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Synthesis and biological evaluation of *cis*-locked vinylogous combretastatin-A4 analogues: Derivatives with a cyclopropyl-vinyl or a cyclopropyl-amide bridge

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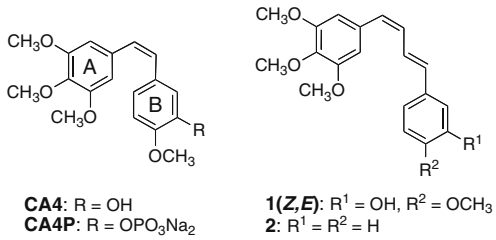
ABSTRACT

A series of novel combretastatin A4 analogues, in which the *cis*-olefinic bridge is replaced by a cyclopropyl-vinyl or a cyclopropyl-amide moiety, were synthesized and evaluated for inhibition of tubulin polymerization and antiproliferative activity. The derivative **9a** with a (*cis*,*E*)-cyclopropyl-vinyl unit is the most promising compound. As expected, molecular docking of **9a** has shown that only one of the *cis*-cyclopropyl enantiomers is a good ligand for tubulin.

Tumour vasculature represents an attractive target for anticancer drug discovery.[1,2] Vascular disrupting agents (VDA), which exploit differences between normal and immature tumour blood vessels, elicit selective shutdown of blood flow resulting in extensive tumour-cell necrosis.[3] The most promising compound, combretastatin A4 (CA4) in the form of a water-soluble phosphate prodrug (CA4P), is currently in phase II and III clinical trials for the treatment of solid tumours.[3–5] CA4 is a natural *cis*-stilbene product isolated from the South African willow tree *Combretum caffrum*, which strongly inhibits tubulin polymerization by binding to the colchicine site.[6] Interference with microtubule dynamics affects the cell signaling pathways involved in regulating and maintaining the cellular cytoskeleton of endothelial cells in tumour vasculature.[7,8] The result for the tumour's blood vessels, is a rapid morphological change of their endothelial cells leading to their occlusion and interruption of blood flow.[9,10]

Extensive studies have been conducted to examine the structure–activity relationships of variously modified CA4 analogues. The 3,4,5-trimethoxy substitutions on the A-ring and the *cis*-orientation between the two aryl rings have been reported as essential requirements for efficient binding to tubulin.[11] Accordingly, many structural variations have been investigated, but few acyclic bridgehead analogues with three (or more)-atom linkers inserted between the two aryl rings have been reported.[12,13] We have previously shown with the vinylogous analogues of CA4, **1** and its (*E*,*Z*)-dienic isomer, that a (*E*,*Z*)-butadiene moiety could replace the *cis*-olefinic bond of CA4 without compromising tubulin polymerization properties.[14] Moreover, derivative **2** with a simple phenyl group as the B-ring, was found to be a more potent inhibitor than CA4 (Fig. 1). But these dienic derivatives are prone to isomerization to the more stable but inactive (*E*,*E*)-isomeric derivatives.

Thus, our attention was focused on the structural modifications that maintained the *cis*-diaryl relationship of **2** (Fig. 2). The cyclo-



CA4: R = OH
CA4P: R = OPO_3Na_2

1(*Z*,*E*): R^1 = OH, R^2 = OCH_3
2: R^1 = R^2 = H

Figure 1. The structures of CA4 and vinylogous analogues.

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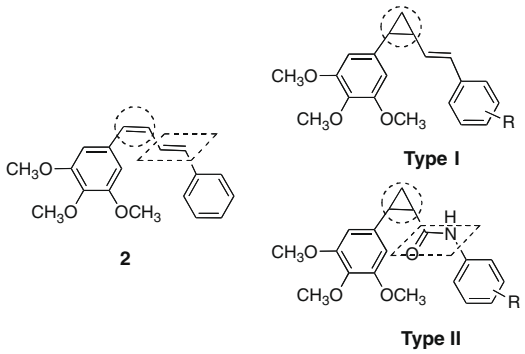


Figure 2. Design concept of new CA4 analogues.

propyl group is generally considered as an alkene bioisostere.[15] In this view, we targeted the synthesis of configurationally *cis*-locked CA4 analogues of type **I**, which are characterized by a (*cis*,*E*)-cyclopropyl-vinyl unit.[16] We were also interested to investigate the biological evaluation of cyclopropyl derivatives (type **II**) for which the *E*-double bond was replaced by an amide group. Indeed, a secondary amide may be considered to have a similar spatial disposition than a *trans*-olefin, despite its different electronic character.[17] The influence of the substitution on the B-ring was also examined.

