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1,5-Disubstituted 1,2,3-triazoles as *cis*-restricted analogues of combretastatin A-4: Synthesis, molecular modeling and evaluation as cytotoxic agents and inhibitors of tubulin

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Dedicated to Professor Lars Skattebøl on the occasion of his 80th birthday.

Abstract—A series of *cis*-restricted 1,5-disubstituted 1,2,3-triazole analogues of combretastatin A-4 (**1**) have been prepared. The triazole **12f**, 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)aniline, displayed potent cytotoxic activity against several cancer cell lines with IC_{50} values in the nanomolar range. The ability of triazoles to inhibit tubulin polymerization has been evaluated, and **12f** inhibited tubulin polymerization with IC_{50} = 4.8 μM. Molecular modeling experiments involving **12f** and the colchicine binding site of α,β-tubulin showed that the triazole moiety interacts with β-tubulin via hydrogen bonding with several amino acids.

1. Introduction

Microtubules are cylindrical protein polymers composed of α- and β-tubulin heterodimers. Microtubules are essential for many cellular processes, such as maintenance of cellular shape, intracellular transport and mitotic spindle assembly during cell division.[1] Inhibition of microtubule formation leads to mitotic arrest and promotes vascular disruption in angiogenic vessels eventually leading to cell death by apoptosis.[2] Hence, tubulin is one of the most common and strategic targets for development of new anticancer drugs.[3,4]

Combretastatin A-4 (CA-4, **1**) was isolated from the bark of the South African tree *Combretum caffrum* in 1989 by Pettit et al.[5] This *cis*-stilbene binds to the colchicine binding site of tubulin and inhibits tubulin polymerization. Combretastatin A-4 is also highly cytotoxic against a variety of human cancer cell lines, including multidrug resistant cell lines. Moreover, this natural product disrupts the cell signalling pathways involved in regulation and maintenance of the cytoskeleton of endothelial cells in tumor vasculature, thereby leading to selective shutdown of blood flow through tumors. These biological effects and the structural simplicity of **1** make this natural product an attractive lead compound in the development of new anticancer agents.[6] Structure–activity relationship (SAR) studies showed that a 3,4,5-trimethoxysubstituted A ring and a 4-methoxysubstituted B ring separated by a double bond (two-atom bridge) with *cis* configuration are important for optimal cytotoxic activity.[7] The major disadvantages of combretastatin A-4 as a drug candidate are low water solubility and isomerization to the less active *trans*-form. A phosphate prodrug of combretastatin A-4 (CA-4P, **2**)

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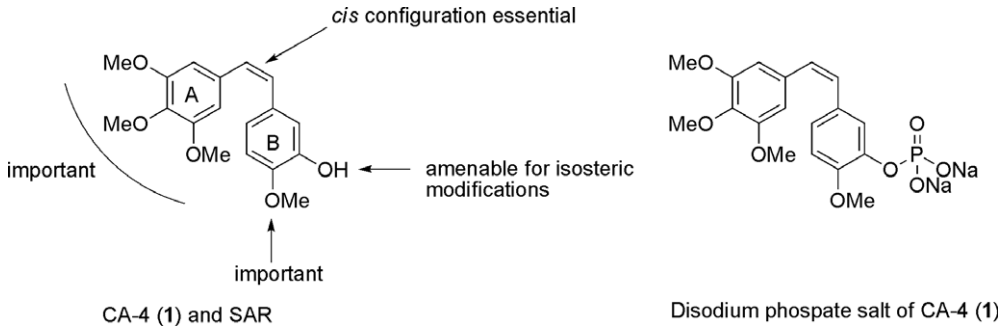


Figure 1. Combretastatin A-4 **1** and its prodrug **2**.

has been prepared, and is currently in clinical trials[8] (Fig. 1).

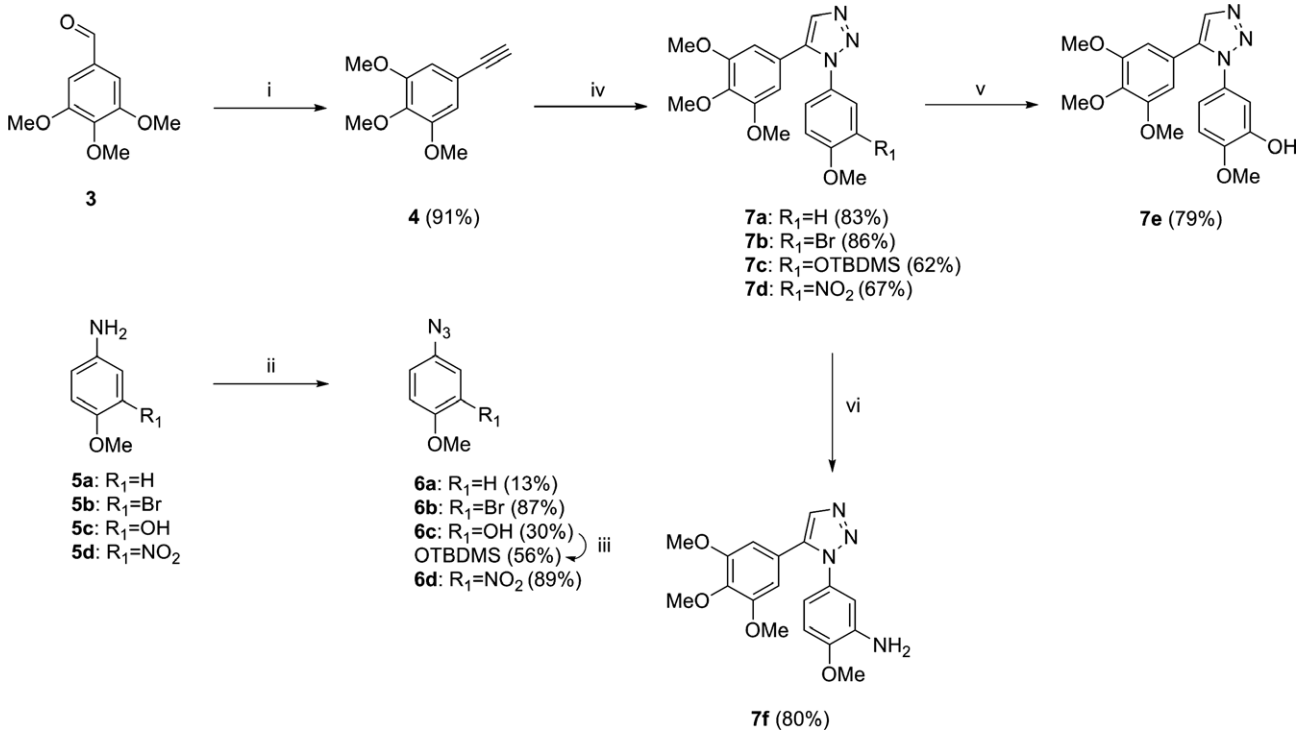
A series of combretastatin analogues with a locked *cis*-type bridge between the two phenyl rings have been prepared to overcome the formation of the less active *trans*-form of **1**. This was achieved by the introduction of a heterocyclic moiety between rings A and B.[9] Hence, we envisioned that a 1,5-disubstituted 1,2,3-triazole heterocyclic ring would serve as a suitable mimic for the *cis* configuration present in **1**. Moreover, some triazole analogues of **1** have been reported.[9a,10] Herein we report the synthesis and in vitro cytotoxic activities of several 1,5-disubstituted 1,2,3-triazole analogues of combretastatin A-4, as well as the inhibition data of tubulin polymerization for the most active compounds. Furthermore, molecular modeling studies of the most active tubulin inhibitor with the colchicine binding site of α,β-tubulin revealed hydrogen-bonding interactions with several amino acids in the colchicine binding site of β-tubulin.

