



Cite this: *Med. Chem. Commun.*,
2018, 9, 843

Synthesis and biological evaluation of new 3-amino-2-azetidinone derivatives as anti-colorectal cancer agents†

Farida Tripodi, ^a Federico Dapiaggi, ^b Fulvia Orsini, ^b Roberto Pagliarin, ^{*b} Guido Sello ^{*b} and Paola Coccetti ^{*a}

Several synthetic combretastatin A4 (CA-4) derivatives were recently prepared to increase the drug efficacy and stability of the natural product isolated from the South African tree *Combretum caffrum*. A group of ten 3-amino-2-azetidinone derivatives, as combretastatin A4 analogues, was selected through docking experiments, synthesized and tested for their anti-proliferative activity against the colon cancer SW48 cell line. These molecules, through the formation of amide bonds in position 3, allow the synthesis of various derivatives that can modulate the activity with great resistance to hydrolytic conditions. The cyclization to obtain the 3-aminoazetidinone ring is highly diastereoselective and provides a *trans* biologically active isomer under mild reaction conditions with better yields than the 3-hydroxy-2-azetidinone synthesis. All compounds showed IC₅₀ values ranging between 14.0 and 564.2 nM, and the most active compound showed inhibitory activity against tubulin polymerization *in vitro*, being a potential therapeutic agent against colon cancer.

Received 16th March 2018,
Accepted 30th March 2018

DOI: 10.1039/c8md00147b

rsc.li/medchemcomm

Introduction

Combretastatins are a group of polyhydroxylated stilbenes isolated from the South African tree *Combretum caffrum*.^{1,2} They are natural-occurring well-known microtubule destabilizing agents that inhibit the polymerization of tubulin by binding to its colchicine binding site. Combretastatin A4 (CA-4, Fig. 1), the main component of this family of natural products, is in advanced clinical trials for cancer therapy because it exhibits potent anticancer activity against a panel of human cancer cells including multi-drug resistant ones.³

CA-4 has anti-angiogenic effects inducing apoptosis in various human tumor cell lines,^{4–7} through blocking of the blood flow, vascular rupture and cellular necrosis. Two problems, however, have limited their use as therapeutic agents for a long time: low water solubility and *cis/trans* CC double bond isomerization, which may occur during storage and administration, causing a dramatic loss of activity. Between the two stereoisomers, the *cis* form is much more active than the *trans* one, due to the pharmacophore of the colchicine binding site, which encompasses the trimethoxybenzene ring in *cis* linked to a methoxy-containing arene.⁸ The low solubility

has been overcome by a water-soluble phosphate prodrug⁹ (Fig. 1), which is currently under investigation in human clinical trials (phase III) as an anticancer drug.^{4,9,10} The low *cis* isomer stability has been approached by designing a variety of conformationally restricted *cis*-locked analogues. Many structural modifications of combretastatin A4 have been proposed by incorporating the stilbene double bond into a carbocyclic or heterocyclic moiety. Carbocyclic derivatives, containing a phenyl ring, or a triatomic or a pentaatomic ring, have to be cited, as well as nitrogen or oxygen-containing heterocyclics,¹¹ triazoles,¹² tetrazoles,¹³ isooxazoles,¹⁴ imidazoles,¹⁵ pyrazoles,¹⁶ and pyridines.¹⁷ Other rings, such as azetidinones, have been inserted between the two phenyl groups of combretastatin A4.^{18,19} In addition, *trans*-3,4-disubstituted azetidinones show strong anti-proliferative activity against several cancer cell lines.^{20,21} Combretastatin-like compounds bearing an azetidinone unit between the A and B rings were presented by N. M. O'Boyle and colleagues.^{22,23} They firstly synthesized a few

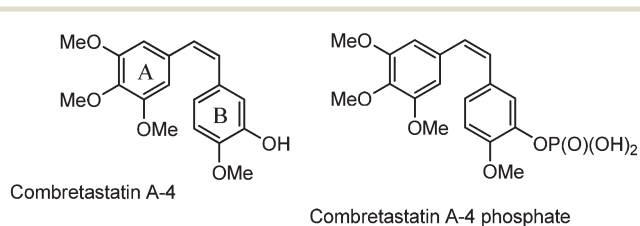


Fig. 1 The main component of combretastatins: combretastatin A4 and its phosphate derivative.

^a Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy. E-mail: paola.coccetti@unimib.it

^b Department of Chemistry, University of Milano, Milano, Italy. E-mail: roberto.pagliarin@unimi.it, guido.sello@unimi.it

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8md00147b

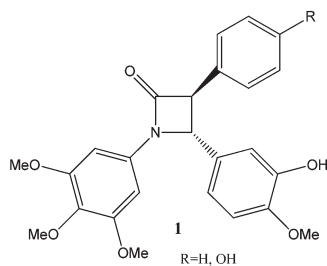


Fig. 2 Triaryl derivatives of azetidinone proposed by O'Boyle and coworkers.²²

derivatives of *trans* 1,3,4 tri-aryl azetidinone **1** (Fig. 2) which inhibited the polymerization of tubulin with a 6-fold rate of inhibition in comparison with the natural compound CA-4.^{22,23}

We recently developed a *trans*-3-hydroxy-2-azetidinone, bearing in position 1 the A ring and in position 4 the B ring of CA-4 (compound **2**) (Fig. 3).²⁴ The racemic mixture of this compound showed both high stability and solubility (1700 μM) in aqueous media due to the presence of a second hydroxyl group in position 3. It was synthesized under thermodynamic control of the reaction at 100 $^{\circ}\text{C}$ with a total yield of 33%. Compound **2** induced inhibition of tubulin polymerization and subsequent G2/M arrest in cancer cells.²⁴ At the same time, activation of AMP-activated protein kinase (AMPK) and induction of apoptosis were also reported.²⁴ Moreover, the drug displayed significant nanomolar cytotoxic activity against different cancer cell lines, as well as a tumor growth delay in a colorectal cancer mouse xenograft model, confirming its potential therapeutic action against colon cancer.²⁵ In the present paper, we report the synthesis of compound **3** (Fig. 3), in which the hydroxyl group of compound **2** was substituted by an amino group, as well as a series of its derivatives. Remarkably, the synthesis of compound **3** was easier than that of compound **2** because in the Staudinger reaction, the *trans* closure of the azetidinone ring took place at room temperature with an overall yield of 50%, with only a few traces of the *cis* isomer.

In order to select derivatives of compound **3** which could have pronounced activity, we performed a preliminary docking study before biological tests. The presence of the amino group permits the easy preparation of a series of derivatives by transforming NH_2 to its corresponding amido group. The variability in size and functions of the introduced substituents can be the source of valuable insights into substrate binding site interactions.

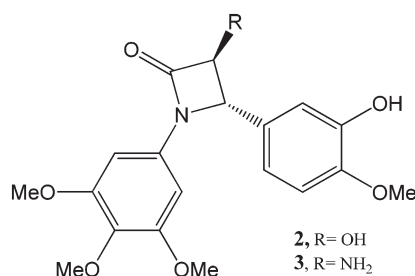


Fig. 3 Azetidinone derivatives proposed by Tripodi and coworkers.²⁴

We hypothesized the preparation of 10 derivatives (compounds **11a–j**) (Table 1), where the R residue derived from the acidic part of the amido group varies from acetic to naphthoic acids. We included short chain, aromatic, hetero-aromatic, cyclic and polycyclic derivatives with diverse functional groups.

