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Synthesis, biological evaluation and molecular modeling of 1,2,3-triazole analogs of combretastatin A-1

Øyvind W. Akselsen [a], Kristin Odlo [a], Jing-Jy Cheng [b], Giorgio Maccari [c], Maurizio Botta [c], Trond Vidar Hansen [a,*]

[a] School of Pharmacy, Department of Pharmaceutical Chemistry, University of Oslo, PO Box 1068, N-0316 Oslo, Norway
[b] National Research Institute of Chinese Medicine, 155-1 Li-Nung Street, Section 2, Shih-Pai, Taipei, Taiwan
[c] Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Via Aldo Moro 2, I-53100 Siena, Italy

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ABSTRACT

The synthesis, cytotoxicity, inhibition of tubulin polymerization data and anti-angiogenetic effects of seven 1,5-disubstituted 1,2,3-triazole analogs and two 1,4-disubstituted 1,2,3-triazole analogs of combretastatin A-1 (**1**) are reported herein. The biological studies revealed that the 1,5-disubstituted 1,2,3-triazoles 3-methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)benzene-1,2-diol (**6**), 3-methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)benzene-1,2-diamine (**8**) and 5-(2, 3-difluoro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**9**) were the three most active compounds regarding inhibition of both tubulin polymerization and angiogenesis. Molecular modeling studies revealed that combretastatins **1** and **2** and analogs **5**–**11** could be successfully docked into the colchicine binding site of α,β-tubulin.

1. Introduction

Within the group microtubule-binding agents (MBAs), natural products and their derivatives have been widely used in cancer chemotherapy.[1,2] There are two classes of MBAs; those that stabilize microtubules and promote polymerization, and those that destabilize the microtubules and promote depolymerization by inhibition of tubulin.[3] Both types interfere with the mitotic spindle assembly during cell division, resulting in cell death. Studies have shown that most MBAs have antivascular effects from anti-angiogenetic or vascular disrupting activities, or both.[4]

The combretastatins, such as combretastatin A-1 (CA-1, **1**) and combretastatin A-4 (CA-4, **2**) depicted in Figure 1, have attracted much interest as anti-cancer agents.[5] The combretastatins were isolated from the bark of the South African bush willow tree *Combretum caffrum* by Pettit et al.[6] The phosphate prodrug of combretastatin A-1 (CA-1P, **3**, OXi4503) has entered clinical trials.[7] Combretastatin A-1 (**1**) and its prodrug **3** are cytotoxic against a variety of human cancer cell lines[8] and is also a potent anti-vascular agent (VDA).[4j] In addition, **1** has been reported to undergo oxidation to its *ortho*-quinone derivative that also exhibits interesting biological effects.[9] Moreover, CA-1P (**3**) is an even more potent VDA than the prodrug CA-4P (OXi2021, **4**) derived from combretastatin A-4 (**2**).[10] Prodrug **4** has entered several clinical trials and attracted more interest as an anticancer agent than **3**.[7a,f] Furthermore, CA-1P (**3**) induces tumor growth delays and regressions resulting in an enhanced in vivo activity compared to **4**, most likely due to a direct mode of action on the tumor mass.[7a,11]

Structure–activity relationship (SAR) studies have shown that a 3,4,5-trimethoxy substituted A ring (TMP) and a 4-methoxysubstituted B ring separated by a double bond with *cis* configuration are important for optimal cytotoxic activity for the combretastatins.[7a] The isomerization to the biologically less active *trans*-form may be problematic for the further development of the combretastatins as drug candidates.[11] Hence, numerous combretastatin analogs with a locked *cis*-type bridge between the two phenyl rings have been prepared.[12,13] Recently we prepared a series of 1,4- and 1,5-disubstituted 1,2,3-triazole analogs of combretastatin A-4 (**2**) that exhibited potent cytotoxicity in the nanomolar range and tubulin inhibitory activity in the low micromolar range.[14a,b] A few other 1,2,3-triazole analogs of combretastatin A-4 (**2**) have been reported.[14c,d] Furthermore, Welsh and co-workers reported that 3, 4-disubstituted-1,2,4-triazole analogs of **2** exhibited potent cytotoxic effects and inhibition of tubulin polymerization.[14e] Lee and co-workers reported a few 4,5-disubstitued NH-1,2,3-triazoles with potent cytotoxic effects.[14f] As of today, a growing number of analogs of the naturally occurring stilbene **1** have been prepared and biologically evaluated.[9,15] Herein we present the synthesis,

* Corresponding author. Tel.: +47 22857450; fax: +47 22855947.
E-mail address: t.v.hansen@farmasi.uio.no (T.V. Hansen).