2. Results and discussion

2.1. Synthesis

1,2,3-Triazoles are available from the thermally induced Huisgen cycloaddition reaction between azides and alkynes.[11] This cycloaddition reaction usually affords mixtures of 1,4- and 1,5-disubstituted 1,2,3-triazoles.[12] Recently, the groups of Sharpless and Meldal reported that 1,4-disubstituted 1,2,3-triazoles are specifically prepared from azides and terminal alkynes under copper-(I) catalysis.[13] The regioisomeric 1,5-disubstituted triazoles are available from azides and terminal alkynes by the use of either magnesium acetylides[14] or ruthenium catalysis.[15]

Our synthetic efforts toward the target triazoles **7a**–**7b** and **7d**–**7f** depicted in Scheme 1, started with the synthesis of the terminal alkyne **4** from the aldehyde **3** using the Colvin rearrangement.[16] The azides **6a**–**6d** were obtained



Scheme 1. Synthesis of 1,5-disubstituted triazoles **7a**–**7f**. Reagents and conditions: (i) LDA, $TMSCHN_2$, THF, −78 °C; (ii) HCl (aq), NaN_3, $NaNO_2$, 0 °C; (iii) *n*-Bu_4NF, THF; (iv) a—EtMgCl, THF, Δ; b—**6a**–**6d**; (v) *n*-Bu_4NF, THF; (vi) H_2, Pd/C, EtOH, H_2SO_4.

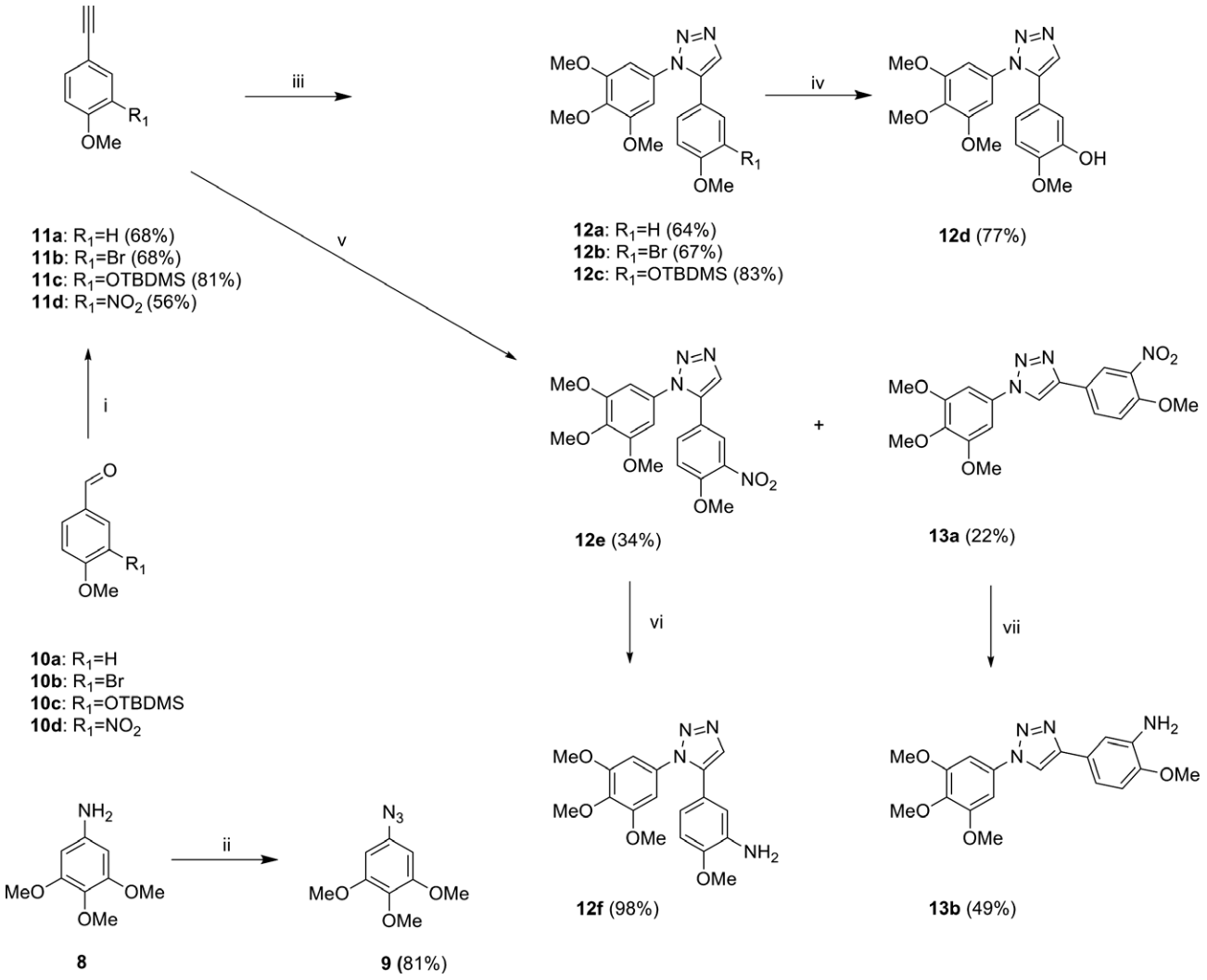
from the anilines **5a**–**5d** employing standard diazotization conditions.[17] The yield of the azide **6a** was improved to 93% yield using a recently published procedure.[18] The terminal alkyne **4** was treated with ethyl magnesium chloride as previously described,[9a] followed by the addition of a THF-solution of the azides **6a**–**6d**. The phenol in azide **6c** was protected in 56% yield as the corresponding TBDMS-ether[19] prior to the cycloaddition reaction. After heating at reflux for 3 h, the 1,5-disubstituted 1,2,3-triazoles **7a**–**7d** were obtained in 62–86% yields after purification by column chromatography. Deprotection[20] of the TBDMS-ether in **7c** yielded triazole **7e** in 79% yield after purification. Reduction of the nitro group in triazole **7d** by catalytic hydrogenation afforded the amino triazole **7f** in 80% yield.