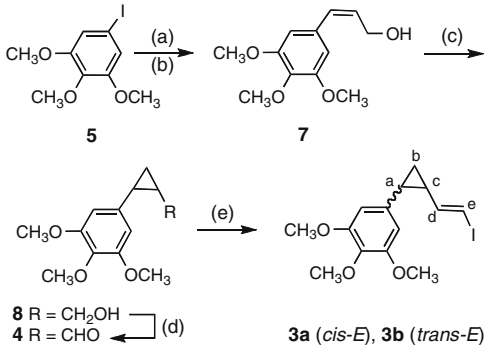
Access to the type **I** derivatives, with a (*cis*,*E*)-cyclopropyl-vinyl moiety, was envisioned via a Suzuki coupling reaction between aromatic boronic acids and the vinyl iodide **3a** which may be obtained by a Takai homologation of the cyclopropane carbaldehyde **4** (Scheme 1). The Sonogashira coupling of aryliodide **5** with propargyl alcohol afforded alkyne **6** which was reduced selectively to the *Z*-olefin **7** by catalytic hydrogenation.[18] According to Cooper[19], the cyclopropanation furnished the cyclopropylcarbinol **8** which was converted, by a ruthenium-catalyzed (TPAP/NMO) oxidation, to the *cis*-cyclopropane carbaldehyde **4** (80% yield).[20,21] Takai homologation of aldehyde **4** was carried out using chromous chloride and iodoform in a ratio of 5.5:1 and was first investigated at 0 °C. A mixture of two *E*-vinyl iodides (J = 14.4 Hz), in a 8:2 ratio, was isolated after chromatography (56% yield). At lower temperature (−10 °C) the ratio of these both isomers was inversed (3:7, 65% yield) while, at −78 °C, the reaction did not occur and the aldehyde was recovered in its *trans*-isomerized form. We hypothesized that, at 0 °C, homologation occurred rapidly, so that the desired (*cis*,*E*)-vinyl iodide **3a** was predominantly formed, but the starting *cis*-cyclopropane aldehyde **4** was in part isomerized into the *trans*-isomer, leading to concomitant formation of the (*trans*,*E*)-product **3b** after Takai reaction. Although the expected vinyl iodide **3a** could not be obtained in a pure form after column chromatography, it was submitted to a Suzuki–Miyaura coupling. Thus, treatment of a mixture of vinyl iodides **3a** and **3b** (9:1) with phenyl boronic acid, in the presence of $Pd(PPh_3)_4$ and EtONa as a base (Scheme 2),[14] afforded the adduct **9a**, which retained the configuration (*cis*,*E*) of the preponderant starting iodide (40% yield, purity 90%). The (*trans*,*E*)-analogue **9b** was obtained, under the same conditions, from a mixture containing mainly the *trans*-isomer **3b**.[22]

These disappointing results led us to examine the formation of the desired (*cis*,*E*)-cyclopropyl-styryl derivative **9a**, via a Wittig olefination with aldehyde **4**. Therefore, by condensation of **4** with phosphorus ylide generated from benzyl tributylphosphonium bromide and *n*-BuLi, a mixture of *Z*- and *E*-olefins (**9a**/**9c** 1:1) was obtained in 70% yield. Nevertheless, isomers **9a** (*cis*,*E*) and **9c** (*cis*,*Z*) could be isolated in pure form for biological assays after normal phase HPLC purification.[23] As expected, unlike the dienic compound **2**, the cyclopropyl-vinyl analogues **9a** and **9c** showed great chemical stability.