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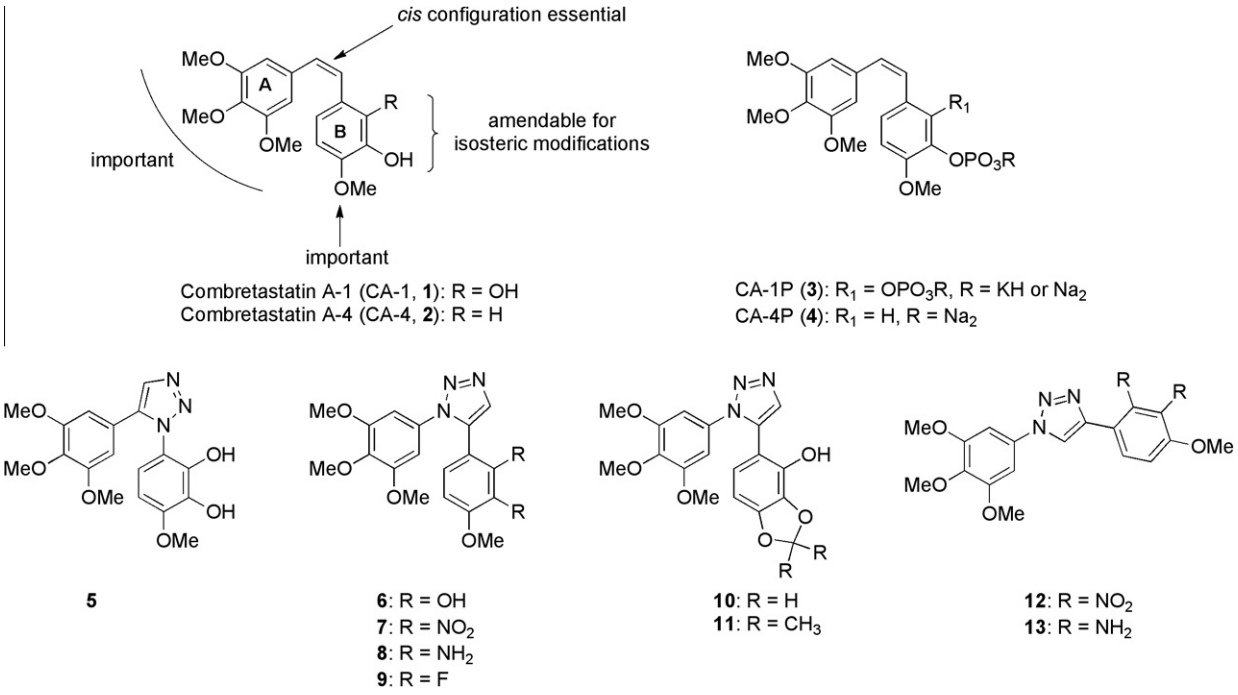


Figure 1. Structures of combretastatin A-1 (**1**) and A-4 (**2**), their prodrugs **3** and **4**, respectively, and 1,2,3-triazole analogs **5**–**13**.

cytotoxicity, inhibition of tubulin polymerization data and anti-angiogenetic effects of several 1,5-disubstituted 1,2,3-triazole analogs of combretastatin A-1 (**1**) as well as two 1,4-disubstituted 1,2,3-triazoles (Fig. 1). Furthermore, molecular modeling studies with the colchicine binding site of α,β-tubulin were performed with all 1,2,3-triazoles **5**–**13**. No molecular docking studies of the natural product **1** or any of its analogs in the colchicine binding site[4a,16] on tubulin have been reported.