Syntheses of the 1,5-disubstituted 1,2,3-triazoles **12a**–**12b** and **12d**–**12f** followed the chemistry described above (Scheme 2). Triazole **12e** and its 1,4-disubstituted isomer **13a** were obtained from the thermally induced Huisgen cycloaddition reaction[21] between azide **9** and alkyne **11d**, since the magnesium acetylide of alkyne **11d** proved difficult to obtain. Reduction of the nitro group in **12e**–**12f** was achieved by catalytic hydrogenation, while **13a** was reduced to **13b** using $NaBH_4/CuSO_4$.[22] Triazoles **12a**–**b**, **12d**–**12f** and **13a**–**13b** were purified by chromatography or recrystallization. All new compounds exhibited spectral data in agreement with their assigned structures.

2.2. Biological evaluation

All prepared triazoles have the four methoxy groups of the A- and B-rings in the correct positions and a two-atom bridge with a *cis*-similar arrangement between the A- and B-rings required for optimal cytotoxicity. Previous studies have shown that the hydroxyl group on the B-ring of combretastatin A-4 is amenable for bioisosteric substitution[9g,23] (Table 1).

The target triazoles were submitted to the MTT assay[24] for the evaluation of their cytotoxic effects against the K562 leukemia cancer cell line. These results showed that compounds **7a**–**7b** and **7d**–**7f**, in which the three



Scheme 2. Synthesis of triazoles **12a**–**12f** and **13a**–**13b**. Reagents and conditions: (i) LDA, $TMSCHN_2$, THF, −78 °C; (ii) HCl (aq), NaN_3, $NaNO_2$, 0 °C; (iii) a—EtMgCl, THF, Δ; b—**9**; (iv) *n*-Bu_4NF, THF; (v) **11d**, H_2O, EtOH, **9**, Δ; (vi) H_2, Pd/C, MeOH; (vii) $NaBH_4$, $CuSO_4$, EtOH, Δ.

Table 1. Cytotoxicity and inhibition of tubulin polymerization by triazoles **7a**–**7b**, **7d**–**7f**, **12a**–**12b**, **12d**–**12f** and **13a**–**13b**

Compound	K562 cell assay, IC_{50}[a] (μM) in vitro cytotoxicity	Inhibition of tubulin polymerization IC_{50}[b] (μM)
7a	4.00	n.d.[c]
7b	3.60	n.d.[c]
7d	>10	n.d.[c]
7e	0.41	14.0
7f	0.57	>20
12a	0.027	7.0
12b	0.057	>20
12d	0.035	12.6
12e	0.46	>20
12f	0.011	4.8
13a	>10	n.d.[c]
13b	1.30	>20
1	0.010	0.6

[a] Results of three experiments.
[b] Results of two experiments.
[c] n.d., not determined.

N atoms in the triazole moiety were derived from the B-ring azides **6a**–**6d**, were less active than the triazoles derived from the A-ring azide **9**. Triazoles **7e** and **7f** revealed the best cytotoxic activities with IC_{50} = 0.41 and 0.57 μM, respectively. For the regioisomeric triazoles **12a**–**12b** and **12d**–**12f**, cytotoxic activities in the nano- to micromolar range were observed for all compounds; **12a** and **12f** showed best cytotoxic activities with IC_{50} = 27 and 11 nM, respectively (**1**, IC_{50} = 10 nM). In triazoles **12a** and **12f** the 3′-hydroxyl group in the lead compound was replaced by a hydrogen atom and an amino group, respectively. Triazole **12d** with a hydroxyl group in the 3′-position on ring B was less active (IC_{50} = 35 nM) than both **12a** and **12f**. The structure of **12f** is more similar to the known 3′-amino analogue of **1**, and this compound has been reported to exhibit increased activity compared to **1** in terms of in vitro cytotoxicity against some human cancer cell lines.[25] Apparently, the position of the 1,2,3-triazole moiety is important, since triazoles **12a** and **12f** were more active than their regioisomers **7a** and **7f**. Interestingly, triazole **13b**, which is the 1,4-regioisomer of **12f**, also exhibited cytotoxic activity (IC_{50} = 1.3 μM) in this assay.

The triazoles that exhibited significant cytotoxic activities in the K562 cell assay were further evaluated for their ability to inhibit tubulin assembly.[26] Triazoles **12a** and **12f** showed inhibition of tubulin assembly with IC_{50} = 7.0 and 4.8 μM, respectively, while the other compounds tested showed IC_{50} values >10 μM. However, the lead compound combretastatin A-4 (**1**) inhibited tubulin assembly with IC_{50} = 0.6 μM. Hence, the antimitotic effects of these triazoles were not investigated further, but triazole **12f** and combretastatin A-4 (**1**) were submitted to several other cancer cell lines (Table 2).

These data showed that both **12f** and combretastatin A-4 (**1**) were cytotoxic in all six human cancer cell lines with comparable activities with IC_{50} values ranging between 3.9–5.1 nM and 2.5–6.0, respectively.

2.3. Molecular modeling

Molecular modeling studies were performed to investigate the binding ability of the triazoles to the colchicine binding site of α,β-tubulin. Docking studies showed that triazole **12f** occupied the colchicine binding site of α,β-tubulin (mostly buried in the β subunit) (Fig. 2a). The binding energies of **12f** for α- and β-tubulin were about 6 and −10 kcal/mol, respectively. The trimethoxyphenyl moiety in ring A of triazole **12f** was positioned in the binding cavity surrounded by the strands S8 and S9, and the helices H7 and H8 of β-tubulin. The thiol group of βC241 in H7 formed a hydrogen bond with the oxygen atom of one of the methoxy groups, and was close (∼4 Å) to the oxygen of another methoxy group. Several amino acids of β-tubulin formed hydrophobic interactions with the trimethoxyphenyl moiety of **12f**. βL248 and βL255 exhibited particular strong van der Waals interactions (distance <3.7 Å) with the phenyl ring (ring A) of the trimethoxyphenyl moiety, while βL242, βI378, and βV318 interacted with the methyl group of the methoxy moieties in ring A (Fig. 2b).