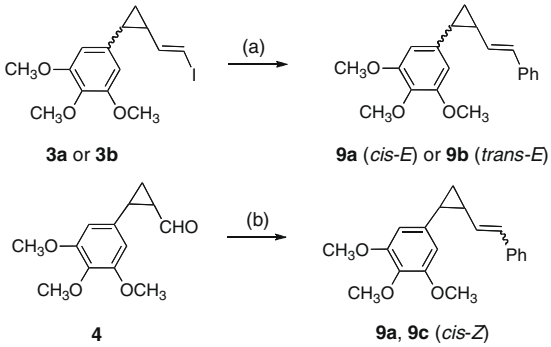
Next, we investigated the replacement of the (*E*)-double bond by an amide group. Type **II** CA4 analogues, bearing a cyclopropane-carboxamide linker, would arise from the coupling reaction of the *cis*-cyclopropane carboxylic acid **10a** with a variety of anilines (Scheme 3). In the presence of copper[II]acetylacetonate, cyclopropanation of the known olefin **11**[24] with ethyl diazoacetate (EDA)[25] afforded a mixture of the *cis*- and *trans*-cyclopropane carboxylates **12a** and **12b** (46% conversion, ratio 3:7). When $Cu(acac)_2$ was added in several batches, to avoid catalyst deactivation,[26] the yield in cyclopropane products was increased (69%). Under these conditions cyclopropanation provided the *cis*-isomer **12a** (18% yield) and the *trans*-isomer **12b** (46% yield) after chromatographic separation.[27] Hydrolysis of the ester *trans*-**12b** with 2 N NaOH at room temperature gave the acid **10b**. Saponification of the *cis*-derivative **12a**, had to be performed at 50 °C during 7 h, affording the desired carboxylic acid **10a**.[19,27]

Under standard EDCI/HOBt conditions, acid **10a** was coupled to aniline and two other amines bearing a phenol function protected by a silyl group,[28,29] to give the corresponding *cis*-amides **13a**, **14a** and **15a**. Removal of the hydroxyl protecting group by the action of TBAF afforded the phenol derivatives **16a** and **17a**. In a similar manner, the carboxamides **13b** and **14b** were obtained by coupling reactions with the *trans*-cyclopropane carboxylic acid **10b**. Treatment of the acids **10a** and **10b** with oxalyl chloride followed by coupling with 3-aminopyridine afforded the *cis*- and *trans*-derivatives **18a** and **18b** in 71 and 63% yields, respectively.[30]

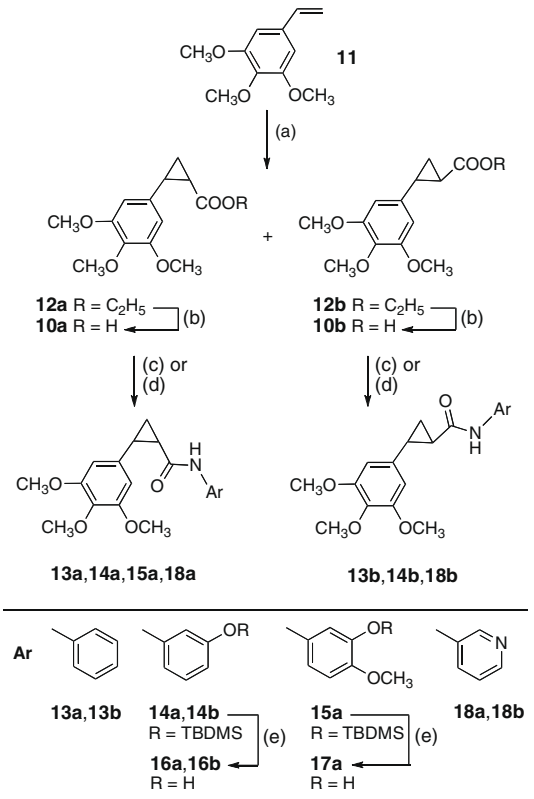
The synthesized cyclopropyl derivatives were evaluated (Table 1) for their ability to inhibit tubulin polymerization and their antiproliferative activities against two types of human cancer cell lines, HCT-116 (colon carcinoma) and MCF-7 (hormone dependent breast carcinoma). Against the MCF-7 breast cancer cells, only



Scheme 1. Reagents and conditions: (a) $CH{\equiv}CCH_2OH$, $Pd(PPh_3)_4$, CuI, piperidine, rt, 1.5 h, 81%; (b) H_2, cat. Lindlar, Et_3N, AcOEt, rt, 1 h, 71%; (c) Et_2Zn, CH_2I_2, CH_2Cl_2, 0 °C to rt, 5 h, 60%; (d) TPAP, NMO, CH_2Cl_2, rt, 30 min, 80%; (e) $CrCl_2$, CHI_3, THF, 0 °C, 1.5 h, 56% (**3a**/**3b** 8:2).

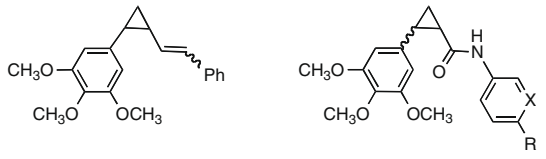


Scheme 2. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, EtONa, THF, reflux, 5 h; (b) $PhCH_2P^+Bu_3Br^-$, *n*-BuLi, THF, −30 °C, 30 min then −78 °C, **4**, 30 min.