2. Results and discussion

2.1. Synthesis

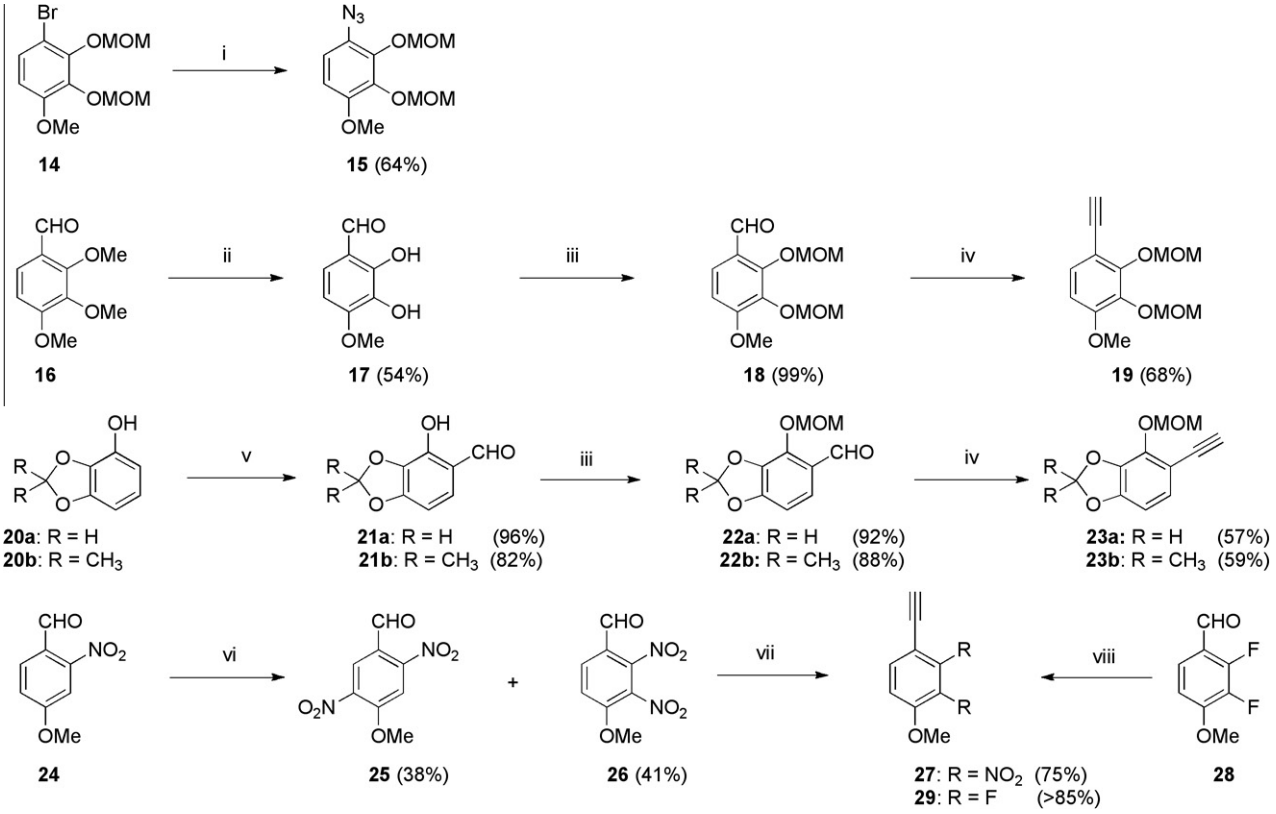
The syntheses of the starting materials are depicted in Scheme 1. The known bromide **14**[17] was converted into the azide **15** after reacting first with *n*-BuLi and then with tosylazide.[18] Unfortunately, purification of this product proved difficult, therefore the crude phenyl azide **15** was used in the cycloaddition reaction. Catechol **17** was prepared from commercially available **16** using a literature procedure.[19] Then **17** was protected as its *bis*-methoxymethyl ether according to a standard protocol[20] affording benzaldehyde **18**. Benzaldehyde **18** was converted to alkyne **19** using a Colvin rearrangement reaction[21] (Scheme 1). The known phenol **20a**[22] reacted in an *ortho*-formylation reaction[23] to the corresponding salicylaldehyde **21a** that was protected using MOMCl and DIPEA,[19] affording the aldehyde **22a**. The Colvin rearrangement reaction was also applied for the synthesis of alkyne **23a**. Using the same three step protocol starting from phenol **20b**, alkyne **23b** was obtained. Alkyne **27** was prepared by nitration of commercially available benzaldehyde **24** affording compounds **25** and **26** in approximately 1:1 ratio.[15e] Compounds **25** and **26** were separated by column chromatography affording the desired benzaldehyde **26** in 41% yield. Neither the Colvin rearrangement nor the Corey–Fuchs reaction[24] converted benzaldehyde **26** into alkyne **27**. However, the Ohira–Bestmann reaction[25] produced the desired alkyne **27** in 75% yield from **26**. The same conditions were applied to aldehyde **28** affording alkyne **29**.