The *N*-2 and *N*-3 atoms of the 1,2,3-triazole ring of **12f** formed hydrogen bonds with the main chain nitrogen atoms of βA250 and βD251 in loop T7 that connects H7 and H8 of β-tubulin. The amino group of the 3-amino-4-methoxy phenyl moiety formed hydrogen bonds with the main chain carbonyl oxygen atom of αT179 of loop T5 (the loop connecting strand 5 and helix 5 of α-tubulin). The methoxy oxygen atom in ring B of **12f** formed hydrogen bonds with the main chain nitrogen atom of αV181. The side chain of βK352 was close both to the amino group and the methoxy oxygen atom (3.8 Å and 4.2 Å, respectively), indicating the possibility of hydrogen bonds with both groups. The side chains of αV181 and βM259 and βK352 (Cγ atom) formed strong van der Waals contacts (distances of 3.5 Å, 3.8 Å, and 3.6 Å, respectively) with the methoxy methyl group.

The binding energies of **12f** with different tubulin subunits indicate that β-tubulin is responsible for the strength of the binding. The suggested binding mode of triazole **12f** is in agreement with the X-ray structure complex of DAMA-colchicine–tubulin[27] (Fig. 2a), a

Table 2. IC_{50} values (nM)[a] for in vitro cytotoxicity data of CA-4 (**1**) and **12f**

Compound	MDA-MB231 cell assay	SK-BR 3 cell assay	SKOV cell assay	OVCAR cell assay	WM35 cell assay	WM239 cell assay
1	6.00	5.10	3.30	3.30	2.50	2.80
12f	>10	4.00	4.90	4.70	3.90	5.10

[a] Data are medians of three experiments.

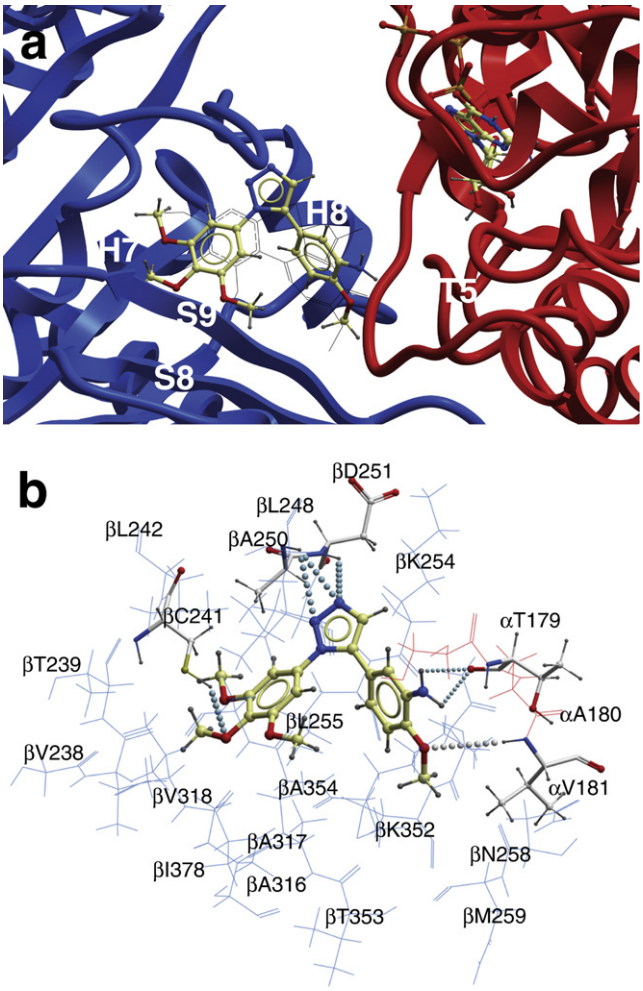


Figure 2. The structure of **12f** docked into the colchicine binding site of tubulin (PDB entry 1SA0). (a) Superposition of the docked conformation of **12f** (stick model with atom type color: carbon-white, nitrogen-blue, oxygen-red, hydrogen-gray) on top of the X-ray structure of DAMA-colchicine (black wire model). The backbone of tubulin is shown as ribbon representation (α-tubulin: red, and β-tubulin: blue). Secondary structures important for ligand binding are labeled. GTP is shown as stick model within α-tubulin. (b) A closer view of **12f** at the binding site. Triazole **12f** and its hydrogen bonding partners are shown as stick models. Hydrogen bonds (distance ⩽3.5 Å) are shown as dotted lines. The amino acids of tubulin within 4.2 Å from **12f** are shown as wire models.

study of 1,2,4-triazole-based tubulin inhibitors,[9a] and with a suggested structure-based pharmacophore model of tubulin.[28] In agreement with the first two studies, different moieties (trimethoxyphenyl, triazole, and 3-amino-4-methoxyphenyl) of **12f** occupied a Cartesian space corresponding to that of colchicine. The binding mode is also in agreement with the pharmacophore model which identified specific receptor–ligand hydrogen bonds and hydrophobic interactions.[27] In addition, we observed the following interactions which have not been reported previously: (a) main chain carbonyl oxygen of αT179 participated in hydrogen bonds to **12f**; (b) strong hydrophobic interactions of several β-tubulin amino acids (βL248, βL255, βL242, βI378, and βV318) to the trimethoxyphenyl moiety; and (c) strong polar and hydrophobic interactions of βK352 to **12f**.

We are currently preparing other triazole mimics of the combretastatins, and these efforts, together with their biological evaluation and docking studies, will hopefully further elucidate the importance of the 1,2,3-triazole moiety with regard to tubulin inhibition and cytotoxicity. These efforts will be reported in due course.

3. Conclusion

Compound **12f** exhibited potent cytotoxic activity in several cancer cell lines as well as moderate inhibition of tubulin polymerization. These activities are most likely, at least in part, due to binding to the colchicine binding site of α,β-tubulin in the β subunit, as supported by our molecular modeling studies.

4. Experimental

4.1. General procedure

Unless noted otherwise, all reagents and solvents were used as purchased without further purification. Melting points are uncorrected. Analytical TLC was performed using silica gel 60 F_{254} glass plates (Merck). Flash column chromatography was performed on silica gel 60 (40–63 μm, Fluka). NMR (^{1}H, ^{13}C) spectra were recorded on a Bruker DPX-300 MHz spectrometer. Coupling constants (J) are reported in hertz, and chemical shifts are reported in parts per million (δ) relative to $CDCl_3$ (7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C) or DMSO-d_6 (2.50 ppm for ^{1}H and 39.43 ppm for ^{13}C). Intermediates **4**,[29] **6a**,[30] **6d**,[31] **9**,[32] **10c**,[33] **11a**,[34] and **11c**[35] are known compounds. Combretastatin A-4 (**1**) was prepared according to a literature procedure.[19b]

4.2. Syntheses of azides: general procedure

The corresponding aniline (10.00 mmol) was suspended in HCl/H_2O (1:1, 34 mL) and cooled to 0 °C. An aqueous solution of sodium nitrite (811 mg, 11.75 mmol) was added dropwise, and the reaction mixture stirred at 0 °C for 1 h. An aqueous solution of sodium azide (780 mg, 11.96 mmol) was added dropwise (caution!). The reaction mixture was stirred at room temperature for 3 h, and extracted with ethyl acetate (2× 150 mL). The combined organic layers were washed with brine (2× 100 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo. The azides were used as such without purification.