First, we examined the docking of compounds **2a** and **2b** showing anticancer activity in previous studies (Fig. 4).²⁴ Molecular docking was performed generating 100 binding poses for each compound. Considering the similarity of the energies obtained from the molecular docking of these compounds (Tables S1 and S2[†]), we performed a cluster analysis on the 100 conformations obtained from each run using an RMSD threshold of 2 \AA . We considered the most populated cluster of each compound as the best docked conformation. Compound **2a** (enantiomer SS) showed the known interaction of the trimethoxyphenyl group that came into contact with Cys β 241 and Val β 238. In addition, it interacted with Asp β 251 through hydrogen bonds. Compound **2b** (enantiomer RR) maintained the interaction of the trimethoxyphenyl group with Cys β 241 and Val β 238. In contrast, it did not interact with Asp β 251, suggesting that the two enantiomers did not share the same docking conformation.

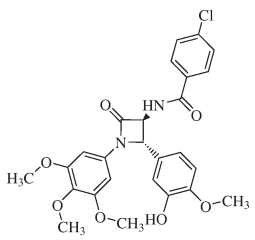
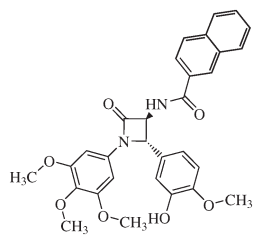
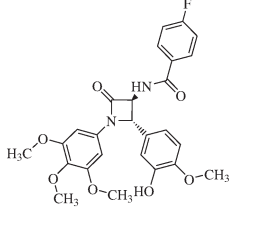
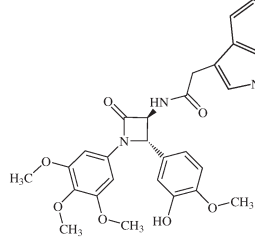
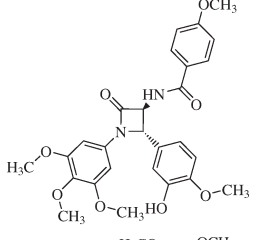
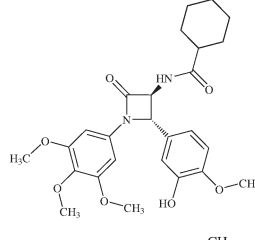
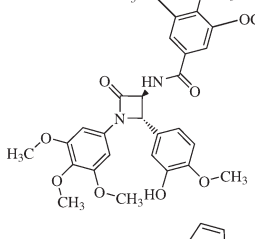
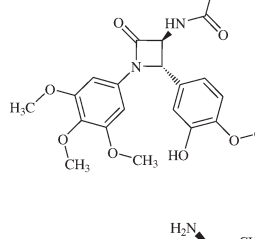
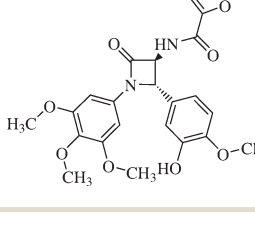
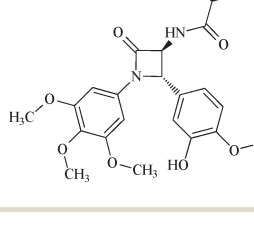
Then, molecular docking studies were carried out on compounds **11a–j** in order to assess their ability to bind to the colchicine site of β tubulin.

RR enantiomers showed low binding capability, displaying a large number of clusters of different conformations. Compound **11i** resulted to be the most interesting compound among the RR enantiomers, displaying a very populated cluster containing 85% of the conformations. Details regarding binding energies and clustering are reported in Table S1.[†]

Much more interesting results were obtained from the molecular docking of SS enantiomers. In this case, most of the docked compounds showed a more highly populated cluster with respect to RR compounds and binding energies around -5 kcal mol^{-1} (Table S2[†]). Compounds **11d**, **11f** and **11g** were not suitable to bind within the colchicine site, due to the steric hindrance of the R substituents of the amide group. This was supported by highly diversified docked conformations. Compounds **11a**, **11b**, **11c**, **11e**, **11h** and **11j** shared the same orientation within the binding site (Fig. 5). Like combretastatin compounds,⁸ the trimethoxyphenyl group came into contact with Cys β 241 and Val β 238. Moreover, the amidic group in position 3 interacted with Met β 259 through a hydrogen bond (Fig. 6). The most interesting compound, however, was **11i**, showing a single cluster of 100 conformations.

This compound showed a different docking pose with respect to the aforementioned ones. It interacted with Asn α 101 and Asp β 251 through hydrogen bonds, while the orientation of the trimethoxyphenyl group was towards residues Cys β 241 and Val β 238 as in the previous cases (Fig. 7). Moreover, compound **11i** showed a very similar docking pose to compounds already studied by our group, namely *trans* 3-hydroxy-1,4-diaryl-2-azetidinone (**2b**) (Fig. 8).

Table 1 Structures and IC₅₀ values of the indicated compounds in SW48 cell lines measured by the MTT assay after 72 h of treatment (0.1% DMSO was used as a vehicle control)

Entry	Compound	IC ₅₀ (nM)	Entry	Compound	IC ₅₀ (nM)
11a		270.7 ± 76.6	11f		220.2 ± 76.5
11b		384.2 ± 117.3	11g		49.4 ± 1.1
11c		108.4 ± 11.6	11h		244.2 ± 64.2
11d		564.2 ± 61.7	11i		14.0 ± 2.1
11e		18.6 ± 1.7	11j		90.6 ± 7.3

In keeping with these results, the syntheses of all the derivatives were performed.

Chemistry

In this work, we focused our attention on the preparation of a small library of 3-amido-1,4-diaryl-2-azetidinones **11a–j** (Table 1), with the goal of improving the biological activity and elucidating the biological effects by the introduction of specific ligands useful to selectively target tumor cells.

3,4-*trans*-3-Amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-azetidin-2-one **9** was synthesized as previously reported by the authors,²⁴ *via* the Staudinger approach be-

tween a properly selected imine **6** and phthalylglycyl chloride as the ketene precursor, followed by hydrolysis with hydrazine dihydrochloride at 60 °C (Scheme 1). The stereochemistry of the products obtained from the Staudinger cycloaddition was affected by the experimental conditions (such as solvent, temperature and order of addition of the reagents) and by the type of the ketene precursor: acetoxyacetyl chloride or phthalylglycyl chloride, used to introduce an OH group or a NH₂ group, respectively, at the position 3 of the azetidin-2-one. In the latter case, the best results were obtained by dropwise addition of a 4-fold excess of acid chloride **7** to a methylene chloride solution of imine **6** and dry triethylamine at 0 °C, followed by stirring at room temperature for twenty-four

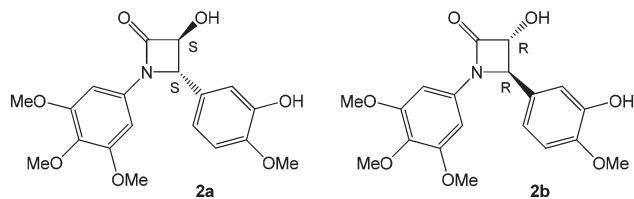


Fig. 4 SS an RR enantiomers of compound 2, previously tested as anticancer agents.

hours. Hydrolysis of the Staudinger adduct with hydrazine afforded only *trans*-diastereoisomer 9 in 61% total yield from the imine. A different approach,²⁶ based on the use of *in situ* generated *N*-chloramines as imine precursors, was tested, without improving the yields of the reaction.