Scheme 3. Reagents and conditions: (a) $N_2CHCO_2C_2H_5$, $Cu(acac)_2$ in several batches, $ClCH_2CH_2Cl$, reflux, 7 h; (b) 2 N NaOH, MeOH: 50 °C, 7 h for **12a**; rt, 7 h for **12b**; (c) $ArNH_2$, EDCI, HOBt, CH_2Cl_2, rt, 3–4 h; (d) $(COCl)_2$, DMF, CH_2Cl_2, rt, 3 h, evaporation then 3-aminopyridine, Et_3N, CH_2Cl_2, rt, 2.5 h; (e) TBAF, THF, rt, 2 h.

Table 1
Inhibition of tubulin polymerization (ITP) and cell proliferation by synthesized cyclopropyl derivatives.



9a, **9c** **13**, **16**, **17**, **18**

Compound	X	R	ITP[a] IC_{50} (μM)	Cytotoxicity IC_{50} (μM)[b]	
				HCT116	MCF7
9a (*cis,E*)	—	—	5.3	0.53	6.49
9c (*cis,Z*)	—	—	38	na[c]	na
13a (*cis*)	CH	H	>100	0.68	na
13b (*trans*)	CH	H	92	3.35	na
16a (*cis*)	C–OH	H	>100	na	na
17a (*cis*)	C–OH	OCH_3	82	na	na
18a (*cis*)	N	H	>100	2.37	na
18b (*trans*)	N	H	>100	na	na
CA4			0.79	0.003	nd[d]
2 (*Z,E*)[e]			0.5	0.50	

[a] Compound concentration inhibiting 50% of about 2.0 mg/mL microtubular protein assembly. For CA4 and derivatives **9a** and **9c** values are the average of three experiments.

[b] Compound concentration required to inhibit human tumor cells proliferation by 50% after 72 h incubation. Values are the average of experiments in duplicate.

[c] Na (not active): inhibition of cell growth did not exceed 20% at concentration of 1 μM (triplicate).

[d] Nd: IC_{50} values could not be determined, as growth inhibition did not exceed 50%. 25% inhibitory concentration: IC_{25} = 0.004 μM.

[e] Values from Ref [14]. ITP value for CA4: 1.2 μM.

the (*cis,E*)-cyclopropyl-vinyl derivative **9a** displayed modest antiproliferative activity (IC_{50} = 6.49 μM). Thus, the discussion will focus on the results obtained with the HCT-116 cell line.

Of the two *cis*-cyclopropyl-vinyl compounds evaluated, **9a** and **9c**, only the (*cis-E*)-isomer **9a** which retained the configuration (*Z,E*) of the lead compound **2**, exhibited some inhibitory activity against tubulin assembly (IC_{50} = 5.3 μM), although weaker than those of CA4 (IC_{50} = 0.79 μM) and diene **2**. The fact that compound **9a** is a racemic mixture and that only one enantiomer has the structural features required to interact with tubulin, may account—at least in part—for its moderate potency (discussed below). This result validates the hypothesis that a *cis*-cyclopropyl group could efficiently replace a *Z*-olefinic bond in this dienic derivatives series. It is not surprising that compound **9a** showed a lower cytotoxic potency in comparison to CA4 (IC_{50} = 0.53 μM vs 0.003 μM), since the same trend was observed with our previously reported vinylogous CA4 analogues.[14] Nevertheless, compounds which display potent ability to inhibit tubulin assembly while showing limited cytotoxicity may be of high interest in the search of new potent VDAs.[31]

Replacement of the *E*-double bond of compound **9a** by an amide group (**13a**) was detrimental to tubulin interaction, nevertheless did not affect cytotoxicity (IC_{50} = 0.68 μM). Additionally, attempts were directed at replacing the phenyl group by different aromatic moieties to explore spatial and electronic requirements. Compounds **16a** and **17a**, respectively, bearing a phenolic or an isovanillic ring, did not interact with tubulin and were devoid of cytotoxic activity. The pyridyl analogue **18a**, which might generate new interactions with tubulin, exhibited weak cytotoxicity (IC_{50} = 2.37 μM) but without any microtubule disrupting activity. Amide compounds **13b** and **18b** bearing a *trans*-cyclopropyl moiety were essentially inactive as tubulin inhibitors.