4.2.1. 1-Azido-4-methoxybenzene (6a). Brown oil (13%). R_f = 0.89 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.74 (s, 3H), 6.96–7.00 (m, 2H), 7.02–7.06 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.30, 115.23, 120.06, 131.32, 156.69. MS (EI): 150.0 (M+1).

4.2.2. 4-Azido-2-bromo-1-methoxybenzene (6b). Dark brown oil (87%). R_f = 0.82 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.87 (s, 3H), 6.86 (d, J = 8.8 Hz, 1H), 6.94 (dd, J = 8.8, 2.6 Hz, 1H), 7.23 (d,

J = 2.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.54, 112.51, 112.67, 118.82, 123.86, 133.27, 153.35. HRMS calcd for $C_7H_6BrN_3O$ (M^+): 226.9694. Found 226.9691.

4.2.3. 5-Azido-2-methoxyphenol (6c). Brown oil (30%). R_f = 0.88 (hexane/EtOAc 1:5). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.74 (s, 3H), 6.48–6.52 (m, 2H), 6.94 (dd, J = 7.4, 1.5 Hz, 1H), 9.40 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.86, 106.34, 109.23, 113.31, 131.57, 145.40, 147.70. HRMS calcd for $C_7H_7N_3O_2$ (M^+): 165.0538. Found 165.0539.

4.2.4. (5-Azido-2-methoxyphenoxy)(*tert*-butyl)dimethylsilane (6c-protected). Brown semisolid (56%). R_f = 0.83 (hexane/EtOAc 2:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.13 (s, 6H), 0.95 (s, 9H), 3.75 (s, 3H), 6.52 (d, J = 2.7 Hz, 1H), 6.71 (dd, J = 8.7, 2.7 Hz, 1H), 7.02 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = −4.85, 18.04, 25.41, 55.59, 111.42, 112.11, 113.40, 131.49, 145.17, 148.18. HRMS calcd for $C_{13}H_{21}N_3O_2Si$ (M^+): 279.1403. Found 279.1404.

4.2.5. 4-Azido-1-methoxy-2-nitrobenzene (6d). Brown solid (89%). Mp 80 °C. R_f = 0.69 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.91 (s, 3H), 7.36–7.44 (m, 2H), 7.64 (d, J = 2.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.89, 115.43, 115.73, 124.75, 131.89, 139.52, 148.98. HRMS calcd for $C_7H_6N_4O_3$ (M^+): 194.0440. Found 194.0439.

4.2.6. 5-Azido-1,2,3-trimethoxybenzene (9). Dark red solid (81%). Mp 42–45 °C. R_f = 0.81 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.62 (s, 3H), 3.78 (s, 6H), 6.40 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.94, 60.02, 96.68, 134.78, 134.87, 153.66. HRMS calcd for $C_9H_{11}N_3O_3$ (M^+): 209.0800. Found 209.0795.

4.3. Syntheses of 1,5-disubstituted 1,2,3-triazoles: general procedure

The corresponding terminal alkyne (0.79 mmol) dissolved in dry THF (1 mL) was added dropwise to an oven dried flask containing a solution of EtMgCl in dry THF (0.36 mL, 2.0 M) under argon at room temperature. After the alkyne was added, the solution was heated to 50 °C for 15 min and cooled to room temperature. The corresponding azide (0.72 mmol) dissolved in dry THF (1 mL) was added dropwise. The reaction mixture was heated to 50 °C for 3 h. After quenching with aqueous NH_4Cl (4 mL), the product was extracted with dichloromethane (3× 40 mL). The combined organic layers were washed with aqueous NH_4Cl (2× 60 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo.

4.3.1. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7a). The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.27) affording a pale orange solid (83%). Mp 98–99 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.61 (s, 6H), 3.66 (s, 3H), 3.82 (s, 3H), 6.57 (s, 2H), 7.10 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 9.0 Hz, 2H), 8.13 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.69, 55.71, 59.98, 105.91, 114.48, 121.47, 127.37, 129.17, 132.50, 137.61, 137.85, 152.74, 159.82. HRMS calcd for $C_{18}H_{19}N_3O_4$ (M^+): 341.1376. Found 341.1379.

4.3.2. 1-(3-Bromo-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7b). The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.26) affording a pale yellow solid (86%). Mp 186–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.64 (s, 6H), 3.66 (s, 3H), 3.91 (s, 3H), 6.62 (s, 2H), 7.26 (d, J = 8.9 Hz, 1H), 7.45 (dd, J = 8.8, 2.6 Hz, 1H), 7.82 (d, J = 2.5 Hz, 1H), 8.13 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.79, 56.65, 60.01, 106.17, 110.35, 112.59, 121.25, 126.80, 129.65, 130.42, 132.60, 137.85, 138.01, 152.77, 156.17. HRMS calcd for $C_{18}H_{18}BrN_3O_4$ (M^+): 419.0481. Found 419.0480.

4.3.3. 1-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7c). The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.42) affording a pale orange solid (62%). Mp 129–130 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.01 (s, 6H), 0.86 (s, 9H), 3.62 (s, 6H), 3.65 (s, 3H), 3.82 (s, 3H), 6.57 (s, 2H), 6.74 (d, J = 2.1 Hz, 1H), 7.12 (dd, J = 8.7, 2.2 Hz, 1H), 7.16 (d, J = 8.7 Hz, 1H), 8.06 (s, 1H). ^{13}C NMR (75 MHz,, DMSO-d_6): δ = −5.17, 17.96, 25.27, 55.68, 59.86, 106.06, 112.36, 117.90, 119.39, 121.64, 128.94, 132.58, 137.54, 137.89, 144.21, 151.39, 152.80. HRMS calcd for $C_{24}H_{33}N_3O_5Si$ (M^+): 471.2189. Found 471.2192.