With respect to previously reported 3-hydroxy-1,4-diaryl-2-azetidinones, 3-amino-1,4-diaryl-2-azetidinones could *a priori* display different and hopefully improved activity, and the nitrogen atom at position 3 can be derivatized to give stable amides, easily prepared by reaction with a properly selected carboxylic acid, RCOOH. In order to obtain a library of compounds able to interact with the receptors in a variety of ways, R has been varied in terms of polarity, size, and ability to act as a hydrogen bond acceptor or donor and to give different types of interactions, namely dipole-dipole, β -stacking and hydrophobic interactions.

Amides 11a-i were obtained by a five-step synthesis. As already described,²⁴ imine 6 was obtained by reaction of compounds 4 and 5 in anhydrous ethyl alcohol: the solid compound 6 was subjected to Staudinger cyclization with phthalyl glycine chloride and was transformed into β -lactam 8, which was easily hydrolyzed to compound 9. 3,4-*trans*-3-Amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-azetidin-2-one 9 was first added to an acetonitrile solution of the selected carboxylic acid, previously activated by reaction with *N,N'*-disuccinimidyl carbonate (overnight at room temperature). A mixture of the *N,O*-diacyl derivative 10 and amide 11 was obtained. Pure samples of 11a-j were obtained by flash-chromatography, whereas, the presence of *N,O*-diacyl derivatives 10a-j was recognized in the MS spectra of the mixtures (10a-j and 11a-j) which were directly treated with hydrazine hydrochloride at room temperature to hydro-

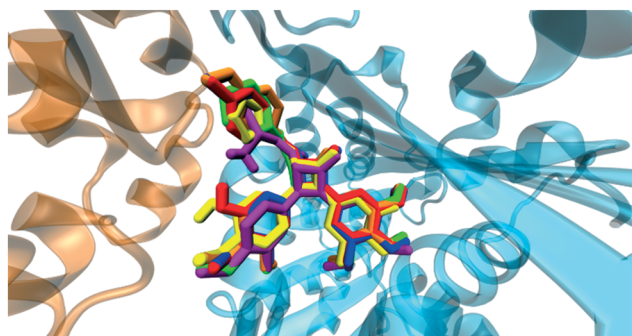


Fig. 5 Superimposition of docking poses of compounds 11a (blue), 11b (red), 11c (orange), 11e (yellow), 11h (green), and 11j (purple).

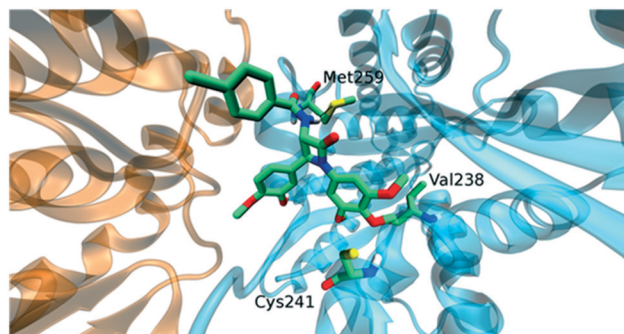


Fig. 6 Interactions of the compound 11a. In the illustration, tubulin α (orange) and β (cyan) subunits are represented. Trimethoxyphenyl group came into contact with residues Cys β 241 and Val β 238, as well as HB with residue Met β 259. This interaction pattern was common to compounds 11a, 11b, 11c, 11e, 11h, and 11j.

lyse the ester moiety. The target amides 11a-j were thus obtained with total yields ranging from 60% to 81% depending on the R substituents of the amide group. The synthesis of the amide 11j required one additional step as *tert*-butoxycarbonyl alanine was used as an acylating reagent: the removal of the protective *tert*-butoxycarbonyl moiety was easily accomplished by treatment with trifluoroacetic acid in methylene chloride.

Biological results

Compounds 11a-j were tested against the SW48 human colon cancer cell line by testing their antiproliferative activity. They retained their nanomolar cytotoxic activity, and among them 11i, with a small alkyl group (methyl), showed the highest activity, comparable to the activity of natural CA-4 and of the previously reported 3-hydroxy azetidinone 2 (Table 1, Fig. S1†). The activity was decreased by the introduction of a primary amino group on the small alkyl chain in 11j and, even more, by the substitution of the methyl group with the more

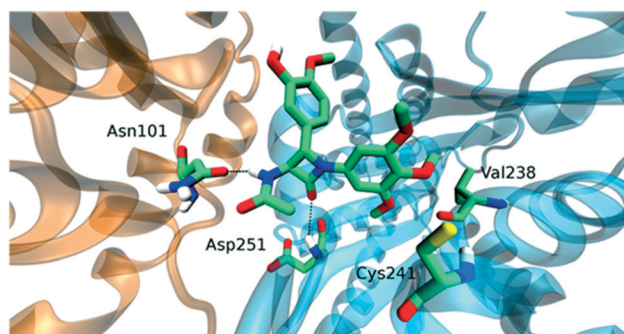


Fig. 7 Interactions of compound 11i. In the illustration, tubulin α (orange) and β (cyan) subunits were represented. Trimethoxyphenyl group is in contact with residues Cys β 241 and Val β 238. Moreover, two hydrogen bonds were observed between the amide group in position 3 and Asn α 101 as well as between the ketone oxygen of the scaffold and Asp β 251. This interaction pattern was common to compounds 11i and 2b.

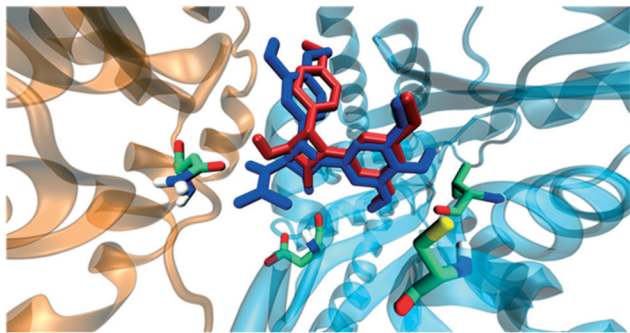


Fig. 8 Superimposition of **11i** (blue) and **2a** (red) docking poses.

sterically demanding cyclohexyl group in **11h**. Hetero-aromatic rings, as in **11e** and **11g**, were approximately comparable to that in **11i**. In the presence of aromatic rings, as in **11a–11d**, the activity was further reduced. The results of the activity tests were consistent with the assessments from the docking study, where the compounds containing bulky aromatic residues did not converge to a single structure.

CA-4 is known to act by inhibiting tubulin polymerization.¹ Thus, we evaluated the effect of compound **11i**, which showed the highest activity against SW48 cells, on the *in vitro* polymerization of purified tubulin, and CA-4 was used as a positive control (Table 2, Fig. S2†). In the assembly assay, **11i** showed an IC₅₀ value of 5.05 μM, higher than that of CA-4, but indicative of the fact that one of the molecular targets of this active compound was tubulin, as reported for other azetidinone compounds.²⁴

Conclusions

Insertions of azetidinone rings into the combretastatin skeleton have been valid modifications because they prevented the isomerization of the *cis* double bond, while preserving the activity of some derivatives comparable to that of the natural compound. They made the natural compound more stable and the synthesis provided good yields. In particular, the 3-aminoazetidinones, compared to the 3-hydroxyazetidinones, which bear a hydroxyl group in position 3 of the β-lactam ring, were synthesized with higher yields since the Staudinger reaction almost exclusively provided the 3,4-*trans* isomer. Furthermore, the derivatization of the amino group afforded amide derivatives which were stable under hydrolytic conditions. Remarkably, these new derivatives showed nanomolar anti-proliferative activity against SW48 cells, probably acting by inhibiting tubulin polymerization, opening the route to a new class of potential therapeutic agents against colon cancer.