Reacting azide **15** with the magnesium acetylide of alkyne **30** as previously reported[14,26] produced the 1,5-disubstituted 1,2,3-triazole **31** in 14% yield (Scheme 2). Deprotection of the *bis*-methoxymethyl functionality in **31** according to a literature procedure generated triazole **5** in 86% yield.[27] Using the previously mentioned cycloaddition conditions for reacting azide **32** with the magnesium acetylide of alkyne **19**, produced triazole **33** in 59% yield. In the same manner, triazole **9** was obtained in excellent 89% yield. Deprotection[27] of the *bis*-methoxymethyl functionality in **33** in aqueous HCl produced triazole **6** in 77% total yield (Scheme 2). Triazoles **7** and **12** were obtained in a thermally induced cycloaddition reaction, as described by Huisgen et al.,[28] between alkyne **27** and azide **32**. The two triazoles **7** and **12** were isolated in a 1:1 ratio. Separation of **7** and **12** was achieved by column chromatography, affording the desired 1,5-disubstituted product **7** in 43% yield. The amino-substituted triazole **8** was obtained in 28% yield by reduction of the nitro groups in **7** using $NaBH_4$ and $CuSO_4$ in EtOH (Scheme 2).[29]

Reacting the magnesium acetylides of alkynes **23a** and **23b** with azide **32** followed by deprotection as described above, afforded the desired triazoles **10** and **11** in 48% and 59% total yield, respectively, over two steps (Scheme 3). The 1,4-disubstituted 1,2,3-triazole **12** was obtained after reacting alkyne **27** and azide **32** using Sharpless and co-workers copper catalysed azide-alkyne cycloaddition (CuAAC) conditions.[30] However, we observed a sluggish reaction that required molar equivalents of $CuSO_4$ for decent conversion of the starting materials. Reduction of the nitro groups in **12** afforded triazole **13** in 13% overall yield from alkyne **27** (Scheme 3).[29]

2.2. Biological evaluation

The prepared 1,2,3-triazoles **5**–**13**, as well as both lead compounds **1** and **2**, were evaluated for their ability to inhibit the growth of four human cancer cell lines (MCF-7; breast cancer cell line, NCIH460; human non-small lung cancer cell line, HT-29; colorectal cancer cell line and CEM; leukemia cancer cell line) and a non-cancerous mammalian fibroblast cell line using the alamar



Scheme 1. Synthesis of starting materials. Reagents and conditions: (i) (a) *n*-BuLi, THF, −78 °C; (b) TsN_3, THF, −78 °C; (ii) BCl_3, CH_2Cl_2; (iii) DIPEA, MOMCl, CH_2Cl_2, 0 °C; (iv) LDA, $TMSCHN_2$, THF, −78 °C; (v) $MgCl_2$, Et_3N, $(CH_2O)_n$, THF, Δ; (vi) HNO_3, H_2SO_4, 0 °C; (vii) (a) separation by chromatography; (b) aldehyde **26**, $CH_3COCH(N_2)P(O)(OMe)_2$, K_2CO_3, MeOH; (viii) $CH_3COCH(N_2)P(O)(OMe)_2$, K_2CO_3, MeOH.

Blue®-assay.[31,32] Triazoles **8**, **9** and **11** inhibited the growth of the MCF-7 cancer cell line with IC_{50}-values in the micromolar range comparable to CA-4 (**2**), and were all more potent than CA-1 (**1**) (Table 1). Noteworthy, compound **7** was more potent than both combretastatins. Triazoles **5**, **6**, **10**, **12** and **13** were all less potent than both CA-1 (**1**) and CA-4 (**2**). Triazole **7** was also the most potent analog (IC_{50} = 0.7 μM) in the H460 lung cancer cell line compared to all compounds tested. In this cell line the 1,4-disubstituted analogs **12** and **13** exhibited IC_{50}-values in the micromolar range, but were less active than the combretastatins and the 1,5-disubstituted 1,2,3-triazoles evaluated. In the HT-29 colorectal cancer cell assay all triazoles **5**–**13** exhibited lower potency than both CA-1 (**1**) and CA-4 (**2**). The most potent triazoles in this cell assay were **6** and **9** with IC_{50} = 1.9 μM for both compounds, compared to IC_{50} = 0.9 μM for CA-1 (**1**). Noteworthy, both combretastatin A-1 (**1**) and A-4 (**2**) as well as all the prepared triazoles **5**, **6** and **8**–**13** were inactive in the leukemia cancer cell line (Table 1).