4.3.4. 1-(4-Methoxy-3-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7d). The crude product was purified by chromatography (hexane/EtOAc 1:5, R_f = 0.45) affording a yellow solid (67%). Mp 192–193 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.65 (s, 6H), 3.67 (s, 3H), 3.99 (s, 3H), 6.64 (s, 2H), 7.52 (d, J = 9.1 Hz, 1H), 7.74 (dd, J = 9.0, 2.7 Hz, 1H), 8.15–8.16 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.84, 57.18, 60.02, 106.32, 115.09, 121.02, 122.62, 128.32, 131.64, 132.86, 138.07, 138.14, 138.82, 152.26, 152.87. HRMS calcd for $C_{18}H_{18}N_4O_6$ (M^+): 386.1226. Found 386.1226.

4.3.5. 5-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (12a). The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.28) affording an orange solid (64%). Mp 126 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.68 (s, 6H), 3.72 (s, 3H), 3.76 (s, 3H), 6.74 (s, 2H), 6.98 (d, J = 8.9 Hz, 2H), 7.27 (d, J = 8.9 Hz, 2H), 8.04 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.18, 56.10, 60.13, 103.70, 114.14, 118.41, 129.74, 131.91, 132.26, 137.50, 137.87, 152.95, 159.72. HRMS calcd for $C_{18}H_{19}N_3O_4$ (M^+): 341.1376. Found 341.1382.

4.3.6. 5-(3-Bromo-4-methoxyphenyl)-1-(3,4,5-tri-methoxyphenyl)-1*H*-1,2,3-triazole (12b). The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.22) affording a pale yellow solid (67%). Mp 76–78 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.71 (s, 6H), 3.72 (s, 3H), 3.85 (s, 3H), 6.80 (s, 2H), 7.14 (d, J = 8.7 Hz, 1H), 7.27 (dd, J = 8.6, 2.2 Hz, 1H), 7.63 (d,

J = 2.2 Hz, 1H), 8.13 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.19, 56.32, 60.16, 104.00, 110.54, 112.58, 119.84, 129.04, 131.69, 132.60, 132.67, 136.23, 138.09, 152.99, 155.82. HRMS calcd for $C_{18}H_{18}BrN_3O_4$ (M^+): 419.0481. Found 419.0467.

4.3.7. 5-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxy-phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (12c). The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.41) affording a brown semi-solid (83%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = −0.05 (s, 6H), 0.84 (s, 9H), 3.68 (s, 6H), 3.71 (s, 3H), 3.77 (s, 3H), 6.60 (d, J = 2.0 Hz, 1H), 6.74 (s, 2H), 7.06 (d, J = 8.5 Hz, 1H), 7.11 (dd, J = 8.4, 2.0 Hz, 1H), 8.04 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = −5.14, 17.85, 25.27, 55.40, 56.02, 59.93, 103.80, 112.51, 118.57, 119.89, 122.48, 131.99, 132.05, 137.32, 137.96, 143.95, 151.28, 153.09. HRMS calcd for $C_{24}H_{33}N_3O_5Si$ (M^+): 471.2189. Found 471.2183.

4.4. Deprotection of TBDMS-ethers: general procedure

Tetra-*n*-butyl ammonium fluoride in THF (1 M, 1.9 mL, 1.9 mmol) was added dropwise to a solution of the corresponding TBDMS-ether (1.44 mmol) in dry THF (2.9 mL). The reaction mixture was stirred at room temperature for 3 h and then treated with water. The mixture was extracted with dichloromethane (3× 30 mL). The combined organic layers were washed with water (60 mL) and brine (2× 60 mL), and dried over anhydrous magnesium sulfate and the solvent removed in vacuo.

4.4.1. 2-Methoxy-5-(5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenol (7e). The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.28) affording a white solid (79%). Mp 202–203 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.62 (s, 6H), 3.66 (s, 3H), 3.82 (s, 3H), 6.59 (s, 2H), 6.82–6.86 (m, 2H), 7.06 (d, J = 8.9 Hz, 1H), 8.11 (s, 1H), 9.61 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.72, 55.80, 59.99, 105.82, 112.04, 113.06, 116.77, 121.53, 129.19, 132.44, 137.37, 137.87, 146.88, 148.57, 152.74. HRMS calcd for $C_{18}H_{19}N_3O_5$ (M^+): 357.1325. Found 357.1323.

4.4.2. 2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)phenol (12d). The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.39) affording a pale yellow solid (77%). Mp 171–172 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.69 (s, 6H), 3.72 (s, 3H), 3.77 (s, 3H), 6.74–6.76 (m, 4H), 6.95 (d, J = 9.0 Hz, 1H), 7.98 (s, 1H), 9.25 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.08, 56.10, 60.11, 103.62, 112.07, 115.34, 118.62, 119.63, 131.98, 132.19, 137.64, 137.87, 146.36, 148.39, 152.93. HRMS calcd for $C_{18}H_{19}N_3O_5$ (M^+): 357.1325. Found 357.1330.

4.5. Synthesis of aminosubstituted triazole 7f

4.5.1. 2-Methoxy-5-(5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)aniline (7f). 1-(4-Methoxy-3-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**7d**) (386 mg, 1 mmol) and Pd/C 10% (107 mg) were added to EtOH/3 M H_2SO_4 9:1 (25 mL). The mixture was stirred under hydrogen at room temperature for 64 h. The mixture was filtered through Celite. Aqueous NaOH (4 M) was added to pH 10, followed by extraction with ethyl acetate (4× 30 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent removed in vacuo affording a pale brown solid (80%). Mp 73–74 °C. R_f = 0.21 (hexane/EtOAc 1:2). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.62 (s, 6H), 3.66 (s, 3H), 3.82 (s, 3H), 5.13 (br s, 2H), 6.55 (dd, J = 8.4, 2.6 Hz, 1H), 6.61 (s, 2H), 6.66 (d, J = 2.5 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 8.11 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.61, 55.70, 60.00, 105.61, 110.28, 110.56, 113.27, 121.63, 129.67, 132.27, 137.21, 137.82, 138.62, 146.79, 152.73. HRMS calcd for $C_{18}H_{20}N_4O_4$ (M^+): 356.1485. Found 356.1478.

4.6. Syntheses of alkynes: general procedure

Trimethylsilyldiazomethane (9.4 mL, 18.8 mmol, 2.0 M hexane solution) was added dropwise to a solution of lithium diisopropylamide (11.5 mL, 20.7 mmol, 1.8 M in heptane/THF/ethylbenzene) at −78 °C under argon, and the mixture was stirred at −78 °C for 1 h. A solution of the corresponding benzaldehyde (15.0 mmol) in dry THF (33 mL) was then added dropwise at −78 °C. The mixture was stirred at −78 °C for 1 h, then at room temperature for 2 h. The reaction was quenched with brine (15 mL), and the mixture extracted with ethyl acetate (4× 30 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous magnesium sulfate and the solvent removed in vacuo.