Experimental section

Docking studies

The tubulin X-ray structure in a complex with combretastatin was obtained from the protein data bank (PDB ID 5LYJ⁸). The pocket analysis and the docking calculations were performed

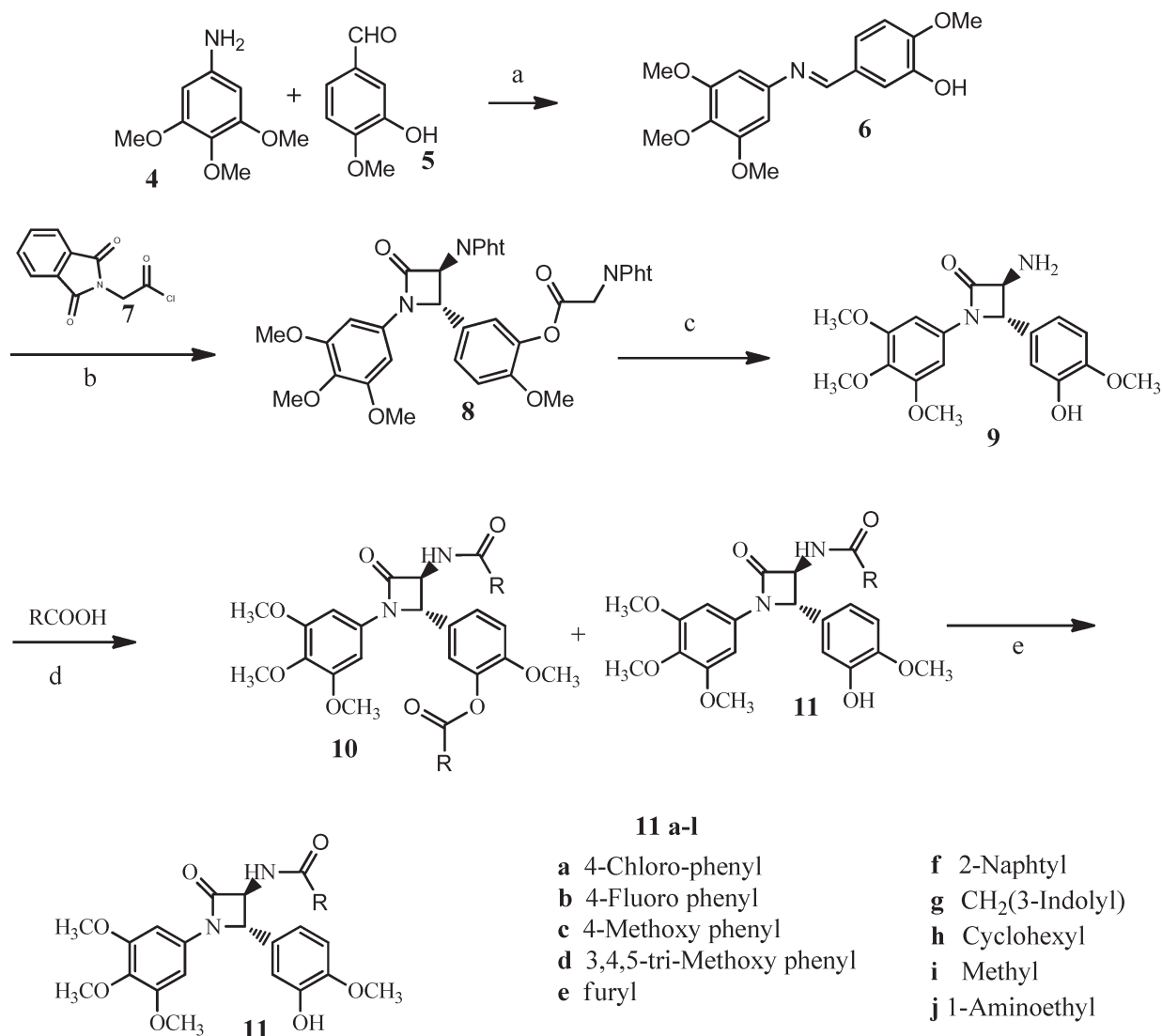
only on one of the dimers in the asymmetric unit, labelled chains A and B. Combretastatin was removed from the structure before docking our compounds. Docking was carried out with Autodock 4.2,²⁷ employing a Lamarckian genetic algorithm.²⁸ 100 independent runs per molecule were performed. In each run, a population of 50 individuals evolved along 27 000 generations and a maximum number of 25 million energy evaluations were performed. The best fit (lowest docked energy) solutions of the 100 independent runs were stored for subsequent analysis. The visual inspection of docked structures was carried out using VMD.²⁹

Chemistry

All commercially available reagents were purchased from Sigma-Aldrich and used without further purification. Preparative separations were usually performed by flash-column chromatography on silica gel (Merck grade 9385). Thin-layer chromatography was conducted on silica gel (Merck, 10 × 5 cm, silica gel 60 F254): zones were detected visually by ultraviolet irradiation (254 nm) or by exposition to iodine vapors. ¹H NMR and ¹³C NMR spectra were recorded at 300 MHz and 400 MHz on Bruker instruments (AC 300 UltrashieldTM 400), using deuteriochloroform solutions, unless otherwise specified, and chemical shifts were represented as δ-values relative to the internal standard TMS. ESI-mass spectra were recorded on a Bruker Esquire 3000 Plus. The synthesized compounds subjected to biological tests have a purity of ≥95% determined by HPLC (Phenomenex, Nucleosil 250 × 3.2 mm column, 5 μm, C18; mobile phase 0.05 M phosphate buffer pH 7/acetonitrile, 7:3; flow rate 1.5 mL min⁻¹), and they have been filtered in sterile atmosphere with 0.20 μm filters.

3-Hydroxy-4-methoxybenzylidene-(3,4,5-trimethoxyphenyl)-amine (6). Under an inert atmosphere, compound **4** (1.9, 10.1 mmol) and compound **5** (1.5 g, 10.1 mmol) were dissolved together at room temperature with stirring, in 10 mL ethanol previously dried on molecular sieves. The reaction was followed by TLC (pre-treatment of the plate with CH₂Cl₂: Et₃N 95:5, then eluted with AcOEt:Esano 1:1). After 2 h, NMR analysis showed the formation of imine **6** which was observable also by the presence of a precipitate. The mixture was filtered to collect the solid product. The filter was washed twice with dichloromethane to dissolve the reaction product. The solvent was removed and compound **6** was collected, pure enough for the next step (yields = 93%). ¹H-NMR (CDCl₃): δ 8.41 (1H, s), 7.63 (1H, s), 7.46 (1H, d, *J* = 7.2 Hz), 6.96 (1H, d, *J* = 7.2 Hz), 6.53 (2H, s), 5.77 (1H, s), 3.99 (3H, s), 3.92 (6H, s), 3.88 (3H, s). EI-MS (*m/z*): 318 (M⁺).

(±)-3,4-*trans*-3-Amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-azetidin-2-one ((±)-*trans*-9). A solution of phthalylglycyl chloride (5.38 g, 24 mmol), in anhydrous methylene chloride (7 mL), was added dropwise at 0 °C under nitrogen to a stirred solution of imine **6** (1.9 g, 6 mmol) and TEA (8.3 mL, 60 mmol) in anhydrous methylene chloride (7 mL). The solution was maintained at 0 °C for 1 h and then allowed to reach room temperature and stirred for



Scheme 1 Reagents and conditions: a) anhydrous EtOH; b) Et₃N/CH₂Cl₂; c) NH₂NH₂·2HCl, Et₃N, MeOH, 60 °C; d) *N,N'*-disuccinimidyl carbonate, Et₃N, CH₃CN; e) NH₂NH₂·2HCl, Et₃N, MeOH, r.t.