In order to investigate the possible binding modes of the subject compounds with tubulin, docking studies of two representative analogues (**9a** and **13a**) were performed by using the reported high-resolution crystal structure of the tubulin/DAMA-colchicine complex.[32] All the simulations were carried out using the GOLD docking tool (Genetic Optimisation for Ligand Docking) algorithm.[33] We firstly docked the CA4 in the colchicine binding site of tubulin and we ensured that a hydrogen bond was favored with Cysβ241, a key residue in the binding of colchicine and CA4.[34]

First, the possible binding modes of the two enantiomeric forms of the cyclopropyl derivative **9a** with tubulin were investigated. Docking of the (*S,S*)-isomeric form of **9a** resulted in a single conformational mode correctly mimicking the CA4 within the tubulin binding site (Figs. 3A and 4A). On the other hand, docking of the **9a**-(*R,R*) isomer yielded two conformer clusters. In the larger group of conformers (8/10), while the trimethoxyphenyl (TMP) ring overlaps well with that of CA4, a different orientation was taken by the remaining part of the molecule, beginning from the cyclopropyl ring (Figs. 3B and 4B). Then, the different poses of both isomeric forms of **9a** were assessed using quantitative criteria. Thereby, we extracted the fitness score provided by GoldScore for each of the ten first poses in both isomers. For the isomer **9a**-(*S,S*) the ten poses had similar scores (comprised between 44 and 48) higher than those of CA4 (35–41). For the **9a**-(*R,R*) isomer, while eight poses had almost the same score values (35–37), the two other positions superimposing with CA4 markedly got lower scores (30 and 32). This is an additional proof of the bad binding of **9a**-(*R,R*) in the colchicine site. These results suggest that the isomer **9a**-(*S,S*) docked in a correct manner in the colchicine binding site and, furthermore, with a stable binding interaction.

Docking of the isomer **9a**-(*S,S*) revealed a consistent set of recurring interactions with the tubulin (Fig. 3A). Two methoxy O

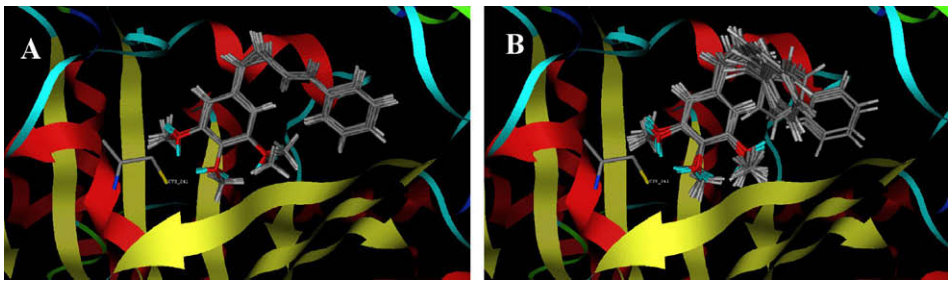


Figure 3. Proposed binding mode of both cyclopropyl isomers of **9a** in the colchicine binding site of tubulin. The ten best scored poses (A) for isomer **9a**-(*S,S*), (B) for isomer **9a**-(*R,R*), Yellow ribbons are β sheets, red ribbons are α-helixes.

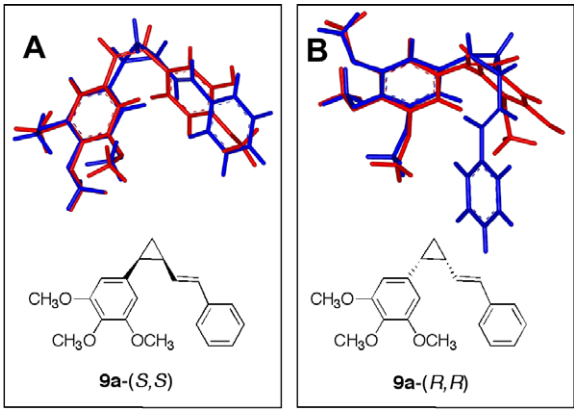


Figure 4. Superimposition of both cyclopropyl isomers of **9a** (blue) and CA4 (red) in the colchicine binding site of tubulin. (A) An overlay of CA4 and **9a**-(*S,S*). (B) An overlay of CA4 and the most representative binding position of **9a**-(*R,R*).