4.6.1. 5-Ethynyl-1,2,3-trimethoxybenzene (4). The crude product was purified by chromatography (hexane/EtOAc 2:1, R_f = 0.65) affording a yellow solid (91%). Mp 69–70 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.03 (s, 1H), 3.85 (s, 9H), 6.73 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.13, 60.93, 76.19, 83.68, 109.34, 117.00, 139.28, 153.03. HRMS calcd for $C_{11}H_{12}O_3$ (M^+): 192.0786. Found 192.0783.

4.6.2. 1-Ethynyl-4-methoxybenzene (11a). The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.72) affording a yellow oil (68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.00 (s, 1H), 3.81 (s, 3H), 6.85 (d, J = 8.9 Hz, 2H), 7.44 (d, J = 8.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.25, 75.76, 83.62, 113.89, 114.10, 133.55, 159.88. MS (EI): 133.0 (M+1).

4.6.3. 2-Bromo-4-ethynyl-1-methoxybenzene (11b). The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.62) affording a white solid (68%). Mp 90–91 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.02 (s, 1H), 3.91 (s, 3H), 6.83 (d, J = 8.5 Hz, 1H), 7.41 (dd, J = 8.5, 2.0 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.27, 76.87, 82.06, 111.28, 111.42, 115.56, 132.58, 136.77, 156.37. HRMS calcd for C_9H_7BrO (M^+): 209.9680. Found 209.9686.

4.6.4. *tert*-Butyl(5-ethynyl-2-methoxyphenoxy)dimethylsilane (11c). 3-(*tert*-Butyldimethylsilyloxy)-4-methoxybenzaldehyde (**10c**) was prepared as previously describe-

d.[19a] The crude product was purified by chromatography (petroleum ether/EtOAc 4:1, $R_f = 0.54$) affording a pale yellow oil (96%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.17$ (s, 6H), 1.00 (s, 9H), 3.89 (s, 3H), 6.95 (d, $J = 8.3$ Hz, 1H), 7.36 (d, $J = 2.0$ Hz, 1H), 7.47 (dd, $J = 8.3$, 2.0 Hz, 1H), 9.82 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = -4.65$, 18.41, 25.63, 55.56, 111.16, 120.07, 126.23, 130.20, 145.55, 156.58, 190.87. MS (EI): 267.2 (M+1). **10c** was used for preparing **11c** according to the general procedure. The crude product was purified by chromatography (petroleum ether/EtOAc 8:1, $R_f = 0.76$) affording a yellow oil (81%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.16$ (s, 6H), 1.00 (s, 9H), 2.97 (s, 1H), 3.81 (s, 3H), 6.77 (d, $J = 8.4$ Hz, 1H), 6.98 (d, $J = 2.0$ Hz, 1H), 7.09 (dd, $J = 8.3$, 2.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = -4.68$, 18.42, 25.67, 55.38, 75.43, 83.69, 111.65, 114.19, 124.46, 126.31, 144.66, 152.04. HRMS calcd for $C_{15}H_{22}O_2Si$ (M^+): 262.1389. Found 262.1393.

4.6.5. 4-Ethynyl-1-methoxy-2-nitrobenzene (11d). The crude product was purified by chromatography (hexane/EtOAc 8:3, $R_f = 0.42$) affording a yellow solid (56%). Mp 101–102 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.08$ (s, 1H), 3.98 (s, 3H), 7.04 (d, $J = 8.7$ Hz, 1H), 7.64 (dd, $J = 8.7$, 2.1 Hz, 1H), 7.96 (d, $J = 2.1$ Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 56.66$, 78.03, 80.95, 113.52, 114.58, 129.28, 137.56, 139.38, 153.03. HRMS calcd for $C_9H_7NO_3$ (M^+): 177.0426. Found 177.0423.

4.7. Huisgen cycloaddition reaction

A mixture of azide **9** (628 mg, 3.0 mmol) and alkyne **11d** (532 mg, 3.0 mmol) was dissolved in ethanol (15 mL). The mixture was refluxed for 24 h. Water (5 mL) was added and the mixture was refluxed for another 24 h. The precipitate was washed with ether. The filtrate was concentrated in vacuo. The products were purified by chromatography (hexane/EtOAc 1:1).

4.7.1. 5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (12e). Pale yellow solid (34%). Mp 79–80 °C. $R_f = 0.13$ (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.71$ (s, 6H), 3.72 (s, 3H), 3.93 (s, 3H), 6.83 (s, 2H), 7.42 (d, $J = 8.9$ Hz, 1H), 7.56 (dd, $J = 8.8$, 2.3 Hz, 1H), 7.93 (d, $J = 2.2$ Hz, 1H), 8.21 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 56.20$, 56.86, 60.13, 104.07, 114.68, 118.50, 124.83, 131.44, 132.92, 133.99, 135.67, 138.21, 138.92, 152.15, 153.08. HRMS calcd for $C_{18}H_{18}N_4O_6$ (M^+): 386.1226. Found 386.1221.

4.7.2. 4-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (13a). Yellow solid (22%). Mp 229–230 °C. $R_f = 0.35$ (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.73$ (s, 3H), 3.90 (s, 6H), 3.99 (s, 3H), 7.25 (s, 2H), 7.53 (d, $J = 8.9$ Hz, 1H), 8.20 (dd, $J = 8.8$, 2.2 Hz, 1H), 8.37 (d, $J = 2.2$ Hz, 1H), 9.34 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 56.20$, 56.80, 60.13, 97.81, 115.13, 119.93, 121.40, 122.81, 130.84, 132.27, 137.38, 139.35, 144.99, 151.73, 153.46. HRMS calcd for $C_{18}H_{18}N_4O_6$ (M^+): 386.1226. Found 386.1234.

4.8. Syntheses of amino substituted triazoles 12f and 13b

4.8.1. 2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)aniline (12f). 5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**12e**) (200 mg, 0.5 mmol) and Pd/C 10% (56 mg) were dissolved in methanol (15 mL). The mixture was stirred under hydrogen (balloon) at room temperature overnight. The mixture was filtered through Celite and the solvent removed in vacuo affording a white solid (98%). Mp 165–166 °C. $R_f = 0.13$ (hexane/EtOAc 1:2). ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (s, 6H), 3.72 (s, 3H), 3.76 (s, 3H), 4.88 (br s, 2H), 6.46 (dd, $J = 8.2$, 2.1 Hz, 1H), 6.63 (d, $J = 2.1$ Hz, 1H), 6.72 (s, 2H), 6.81 (d, $J = 8.3$ Hz, 1H), 7.90 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.26$, 56.05, 60.11, 103.33, 110.30, 113.19, 116.45, 118.64, 132.06, 132.10, 137.75, 137.80, 138.22, 146.87, 152.85. HRMS calcd for $C_{18}H_{20}N_4O_4$ (M^+): 356.1485. Found 356.1485.