24 h. HCl (1 N) was added (40 mL), and the two phases were stirred for 40 min. The aqueous phase was separated and extracted twice with dichloromethane. Organic phases were washed with a saturated solution of NaHCO₃, dried with sodium sulfate, filtered, and concentrated under reduced pressure, affording a brown oil which was purified by flash column chromatography (silica gel; eluent gradient of *n*-hexane/ethyl acetate from 7/3 to 4/6). The isomer (\pm)-*trans*-8 only was isolated with a 65% yield. The isomer (\pm)-*cis*-8 was formed only in traces from the reaction. (\pm)-*trans*-8: ¹H-NMR (CDCl₃) δ 8–7.6 (8H, m), 7.29 (1H, dd, *J*₁ = 8.4 Hz, *J*₂ = 2.2 Hz), 7.19 (1H, d, *J* = 2.2 Hz), 7.00 (1H, d, *J* = 8.4 Hz), 6.57 (2H, s), 5.28 (2H, s), 4.71 (2H, s), 3.85 (3H, s), 3.76 (3H, s), 3.70 (6H, s). ¹³C NMR (CDCl₃): δ 167.46, 166.96, 165.41, 162.03, 140.09, 134.79, 134.53, 133.40, 132.15, 131.86, 128.37, 126.40, 125.08, 124.27, 123.87, 121.47, 113.64, 95.50, 67.78, 61.07, 56.24, 38.89. ESI-MS: *m/z* 691 (M⁺).

Hydrazine dihydrochloride (752.5 mg, 6.223 mmol) was added, at 0 °C and under nitrogen, to a stirred suspension of (\pm)-*trans*-8 (870 mg, 1.245 mmol) in methanol (10 mL). TEA (3.5 mL, 24.89 mmol) was then added dropwise. The mixture was allowed to reach room temperature and then warmed at 50 °C for 5 h. The solvent was removed at reduced pressure, and the residue was treated with 1 N HCl (40 mL) and extracted with dichloromethane (3 × 10 mL). The aqueous phase was made alkaline by 3 N NaOH and extracted with dichloromethane (3 × 10 mL). The organic phase was dried (Na₂SO₄), and the solvent was removed at reduced pressure. A solid was obtained, which was purified by flash chromatography (silica gel; eluent *n*-hexane/ethyl acetate, 2/8) to afford the stereoisomer (\pm)-*trans*-9 (0.437 g, 1.17 mmol, 94% yield) as a yellow solid. ¹H-NMR (CDCl₃): δ 6.92 (1H, s), 6.89 (2H, s), 6.54 (2H, s), 4.54 (1H, d, *J* = 2.2 Hz), 4.05 (1H, d, *J* = 2.2 Hz), 3.88 (3H, s), 3.76 (3H, s), 3.71 (6H, s). ¹³C NMR (CDCl₃): δ 168.05, 153.65, 147.11, 146.51, 134.80, 133.80,

Table 2 *In vitro* inhibition of tubulin polymerization

Compound	IC ₅₀ μM tubulin polymerization
11i	5.05 ± 1.2
CA-4	1.32 ± 0.2

130.14, 118.14, 117.99, 112.33, 111.22, 95.38, 69.70, 66.70, 61.09, 56.22. ESI-MS: *m/z* 374 (M⁺).

General procedure for the preparation of compounds 11a–h and 11j. To a stirred solution of the selected carboxylic acid RCOOH (0.88 mmol) in dry acetonitrile (6 mL), *N,N*-disuccinimidyl carbonate DSC (0.88 mmol) and dry triethylamine (1.603 mmol) were added at 0 °C, under a nitrogen atmosphere. The stirring was continued overnight and 3,4-*trans*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-azetidin-2-one **9** (0.4 mmol) was added. The reaction was monitored by TLC (silica gel, eluted with methylene chloride–methanol), treated with a saturated solution of NaHCO₃, and extracted with methylene chloride. The combined organic extracts were dried (Na₂SO₄) and the solvent was removed under reduced pressure. A mixture of **11a–i** and **10a–i** in varying amounts was obtained (the latter generally detected in the MS and/or NMR spectrum of the crude material) and directly hydrolyzed to **11a–i**.

When Boc-*N*-methyl-L-alanine was used as an acyl donor, a mixture of Boc-protected *N*-acylated and *N,O*-di-acylated derivatives was obtained, directly hydrolyzed to the protected Boc-*N*-acyl derivative and deprotected with trifluoroacetic acid to afford **11j**.

Hydrolysis of the ester group was achieved by addition of hydrazine dichloride (0.174 mmol) and dry triethylamine (0.34 mmol) to a stirred solution of the mixture of **10a–j** and **11a–j** (0.087 mmol) in methanol (4 mL), under a nitrogen atmosphere. The reaction was monitored by TLC analysis (silica-gel). The reaction was then treated with KHSO₄ solution (5%) and extracted with methylene chloride. The combined organic extracts were dried and the solvent was removed under reduced pressure. The crude material was flash chromatographed on silica gel, eluting with a gradient of methylene chloride–methanol affording pure **11a–j**.

***N,O*-(Diacetyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one (10i).** The title compound was prepared as well by addition of acetic anhydride (2 mL) to 3,4-*trans*-3-amino-4-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-azetidin-2-one **1**. Removal of acetic anhydride under reduced pressure afforded the diacyl derivative in quantitative yields.

10i ¹H NMR (300 MHz, CDCl₃): δ ppm 7.19 (dd, 1H, *J* = 8.5, 1.7 Hz), 7.05 (d, 1H, *J* = 1.7 Hz), 6.95 (d, 1H, *J* = 8.5 Hz), 6.80 (d, 1H, *J* = 7.5 Hz), 6.48 (s, 2H), 4.94 (d, 1H, *J* = 1.7 Hz), 4.53 (dd, 1H, *J* = 7.4, 1.7 Hz), 3.81 (s, 3H), 3.75 (s, 3H), 3.69 (s, 6H), 2.28 (s, 3H), 2.06 (s, 3H). ¹³C (300 MHz, CDCl₃): 171.45, 169.45, 164.88, 154.08, 152.16, 140.84, 135.12, 134.10, 129.46, 125.23, 121.82, 113.64, 95.73, 66.55,

63.27, 61.57, 56.67, 23.35, 21.25. MS (ESI): *m/z* 481 (M + Na).

Spectroscopic data of compounds 11a–j

***N*-(4-Chlorobenzoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11a** (65% yield). ¹H NMR (400 MHz, CDCl₃): δ ppm 7.79 (d, 2H, *J* = 7.5 Hz), 7.43 (d, 2H, *J* = 7.5 Hz), 7.23 (d, 1H, *J* = 6.5 Hz, NH), 6.98 (d, 1H, *J* = 2.5 Hz), 6.94 (dd, 1H, *J* = 7.5, 2.5 Hz), 6.88 (d, 1H, *J* = 7.5 Hz), 6.55 (s, 2H), 5.00 (d, 1H, *J* = 2.0 Hz), 4.81 (dd, 1H, *J* = 7.5 Hz, 2.0 Hz), 3.92 (s, 3H), 3.77 (s, 3H), 3.72 (s, 6H). ¹³C (400 MHz, CDCl₃): 168.13, 165.29, 154.31, 147.43, 146.57, 136.15, 135.06, 130.76, 130.86, 129.90, 129.15, 118.92, 116.33, 116.14, 112.57, 111.63, 95.72, 66.83, 63.93, 61.51, 56.25, 56.13, 51.44. MS (ESI): *m/z* 535–537 (M + Na).