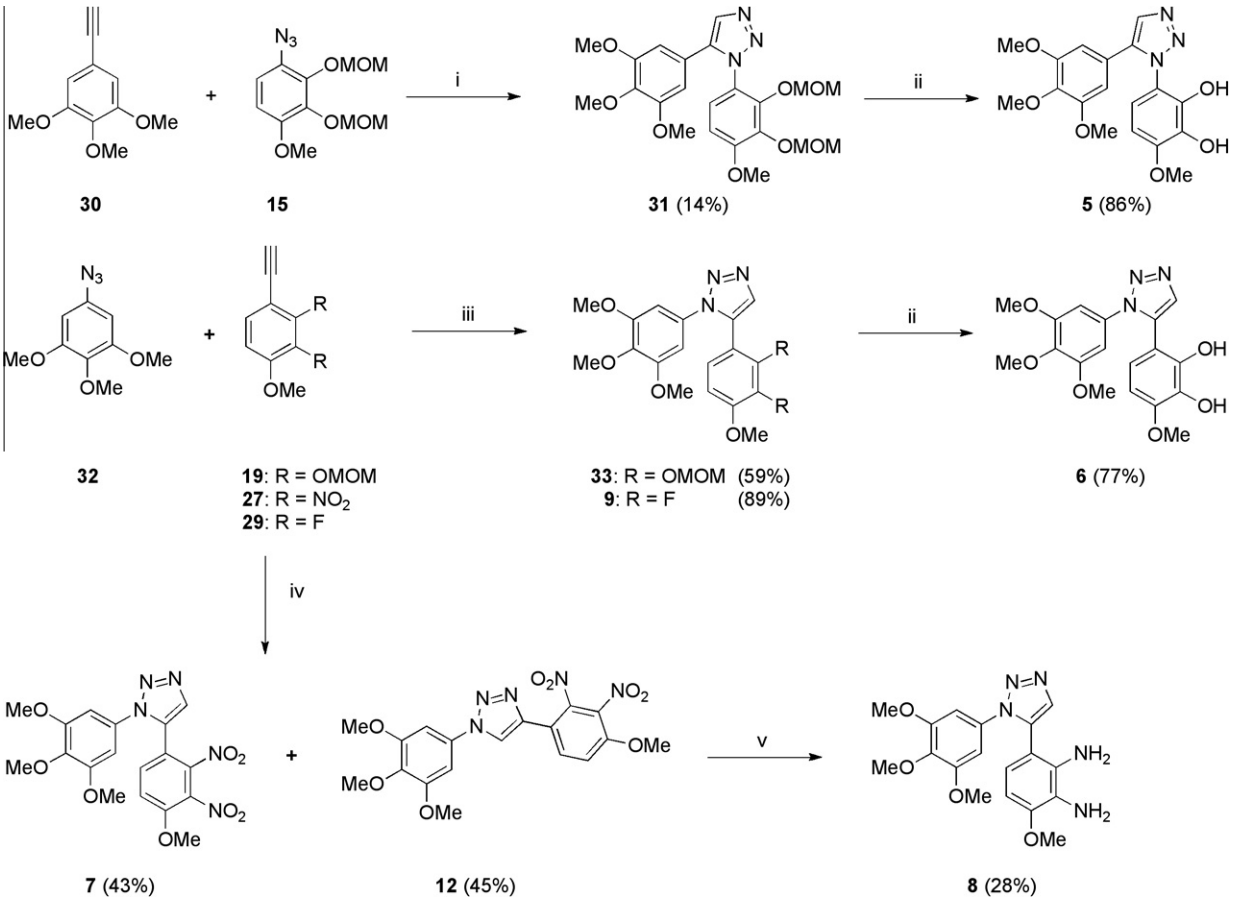
Only triazole analogue **7** exhibited activity in the non-cancerous cell line. Interestingly, none of the other 10 triazoles and neither lead compound **1**, nor **2** displayed any growth inhibition activity in the non-cancerous cell line, thus indicating greater selectivity for cancer cells. The biological results for the 1,2,3-triazole analogs of CA-1 (**1**) reported herein, support the previous findings that 1,5-disubstituted 1,2,3-triazoles with the A ring derived from a phenyl azide, are the most cytotoxic analogs.[14a,b]