4.8.2. 2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)aniline (13b). Saturated copper sulfate solution (0.9 mL) was added to 4-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**13a**) (750 mg, 1.94 mmol) dissolved in ethanol (11.5 mL). The mixture was cooled to 0 °C, and a solution of sodium borohydride (435 mg, 11.5 mmol) in ethanol/H_2O 1:1 (11.5 mL) was added dropwise to the reaction mixture. The mixture was refluxed for 4 h. After cooling, saturated copper sulfate solution (0.9 mL) and a solution of sodium borohydride (435 mg, 11.5 mmol) were added. The mixture was refluxed overnight. After cooling, ethyl acetate was added and the mixture extracted with 1 M HCl (3× 45 mL). The pH of the combined aqueous layers was adjusted to pH 10 using 4 M NaOH, and extracted with ethyl acetate (3× 50 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Recrystallization in methanol and ether afforded a pale brown solid (49%). Mp 166–168 °C. $R_f = 0.25$ (hexane/EtOAc 1:2). ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.72$ (s, 3H), 3.81 (s, 3H), 3.90 (s, 6H), 4.88 (br s, 2H), 6.90 (d, $J = 8.4$ Hz, 1H), 7.08 (dd, $J = 8.2$, 2.1 Hz, 1H), 7.26 (s, 2H), 7.28 (d, $J = 2.1$ Hz, 1H), 9.05 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.27$, 56.20, 60.11, 97.70, 110.61, 113.58, 118.37, 122.91, 132.53, 137.11, 137.88, 146.43, 147.81, 153.42. HRMS calcd for $C_{18}H_{20}N_4O_4$ (M^+): 356.1485. Found 356.1477.

4.9. Biological assays

4.9.1. Cancer cell growth inhibition on K562 human leukemia cell line. The method applied was that described by Edmondson et al.[24] K562 human chronic myelogeneous leukemia cells were cultivated in RMPI medium, free of antibiotics and containing 2-mercaptoethanol (2 μM) and L-glutamine (2 mM), supplemented with fetal calf serum (FCS) (10% v/v). The cells were adjusted to a concentration depending on their observed doubling time, (ca 40,000 cells/mL), in RPMI medium supplemented with FCS (10% v/v). The candidate drug was dissolved in DMSO. A drug solution of 100 μl in medium was added to 100 μl of cell

solution (40,000 cells/mL) in a 96-well microtiter testplate (4 μl of the drug solution diluted in medium in order to reach decreasing concentrations). This series of dilutions was continued to afford samples at different concentrations leaving one cell solution free of drug acting as a control. The plates were incubated at 37 °C (5% CO_2 in air) for 5 days. The plate was then removed from the incubator and 50 μl of a solution of MTT (3 mg/mL in PBS) was added to each well. After incubation (37 °C, 5% CO_2 in air, 3 h) the medium was carefully removed from each well by suction and the resulting formazan precipitate redissolved in 200 μl DMSO. The optical density of each well was read at two wavelengths (λ 540 and 690 nm) using a Titretek Multiscan MCC/340 platereader. After processing and analysis through the application of an 'in-house' software package, the results enabled the calculation of the drug dose required to inhibit cell growth by 50% (IC_{50} value), determined by graphical means as percentage of the control growth.

4.9.2. Inhibition of tubulin assembly. The method applied was that described by Lawrence et al.[26] Tubulin was isolated from porcine brain and stored at −78 °C. Samples were prepared directly in a 96-well microtiter testplate that was preincubated at 4 °C in the fridge for 30 min and contained Mes buffer [128 μl (0.1 M Mes, 1 mM EGTA, 0.5 mM $MgCl_2$, distilled water, pH 6.6)], GTP (20 μl, 5 mM in Mes buffer), tubulin (50 μl, 11 mg/mL in Mes buffer) and the candidate drug (20 μl, C_{sample} in DMSO). The tubulin/drug samples were immediately placed in a 96-well plate reader, alongside blank samples containing Mes buffer (198 μl) and the candidate drug (10 μl, same concentration). The absorbance (λ 350 nm) was recorded at 25 °C temperature for a period of 60 min, and the results were compared to untreated controls to evaluate the relative degree of change in optical density. The results enabled the calculation of the drug dose required to inhibit the assembly of tubulin by 50% (IC_{50} value), determined by graphical means as percentage of the control assembly.

4.9.3. Cancer cell growth inhibition of human cancer cell lines. The human melanoma (WM35 and WM239), ovarian (SKOV, OVCAR) and breast carcinoma cell lines (MDA-MB231, SK-BR 3) were cultivated in RPMI 1640 medium (BioWhittaker Europe, Verviers, Belgium) containing 5% (melanoma) and 10% FCS (PAA laboratories, Pasching, Austria) and 2 mM L-glutamine (BioWhittaker Europe, Verviers, Belgium). The SK-BR 3 cell line medium was in addition supplemented with 12,5 μM insulin (Sigma–Aldrich, St. Louis, MO, USA). The WM35 and WM239 cell lines were kindly provided by Dr. Meenhard Herlyn (Wistar Institute, Philadelphia, PA, USA) whereas the ovarian and breast carcinoma cell lines were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). Five thousand cells in 100 μl regular growth medium were plated per well in 96-well plates and left to attach overnight. Thereafter, a dilution series of candidate drug (in 100 μl) was added. Proliferation was measured after 72 h following labeling of the cells with 1 μCi [^{3}H]thymidine (American Radiolabeled Chemicals, Inc., St. Louis, MO) for the last 24 h before harvesting using a Filtermate harvester (Packard Instrument Company, Meriden, CT). [^{3}H]Thymidine incorporation was assessed in a Packard Microplate Scintillation Counter (Packard Instrument Company). Controls were incubated with medium containing solvent only.

4.10. Molecular modeling

The X-ray crystallographic structures of α- and β-tubulin (PDB entry 1SA0[27]; chain A and chain B, respectively) in complex with guanosine-5′-triphosphate (GTP) were loaded into the Internal Coordinate Mechanics[36,37] (ICMv.3.5) program and hydrogen atoms were subsequently added. ECEPP/3 atom charges were assigned,[38] and followed by energy minimization to relieve atomic clashes. A grid map including the DAMA-colchicine binding site of 1SA0 was calculated. Triazole **12f** was modeled, optimized, and assigned MMFF94[39] partial charges, and docked as a flexible ligand into the rigid α,β-tubulin conformation. The ligand–tubulin complex showing the most favorable docking energy was further refined using the flexible-ligand, flexible-target method of ICM.[40] The free energy of binding of the refined target-ligand complex was calculated in order to predict the contributions of different subunits for binding the triazole **12f**.

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