10a. MS (ESI): *m/z* 673–675 (M + Na).

***N*-(4-Fluorobenzoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11b** (66% yield). ¹H NMR (400 MHz, CDCl₃): δ ppm 7.90 (dd, 2H, *J* = 8.5, *J*_{H-F} = 5.5 Hz), 7.82 (d, 1H, *J* = 7.4 Hz, NH), 7.08 (dd, 2H, *J* = 8.5, *J*_{H-F} = 8.4 Hz), 6.95 (d, 1H, *J* = 1.5 Hz), 6.90 (dd, 1H, *J* = 8.1, 1.5 Hz), 6.84 (d, 1H, *J* = 8.1 Hz), 6.52 (s, 2H), 5.01 (s, 1H), 4.85 (d, 1H, *J* = 7.4 Hz), 3.89 (s, 3H), 3.74 (s, 3H), 3.68 (s, 6H). ¹³C (400 MHz, CDCl₃): 168.05, 166.71 (*J*_{C-F} = 253 Hz), 165.44, 154.95, 148.63, 147.87, 135.97, 134.87, 131.31 (*J*_{C-F} = 9.1 Hz), 130.77, 130.44, 120.70, 116.98 (*J*_{C-F} = 21.9 Hz), 113.55, 112.41, 95.82, 66.73, 63.73, 61.58, 56.65, 56.64, 51.45. MS (ESI): *m/z* 519 (M + Na). **10b.** MS (ESI): *m/z* 641 (M + Na).

***N*-(4-Methoxybenzoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11c** (64% yield). ¹H NMR (400 MHz, CDCl₃): δ ppm 7.82 (d, 2H, *J* = 7.6 Hz), 6.98 (s, 1H), 6.93 (dd, 1H, *J*₁ = 7.5 Hz), 6.86 (d, 1H, *J* = 7.5 Hz), 6.56 (s, 2H), 5.25 (s, 1H), 4.79 (d, 1H, *J* = 6.4 Hz), 5.85 (d, 1H, *J* = 6.4 Hz, NH), 3.89 (s, 3H), 3.87 (s, 3H), 3.77 (s, 3H), 3.70 (s, 6H). ¹³C (300 MHz, CDCl₃): δ ppm 167.38, 164.82, 162.61, 153.34, 147.27, 146.37, 134.54, 133.43, 129.88, 129.2, 125.04, 118.17, 113.78, 112.49, 111.19, 95.24, 66.2, 63.17, 60.88, 60.03, 55.95, 55.38, 50.55. MS (ESI): *m/z* 531 (M + Na).

10c. MS (ESI): *m/z* 665 (M + Na).

***N*-(3,4,5-Trimethoxybenzoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11d** (60% yield). ¹H NMR (400 MHz, CDCl₃): δ ppm 8.34 (d, 1H, *J* = 6.5 Hz), 7.11 (s, 2H), 6.87 (d, 1H, *J* 0 1.0 Hz), 6.82 (dd, 1H, *J* = 8.8, 1.0 Hz), 6.78 (d, 1H, *J* = 8.8 Hz), 6.50 (s, 2H), 5.02 (d, 1H, *J* = 2.2 Hz), 4.71 (dd, 1H, *J* = 6.5), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 6H), 3.73 (s, 3H), 3.69 (s, 3H), 3.62 (s, 3H). ¹³C (300 MHz, CDCl₃): δ ppm 167.87, 165.82, 154.07, 153.99, 152.34, 148.09, 147.21, 135.08, 134.22, 129.99, 129.74, 128.58, 124.45, 118.75, 113.14, 111.94, 108.12, 105.47, 95.74, 93.33, 66.88, 63.34, 61.55, 56.77, 56.67, 56.70, 51.00. MS (ESI): *m/z* 591 (M + Na).

10d. MS (ESI): *m/z* 785 (M + Na).

***N*-(2-Furoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11e** (76% yield).

^1H NMR (400 MHz, CDCl_3): δ ppm 8.07 (sdd, 1H, $J = 1.8, 0.9$ Hz), 7.53 (dd, 1H, $J = 3.5, 0.9$ Hz), 7.92 and 7.91 (AA' system, 2H, $J = 7.3$ Hz), 6.90 (s, 1H), 6.79 (dd, 1H, $J = 3.5, 1.7$ Hz), 6.61 (s, 2H), 5.10 (s, 1H), 4.72 (s, 1H), 3.87 (s, CH_3), 3.72 (s, CH_3), 3.70 (s, $2 \times \text{CH}_3$). ^{13}C (300 MHz, CDCl_3): δ ppm 165.52, 163.7, 153.91, 153.89, 147.87, 147.02, 146.41, 144.36, 135.02, 133.98, 129.76, 122.15, 118.89, 112.92, 111.83, 109.12, 95.76, 66.11, 63.92, 61.53, 56.59, 56.51. MS (ESI): m/z 491 (M + Na).

10e. MS (ESI): m/z 585 (M + Na).

***N*-(2-Naphthoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11f** (60% yield). ^1H NMR (400 MHz, CDCl_3): δ ppm 8.39 (s, 1H), 8.08 (d, 1H, $J = 5.9$ Hz, NH), 7.90–7.82 (4H), 7.55 (t, 1H, $J = 7.4$ Hz), 7.50 (t, 1H, $J = 7.4$ Hz), 6.97 (s, 1H), 6.89 (d, 1H, $J = 7.5$ Hz), 6.81 (d, 1H, $J = 7.5$ Hz), 6.52 (s, 2H), 5.04 (s, 1H), 4.93 (d, 1H, $J = 5.9$ Hz), 3.85 (s, 3H), 3.73 (s, 3H), 3.65 (s, 6H). ^{13}C (400 MHz, CDCl_3): 168.28, 165.35, 153.98, 147.81, 147.02, 135.58, 135.08, 134.17, 133.15, 130.69, 130.00, 129.79, 129.07, 128.94, 128.58, 128.34, 127.40, 124.25, 118.96, 113.02, 111.79, 95.81, 95.69, 66.87, 63.82, 61.59, 56.63, 56.59, 56.56. MS (ESI): m/z 551 (M + Na).

10f. MS (ESI): m/z 705 (M + Na).

***N*-(Indolo-3-acetyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11g** (75% yield). ^1H NMR (400 MHz, CDCl_3): δ ppm 7.60 (d, 1H, $J = 7.34$ Hz), 7.41 (d, 1H, $J = 7.34$ Hz), 7.28 (s, 1H), 7.26 (t, 1H, $J = 7.34$ Hz), 7.21 (t, 1H, $J = 7.34$ Hz), 7.16 (s, 1H, NH), 6.89 (s, 1H), 6.83 (s, 2H), 6.51 (s, 2H), 6.37 (d, 1H, $J = 6.13$ Hz, NH), 4.86 (s, 1H), 4.51 (d, 1H, $J = 6.13$ Hz), 3.89 (s, 3H), 3.80 (s, 2H), 3.76 (s, 3H), 3.69 (s, 6H). ^{13}C (400 MHz, CDCl_3): 172.69, 164.67, 154.07, 147.61, 146.69, 137.08, 135.3, 134.10, 130.12, 127.57, 123.44, 120.96, 119.24, 118.80, 112.87, 112.72, 112.27, 111.72, 108.87, 95.93, 66.60, 63.50, 61.58, 56.74, 56.70, 33.90. MS (ESI): m/z 554 (M + Na).