The anti-angiogenetic activity of the most cytotoxic triazole analogs **5**–**11** was also evaluated (Table 1).[33] The 1,5-disubstitued 1,2,3-triazoles **6**–**11** with the A-ring derived from the azide **32** exhibited anti-angiogenetic activity in the low micromolar range with IC_{50}-values between 0.3 and 4.4 μM; the two most potent triazoles were **8** and **9** with IC_{50} = 0.3 μM for both compounds. Interestingly, these two triazoles were about 10-fold more potent than CA-1 (**1**) (IC_{50} = 3.2 μM). However, CA-4 (**2**) was 10-fold more potent than **8** and **9** with IC_{50} = 0.01 μM. Triazole **5**, with the A-ring derived from the alkyne **30**, was inactive as an inhibitor in this angiogenesis assay (IC_{50} > 10 μM). Triazole **11**, with the dimethyl acetal moiety in the B-ring, was also inactive in this assay. None of the 1,4-disubstituted 1,2,3-triazoles **12** and **13** exhibited any activity (IC_{50} > 10 μM).

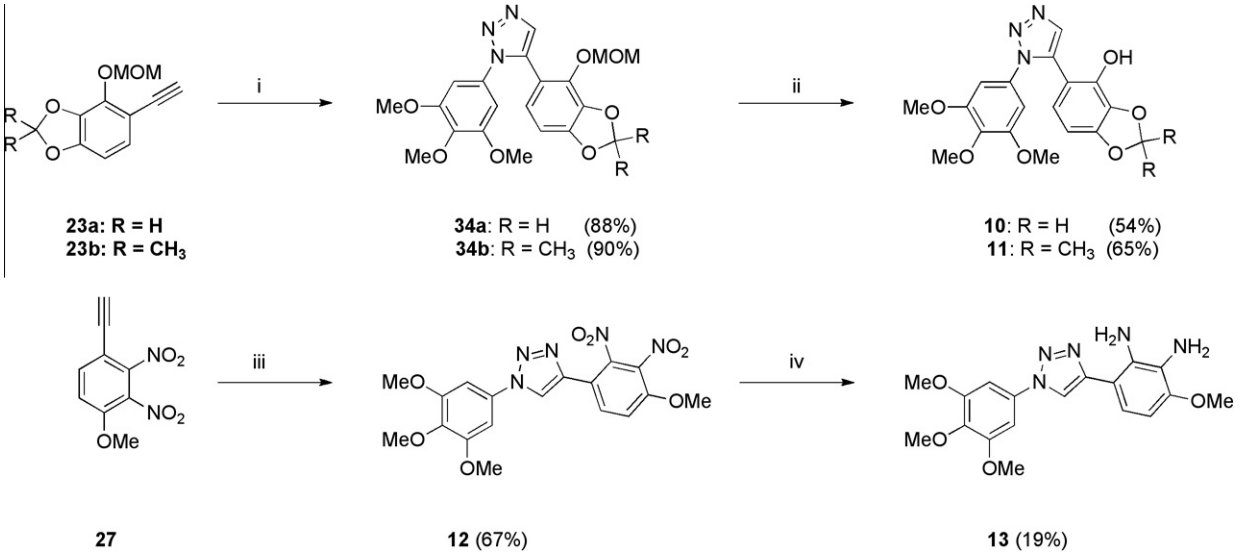
Next we investigated the inhibition of tubulin assembly.[34] All of the tested 1,5-disubstituted 1,2,3-triazoles exhibited lower activities than CA-1 (**1**). This was also observed for 1,2,3-triazole analogs of CA-4 (**2**) in our previous study.[14a,b] Furthermore, all of the prepared triazoles exhibited significant lower inhibition of tubulin assembly compared to CA-4 (**2**) (Table 1). The most potent triazole in the tubulin inhibition assay was the diamino triazole **8** with IC_{50} = 5.2 μM compared to IC_{50} = 3.5 and 0.6 μM for CA-1 (**1**) and CA-4 (**2**), respectively. Triazoles **6**, **9** and **10** exhibited weak tubulin inhibition activities with IC_{50}-values of 10.1, 15.6 and 17.2 μM, respectively. The 1,2,3-triazoles **5**, **7** and **11** were all inactive as tubulin inhibitors (IC_{50} > 20 μM).

