 159.4 (C), 153.2 (2× C), 150.5 (C), 148.6 (C), 143.4 (C), 130.6 (C), 129.2 (2× CH), 128.7 (C), 127.6 (C), 119.2 (CH), 114.0 (2× CH), 111.7 (CH), 110.9 (CH), 105.9 (2× CH), 70.9 (CH_2), 61.2 (CH), 61.0 (CH_3), 59.4 (CH), 56.4 (2× CH_3), 56.0 (CH_3), 55.3 (CH_3). Anal. Calcd for $C_{27}H_{28}O_8$: C, 67.49; H, 5.87. Found: C 66.55; H, 5.72.

4.11. 4-(3-Hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)isoxazole (35)

$BF_3.Et_2O$ (1.25 mL, 10 mmol) was added to a solution of ketoepoxide **34** (480 mg, 1.0 mmol) in dry THF (10 mL) under argon. After reflux for 45 min, the mixture was diluted with Et_2O and washed with water. The combined organic layers were dried, filtered and concentrated under reduced pressure. The residue was dissolved in EtOH (6 mL) and then pyridine (160 μL) and $NH_2OH.HCl$ (140 mg, 2 mmol) were added and the resulting mixture was refluxed for 6 h. The solvent was removed in vacuo, CH_2Cl_2 was added and this solution was washed with water. The combined organic layers were dried, filtered, concentrated under reduced pressure and the residue was chromatographed (CH_2Cl_2/MeOH 99:1) to give **35** as a white solid, which was crystallized in EtOAc (114 mg, 32%). Mp 167–168 °C; ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H, H-3″), 7.01 (d, 1H, J = 1.3 Hz, H-2′), 6.92 (s, 2H, H-2, H-6), 6.89 (m, 2H, H-5′, H-6′), 5.72 (br, 1H, OH), 3.92 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.74 (s, 6H, 2× OCH_3); ^{13}C NMR ($CDCl_3$): δ 163.3 (C-Het), 153.3 (2× C), 152.0 (CH-Het), 146.5 (C), 145.9 (C), 139.5 (C), 123.3 (C), 122.8 (C), 120.8 (CH), 115.4 (C-Het), 115.1 (CH), 110.9 (CH), 104.5 (2× CH), 60.9 (CH_3), 56.0 (3× CH_3). HRMS (DCI/CH_4) m/z 358.1287 [$(M + H)^+$ calcd for $C_{19}H_{20}NO_6$: 358.1291].

4.12. Tubulin assays[53]

Porcin brain tubulin was prepared as previously reported. Tubulin polymerization was monitored by turbidimetry at 350 nM with a Uvikon 931 spectrophotometer (Kontron) fitted with a thermostatically regulated cuvette holder. The products are dissolved at 10 mM in DMSO and added at variable concentrations (0.5–10 μM) to the tubulin solution before polymerization. The IC_{50} value is defined as the concentration of product which inhibits the rate of polymerization by 50%.

4.13. Cytotoxicity assays[50]

HT29 cells (human colon adenocarcinoma, ATCC HTB 38) and SVEC 4–10 cells (SV40 transformed endothelial cells from mouse axillary lymph node, ATCC CRL-2181) were cultivated in Dulbecco's MEM supplemented with 10% FCS. Cells from log-phase culture were seeded in 24-microwell plates (1 mL–5×10^4 cells/well) and incubated for two days at 37 °C in a water-jacketed CO_2 incubator. Tested compounds, in DMSO solution, were added under the minimum volume (5 μL) in increasing concentration. Control cells received 5 μL DMSO alone. Plates were incubated for 24 h, then medium was removed and cells were washed twice with phosphate-buffered saline (PBS) before addition of fresh medium. Plates were reincubated for three days before evaluation of the cell survival using the MTT assay[51] using 30 min incubation with 100 μg/well of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After removal of the medium, formazan crystals were taken up with 100 μL DMSO and absorbance at 540 nm was measured with a microplate reader (Model 450 Bio-Rad). Survival was expressed as percentage of DMSO-treated controls.

Cytotoxic activities were expressed as IC_{50}, the concentration that reduced by 50% the number of treated cells relative to controls. IC_{50} values were extracted from regression curves obtained with experimental points.

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