10g. MS (ESI): m/z 711 (M + Na).

***N*-(Cyclohexanoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11h** (69% yield). ^1H NMR (400 MHz, CDCl_3): δ ppm 6.92 (d, 1H, $J = 6.7$ Hz, NH), 6.88 (s, 1H), 6.83 (d, 1H, $J = 8.4$ Hz), 6.80 (d, 1H, $J = 8.4$ Hz), 6.44 (s, 2H), 4.87 (d, 1H, $J = 1.5$ Hz), 4.54 (dd, 1H, $J = 6.7, 1.5$ Hz), 3.84 (s, 3H), 3.76 (s, 3H), 3.66 (s, 6H), 2.15 (m, 1H), 1.86 (m, 2H), 1.75 (m, 2H), 1.64 (m, 2H), 1.43 (m, 2H), 1.25 (m, 2H). ^{13}C (400 MHz, CDCl_3): 177.63, 165.29, 153.98, 147.85, 147.02, 135.06, 134.20, 130.06, 118.87, 113.04, 111.84, 95.83, 66.41, 63.79, 61.53, 56.74, 56.68, 56.61, 45.44, 41.17, 30.06, 26.29, 26.24. MS (ESI): m/z 507 (M + Na).

11h. MS (ESI): m/z 617 (M + Na).

***N*-(Acetyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11i** (80% yield). ^1H NMR (400 MHz, CDCl_3): δ ppm 6.95 (s, 1H), 6.89 and 6.87 (AB system, 2H, $J = 7.4$ Hz), 6.55 (s, 2H), 6.33 (d, 1H, $J = 7.5$ Hz, NH), 4.91 (d, 1H, $J = 1.5$ Hz), 4.62 (dd, 1H, $J = 7.4, 1.5$ Hz), 3.91 (s, 3H), 3.86 (s, 3H), 3.72 (s, 6H), 2.10 (s, 3H). ^{13}C (400 MHz, CDCl_3): 171.52, 165.24, 153.95, 153.93, 147.83, 147.02, 134.89, 134.18, 129.88, 118.79, 112.98, 111.80, 95.74, 66.18, 63.80, 61.57, 56.61, 23.36. MS (ESI): m/z : 439 (M + Na).

***N*-(*S*-2-amino-propanoyl)-3,4-*trans*-3-amino-1-(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-azetidin-2-one. 11j** (80% yield). ^1H NMR (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ ppm 8.26 (bs, 2H), 6.91 (s, 1H), 6.84 and 6.79 (AB system, 2H, $J = 8.2$ Hz), 6.52 (s, 2H), 4.92 (s, 1H), 4.59 (s, 1H), 3.84 (s, 3H), 3.75 (s, 3H), 3.66 (s, 6H), 3.65 (m, 1H), 1.35 (d, 3H, $J = 7.5$ Hz). ^{13}C (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): 170.01, 168.29, 154.07, 147.79, 147.10, 135.31, 134.18, 130.32, 118.43, 112.94, 111.80, 96.06, 95.86, 69.87, 66.89, 61.54, 56.67, 56.64, 51.17, 49.99, 20.60. MS (ESI): m/z 446 (M + 1).

***N*-Boc-11j.** ^1H NMR (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): 7.64 (s, 1H), 6.88 (s, 1H), 6.88 and 6.86 (AB system, 2H, $J = 7.4$ Hz), 6.57 (s, 2H), 4.95 (s, 1H), 4.59 (s, 1H), 3.86 (s, 3H), 3.72 (s, 3H), 3.70 (s, 6H), 3.6 (q, 1H, $J = 7.4$ Hz), 1.45 (s, 9H), 1.34 (d, 3H, $J = 7.4$ Hz). MS (ESI): m/z 568 (M + Na).

Biology

Reagents. RPMI-1640 medium, fetal bovine serum (FBS), L-glutamine, penicillin, and streptomycin were obtained from Lonza (Basel, Switzerland). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Cell cultures. Colorectal adenocarcinoma SW48 cells were purchased from ATCC, were cultured using RPMI-1640 medium supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 100 U mL^{-1} penicillin, and 100 $\mu\text{g mL}^{-1}$ streptomycin and were maintained at 37 °C in a humidified 5% CO_2 incubator.

Growth inhibition assay. The antiproliferative activity of the azetidinone derivatives was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 8000 cells were seeded in 96-well plates and treated with different concentrations of each compound (dissolved in DMSO). After 72 h, the MTT solution was added and then plates were incubated for 2 h at 37 °C. The purple formazan crystals were solubilized and the plates were read on a Model 550 microplate reader (BioRad Laboratories, Hercules, CA) at 570 nm. Assays were performed in triplicate in three independent experiments and data were analyzed using Sigma Plot software (using the four parameter logistic equation) to estimate IC_{50} values, defined as the concentration of drug causing 50% inhibition in absorbance compared to control cells (in which 0.1% DMSO was used).

Tubulin polymerization assay. The effect of compounds on tubulin polymerization was determined spectrophotometrically, essentially as previously described.²⁴ Lyophilized purified porcine brain tubulin (Cytoskeleton, Denver, CO) was resuspended in assembly buffer (80 mM PIPES, pH 6.9, 1 mM MgCl_2 , 2 mM EGTA, 10% glycerol) at 2.5 mg mL^{-1} and mixed with 1 mM GTP and varying concentrations of the indicated compounds. DMSO (0.2% v/v) was used as a vehicle control. Tubulin assembly was monitored at 340 nm at 37 °C for 15 minutes on a Jasco V-530 spectrophotometer (Jasco Europe, Italy). The IC_{50} values are the compound concentrations required to inhibit tubulin polymerization by 50% and were estimated using the Sigma Plot software (using the four parameter logistic equation).

Conflicts of interest

The authors declare no competing interest.

Acknowledgements

This work was supported by grants to P. C. and F. O. from MIUR and “Piano Sostegno Ricerca 2015-17-Linea 2-Azione B” from the University of Milano.