2.3. Molecular modeling

Molecular modeling studies were performed to investigate the binding ability of the 1,2,3-triazoles to the colchicine binding site of α,β-tubulin (pdb: 1SA0).[35] Docking studies showed that 1,5-disubstituted 1,2,3-triazoles **5**–**11** as well as CA-1 (**1**) and CA-4 (**2**) occupied the colchicine binding site of α,β-tubulin mostly buried in the β subunit (Fig. 2). The docking scores varied from −8.53 to −7.50 kcal/mol. The colchicine-binding pocket could not accommodate compounds **12** and **13**, and the docking score values were 2 kcal/mol lower than the average 1,5-disubstituted 1,2,3-triazole score values.



Scheme 2. Synthesis of 1,2,3-triazoles **5**–**9**. Reagents and conditions: (i) Alkyne **30**, then EtMgCl, THF, Δ; (ii) HCl, H_2O, THF; (iii) alkyne **19** or alkyne **29**, then EtMgCl, THF, Δ; (iv) alkyne **27**, toluene, H_2O, Δ; (v) (a) separation by chromatography; (b) triazole **7**, then $NaBH_4$, $CuSO_4{\cdot}5H_2O$, EtOH.



Scheme 3. Synthesis of 1,2,3-triazoles **10**–**13**, **34a** and **34b**. Reagents and conditions: (i) (a) EtMgCl, THF, Δ; (b) azide **32**; (ii) HCl, H_2O, THF; (iii) azide **32**, Na-ascorbate, $CuSO_4{\cdot}5H_2O$, *t*-BuOH/H_2O (1:1); (iv) $NaBH_4$, $CuSO_4{\cdot}5H_2O$, EtOH.

There was no correlation between the docking scores and the IC_{50}-values for the compounds that exhibited tubulin inhibition properties. Since the compounds are structural similar, this observation was not unexpected as scoring of such structural similar compounds still remains a challenge.[36] In general, after energy minimization, the TMP moiety and B-ring of the 1,5-disubstituted 1,2,3-triazole analogs were superimposed over DAMA-colchicine in the 1SA0 tubulin structure. The 4-methoxy group is a possible

Table 1
Cytotoxicity, inhibition of angiogenesis and inhibition of tubulin

Compound	MCF-7 cell assay IC_{50}[a] (μM)	H460 cell assay IC_{50}[a] (μM)	HT-29 cell assay IC_{50}[a] (μM)	CEM cell assay IC_{50}[a] (μM)	Fibroblast cell assay IC_{50}[a] (μM)	Inhibition of angiogenesis[a] (μM)	Tubulin inhibition[b] (μM)
1	75.9	1.8	0.9	>100	>100	3.2	3.5
2	48.9	4.4	0.4	>100	>100	0.01	0.6
5	>100	26.6	64.3	>100	>100	>10	>20
6	>100	6.4	1.9	>100	>100	3.2	10.1
7	6.1	0.7	12.0	26.0	31.5	4.4	>20
8	38.8	17.3	17.2	>100	>100	0.3	5.2
9	26.7	4.9	1.9	>100	>100	0.3	15.6
10	>100	7.4	8.8	>100	>100	3.4	17.1
11	46.3	16.8	>100	>100	>100	>10	>20
12	>100	18.2	>100	>100	>100	>10	n.d.
13	>100	29.8	>100	>100	>100	>10	n.d.

[c] n.d. = not determined.
[a] Results of three experiments performed as triplicates.
[b] Results of two experiments performed as triplicates.

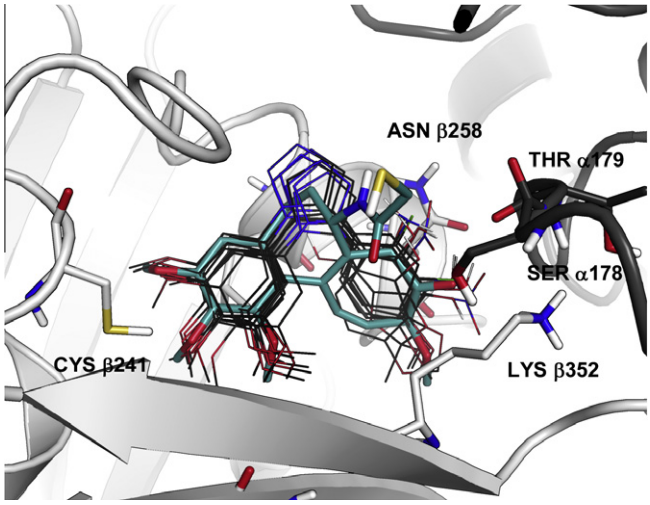


Figure 2. Compounds **5**–**11**, CA-1 (**1**) and CA-4 (**2**) occupy the same Cartesian space as colchicine (teal); α-tubulin (dark gray); β-tubulin (light gray).