atoms of the A-ring participate in a hydrogen bond network with the Cysβ241. The TMP moiety occupies a hydrophobic pocket bounded by Leu248, Val318, and Ile358 from the β sub-unit, while the B-phenyl ring establishes hydrophobic contacts with Alaα180 and Valα181. Moreover, the lower part of the phenyl ring overlays well with the methoxy methyl group of the CA4 B-ring reported as an important hydrophobic center[33] for the interaction of many colchicine site inhibitors within tubulin. It is noteworthy that the region at the vicinity of the cyclopropyl ring is an open space region that should authorize steric extensions.[35]

In an effort to understand the reason of the discrepancy between the design of the amide derivatives and their poor biological activity, we undertook the docking study of the benzanilide **13a** (see Supplementary data). As the amide function adopts a *s-trans* conformation, we infer that the inability for **13a** and the other (*cis*)-cyclopropyl-amide derivatives to inhibit the tubulin assembly is related to the electronic effects of the amide function.

In summary, we have synthesized and biologically evaluated a novel series of CA4 analogues related to the 1,4-diarylbutadiene **2**. Our results validate the feasibility of replacing a *cis* double bond with a *cis*-cyclopropyl moiety. But, contrary to our expectation, the amide moiety was not a good surrogate for the *trans* double bond of vinylogous CA4 analogues. We can presume that the electronic nature, and not the geometry of the amide linkage, is unsuitable for direct tubulin binding. The docking results obtained for derivatives **9a** and **13a** correlate well with the observed biological data. On the basis of the qualitative and quantitative docking study of the racemic derivative **9a**, we could assure that the (*S,S*)-cyclopropyl enantiomeric form displays the highest binding affinity for tubulin. Therefore, asymmetric synthesis of both the pure enantiomers of **9a** and other congeners are currently under investigation to identify new vascular disrupting agents.

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Supplementary data

Experimental procedures, spectroscopic data for new compounds and molecular modeling of derivative **13a**, are available on line. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.062.

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16. *Cis* or *trans* refer to the cyclopropane ring stereochemistry, *E* or *Z* to the olefinic bond configuration. All the synthesized cyclopropyl derivatives are *cis* or *trans* racemates.
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20. The *cis*-cyclopropane structure (J = 8.7 Hz) for aldehyde **4** was confirmed by NOESY analysis and by analogy with ^{1}H NMR data of the phenyl analogue.[21] Moreover, ^{1}H NMR values for the aldehyde proton allowed easy attribution of the *cis* or *trans* stereochemistry for the cyclopropane ring (δ = 8.66 ppm, J = 6.9 Hz/δ = 9.33 ppm, J = 4.6 Hz, respectively).
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22. For the cyclopropyl-vinyl derivatives synthesized (vinyl iodides **3a** and **3b**, styryl derivatives **9a** and **9b**) the stereochemistry *cis* or *trans* of the cyclopropane ring was confirmed by ^{1}H NMR chemical shifts analysis. For derivatives **3a** and **9a** with a *cis*-cyclopropane unit, the cyclopropyl protons H-a and H-c resonate at lower field than in the *trans*-compounds (~0.35 and 0.20 ppm, respectively), while the olefinic proton H-d is shielded (~0.35 ppm). For atom numbering see Scheme 1.
23. Characterization of compounds **9a** and **9c**. Compound **9a**: colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (m, 5H, Ph), 6.53 (d, 1H, J = 15.7 Hz, H-e), 6.49 (s, 2H, H-2, H-6), 5.54 (dd, 1H, J = 15.7, 9.4 Hz, H-d), 3.84 (s, 6H, 2× OCH_3), 3.82 (s, 3H, OCH_3), 2.40 (m, 1H, H-a), 1.99 (m, 1H, H-c), 1.35 (m, 1H, H-b), 1.04 (m, 1H, H-b); HRMS (DCI/CH_4): calcd. for $C_{20}H_{23}O_3$ (MH^+) 311.1647, found 311.1642; compound **9c**: colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 2H, Ph), 7.36 (m, 2H, Ph), 7.23 (m, 1H, Ph), 6.47 (s, 2H, H-2, H-6), 6.36 (d, 1H, J = 11.6 Hz, H-e), 5.01 (dd, 1H, J = 11.6, 9.6 Hz, H-e), 3.84 (s, 9H, 3× OCH_3), 240 (m, 1H, H-a), 2.27 (m, 1H, H-c), 1.39 (m, 1H, H-b), 1.03 (m, 1H, H-b); HRMS (DCI/NH_3): calcd for $C_{20}H_{23}O_3$ (MH^+) 311.1647, found 311.1643.
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35. Such cavity had already been reported. See, for example Bellina, F.; Cauteruccio, S.; Monti, S.; Rossi, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5757.