Notes and references

- Q.-X. Yue, X. Liu and D.-A. Guo, Microtubule-binding natural products for cancer therapy, *Planta Med.*, 2010, **76**, 1037–1043, DOI: 10.1055/s-0030-1250073.
- C. M. Lin, H. H. Ho, G. R. Pettit and E. Hamel, Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicine to tubulin, *Biochemistry*, 1989, **28**, 6984–6991.
- A. T. McGown and B. W. Fox, Differential cytotoxicity of combretastatins A1 and A4 in two daunorubicin-resistant P388 cell lines, *Cancer Chemother. Pharmacol.*, 1990, **26**, 79–81.
- G. C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca and A. A. Genazzani, Medicinal chemistry of combretastatin A4: present and future directions, *J. Med. Chem.*, 2006, **49**, 3033–3044, DOI: 10.1021/jm0512903.
- G. R. Pettit, S. B. Singh, E. Hamel, C. M. Lin, D. S. Alberts and D. Garcia-Kendall, Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4, *Experientia*, 1989, **45**, 209–211.
- N.-H. Nam, Combretastatin A-4 analogues as antimitotic antitumor agents, *Curr. Med. Chem.*, 2003, **10**, 1697–1722.
- H. Quan, Y. Xu and L. Lou, p38 MAPK, but not ERK1/2, is critically involved in the cytotoxicity of the novel vascular disrupting agent combretastatin A4, *Int. J. Cancer*, 2008, **122**, 1730–1737, DOI: 10.1002/ijc.23262.
- R. Gaspari, A. E. Prota, K. Bargsten, A. Cavalli and M. O. Steinmetz, Structural Basis of *cis*- and *trans*-Combretastatin Binding to Tubulin, *Chem*, 2017, **2**, 102–113, DOI: 10.1016/j.chempr.2016.12.005.
- C. M. L. West and P. Price, Combretastatin A4 phosphate, *Anti-Cancer Drugs*, 2004, **15**, 179–187.
- www.clinicaltrials.gov.
- A. Siebert, M. Gensicka, G. Cholewinski and K. Dzierzbicka, Synthesis of Combretastatin A-4 Analogs and their Biological Activities, *Anti-Cancer Agents Med. Chem.*, 2016, **16**(8), 942–960, DOI: 10.2174/1871520616666160204111832.
- N. R. Madadi, N. R. Penthala, K. Howk, A. Ketkar, R. L. Eoff, M. J. Borrelli and P. A. Crooks, Synthesis and biological evaluation of novel 4,5-disubstituted 2H-1,2,3-triazoles as *cis*-constrained analogues of combretastatin A-4, *Eur. J. Med. Chem.*, 2015, **103**, 123–132, DOI: 10.1016/j.ejmech.2015.08.041.
- T. M. Beale, D. M. Allwood, A. Bender, P. J. Bond, J. D. Brenton, D. S. Charnock-Jones, S. V. Ley, R. M. Myers, J. W. Shearman, J. Temple, J. Unger, C. A. Watts and J. Xian, A-ring dihalogenation increases the cellular activity of combretastatin-templated tetrazoles, *ACS Med. Chem. Lett.*, 2012, **3**, 177–181, DOI: 10.1021/ml200149g.
- T. Liu, X. Dong, N. Xue, R. Wu, Q. He, B. Yang and Y. Hu, Synthesis and biological evaluation of 3,4-diaryl-5-aminoisoxazole derivatives, *Bioorg. Med. Chem.*, 2009, **17**, 6279–6285, DOI: 10.1016/j.bmc.2009.07.040.
- Q. Guan, F. Yang, D. Guo, J. Xu, M. Jiang, C. Liu, K. Bao, Y. Wu and W. Zhang, Synthesis and biological evaluation of novel 3,4-diaryl-1,2,5-selenadiazol analogues of combretastatin A-4, *Eur. J. Med. Chem.*, 2014, **87**, 1–9, DOI: 10.1016/j.ejmech.2014.09.046.
- A. W. Brown, M. Fisher, G. M. Tozer, C. Kanthou and J. P. A. Harrity, Sydnone Cycloaddition Route to Pyrazole-Based Analogs of Combretastatin A4, *J. Med. Chem.*, 2016, **59**, 9473–9488, DOI: 10.1021/acs.jmedchem.6b01128.
- D. Simoni, G. Grisolia, G. Giannini, M. Roberti, R. Rondanin, L. Piccagli, R. Baruchello, M. Rossi, R. Romagnoli, F. P. Invidiata, S. Grimaudo, M. K. Jung, E. Hamel, N. Gebbia, L. Crosta, V. Abbadessa, A. Di Cristina, L. Dusonchet, M. Meli and M. Tolomeo, Heterocyclic and phenyl double-bond-locked combretastatin analogues possessing potent apoptosis-inducing activity in HL60 and in MDR cell lines, *J. Med. Chem.*, 2005, **48**, 723–736, DOI: 10.1021/jm049622b.
- M. K. Gurjar, R. D. Wakharkar, A. T. Singh, M. Jaggi, H. B. Borate, P. D. Shinde, R. Verma, P. Rajendran, S. Dutt, G. Singh, V. K. Sanna, M. K. Singh, S. K. Srivastava, V. A. Mahajan, V. H. Jadhav, K. Dutta, K. Krishnan, A. Chaudhary, S. K. Agarwal, R. Mukherjee and A. C. Burman, Synthesis and evaluation of 4/5-hydroxy-2,3-diaryl(substituted)-cyclopent-2-en-1-ones as *cis*-restricted analogues of combretastatin A-4 as novel anticancer agents, *J. Med. Chem.*, 2007, **50**, 1744–1753, DOI: 10.1021/jm060938o.
- N. Ty, R. Pontikis, G. G. Chabot, E. Devillers, L. Quentin, S. Bourg and J.-C. Florent, Synthesis and biological evaluation of enantiomerically pure cyclopropyl analogues of combretastatin A4, *Bioorg. Med. Chem.*, 2013, **21**, 1357–1366, DOI: 10.1016/j.bmc.2012.11.056.
- D. M. Smith, A. Kazi, L. Smith, T. E. Long, B. Heldreth, E. Turos and Q. P. Dou, A novel beta-lactam antibiotic activates tumor cell apoptotic program by inducing DNA damage, *Mol. Pharmacol.*, 2002, **61**, 1348–1358.
- B. Xing, J. Rao and R. Liu, Novel beta-lactam antibiotics derivatives: their new applications as gene reporters, antitumor prodrugs and enzyme inhibitors, *Mini-Rev. Med. Chem.*, 2008, **8**, 455–471.
- N. M. O'Boyle, M. Carr, L. M. Greene, O. Bergin, S. M. Nathwani, T. McCabe, D. G. Lloyd, D. M. Zisterer and M. J. Meegan, Synthesis and evaluation of azetidinone analogues of combretastatin A-4 as tubulin targeting agents, *J. Med. Chem.*, 2010, **53**, 8569–8584, DOI: 10.1021/jm101115u.
- A. M. Malebari, L. M. Greene, S. M. Nathwani, D. Fayne, N. M. O'Boyle, S. Wang, B. Twamley, D. M. Zisterer and M. J. Meegan, β -Lactam analogues of combretastatin A-4 prevent metabolic inactivation by glucuronidation in chemoresistant

- HT-29 colon cancer cells, *Eur. J. Med. Chem.*, 2017, **130**, 261–285, DOI: 10.1016/j.ejmech.2017.02.049.
- 24 F. Tripodi, R. Pagliarin, G. Fumagalli, A. Bigi, P. Fusi, F. Orsini, M. Frattini and P. Coccetti, Synthesis and biological evaluation of 1,4-diaryl-2-azetidinones as specific anticancer agents: activation of adenosine monophosphate activated protein kinase and induction of apoptosis, *J. Med. Chem.*, 2012, **55**, 2112–2124, DOI: 10.1021/jm201344a.
- 25 S. Valtorta, G. Nicolini, F. Tripodi, C. Meregalli, G. Cavaletti, F. Avezza, L. Crippa, G. Bertoli, F. Sanvito, P. Fusi, R. Pagliarin, F. Orsini, R. M. Moresco and P. Coccetti, A novel AMPK activator reduces glucose uptake and inhibits tumor progression in a mouse xenograft model of colorectal cancer, *Invest. New Drugs*, 2014, **32**, 1123–1133, DOI: 10.1007/s10637-014-0148-8.
- 26 A. P. Suvi, H. M. Rajamaki, L. De Luca and F. Capitta, A telescopic one-pot synthesis of beta-lactam rings using amines as a convenient source of imines, *RSC Adv.*, 2016, **6**, 38553–38557.
- 27 G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J. Comput. Chem.*, 2009, **30**, 2785–2791, DOI: 10.1002/jcc.21256.
- 28 G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function, *J. Comput. Chem.*, 1639, **19**, 1639–1662.
- 29 W. Humphrey, A. Dalke and K. Schulten, VMD: visual molecular dynamics, *J. Mol. Graphics*, 1996, **14**, 33–38, 27–28.