hydrogen bond acceptor of the thiol proton of Cys β241. The amide side chain of Asn β258 can be seen as coplanar to the aromatic B-ring that could indicate aliphatic interaction between the two. The triazole moiety was observed in a hydrophobic pocket consisting of the following residues; Leu β248, Ala β250, Lys β254, Leu β55 and Asn β258 as reported earlier.[14a,37]

3. Conclusion

The 1,5-disubstiuted 1,2,3-triazole analogs **6**, **8** and **9** of combretastatin A-1 (**1**) exhibited cytotoxic effects against several cancer cell lines in the low micromolar range. In addition, these triazoles exhibited modest inhibition of tubulin assembly and anti-angiogenetic effects with IC_{50}-values in the low micromolar range. Based on the data from our biological evaluations, triazoles **6**, **8** and **9**, most likely effect their mode of actions, at least in part, due to binding to the colchicine binding site of α,β-tubulin. This is the same mode of action as the lead compounds **1** and **2**. The initial molecular modeling studies also support these observations. The ability to induce vascular disrupting effects is one of the most interesting biological features of the combretastatins A-1 (**1**) and A-4 (**2**) as potential new remedies against cancer. The triazoles reported herein are currently undergoing biological evaluations as VDAs. These studies will be reported in due time.

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} plates (Merck) or RP-18 F_{254s} 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 or DPX-200 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), DMSO-d_6 (2.50 ppm for ^{1}H and 39.43 ppm for ^{13}C), acetonitrile-d_3 (1.94 ppm for ^{1}H and 1.32 ppm for ^{13}C), methanol-d_4 (4.87 ppm for ^{1}H and 49.15 for ^{13}C), acetone-d_6 (2.05 ppm for ^{1}H and 29.84 ppm for ^{13}C). Starting materials **14**,[17] **20a**[38] and **20b**[39] as well as intermediates **17**,[6a] **18**,[15h] **21a**,[21] **21b**,[40] **22a**,[41] **25**,[15e] **26**[15e] and **32**[14b] are known compounds. Aldehyde **28** was purchased from Alfa Aesar. Combretastatins A-1 (**1**)[17] and A-4 (**2**)[42] were synthesized as previously reported and used as positive controls for all biological evaluations.

4.2. Syntheses of aryl azides

4.2.1. 1-Azido-4-methoxy-2,3-bis(methoxymethoxy)benzene (15)

n-BuLi (1.6 M, 3.3 mL, 5.12 mmol) was added dropwise to a solution of 1-bromo-4-methoxy-2,3-bis(methoxymethoxy)-benzene (**14**) (0.86 g, 2.80 mmol) in dry THF (35 mL) at −78 °C under argon, and the mixture was stirred at −78 °C for 0.5 h. A solution of tosylazide (1.174 g, 5.95 mmol) in dry THF (20 mL) was added dropwise at −78 °C, and the mixture was stirred at −78 °C for 1 h, followed by stirring at room temperature for 14 h. Water (50 mL) was added, followed by extraction with dichloromethane (3 × 50 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent removed in vacuo affording a brown oil. The crude product was partially purified by chromatography (hexane/EtOAc 1:2, R_f = 0.64) affording a red oil of **15** (64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.04 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 5.19 (s, 2H), 5.14 (s, 2H), 3.86 (s, 3H), 3.57 (s, 3H), 3.55 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.08, 149.82, 138.72, 125.03, 122.23, 107.89, 99.98, 98.74, 57.88, 57.54, 56.11.

4.3. 4-(Methoxymethoxy)-2,2-dimethylbenzo[*d*][1,3]-dioxole-5-carbaldehyde (22b)

The benzaldehyde **21b** (550 mg, 2.84 mmol) was dissolved in dry dichloromethane under argon and cooled to 0 °C. Diisopropylethylamine (1.50 ml, 8.50 mmol) was added dropwise. Chloromethyl

methyl ether (0.43 ml, 5.68 mmol) was added dropwise. The reaction was stirred at 0 °C for 2 h, and then warmed to room temperature over night. Brine (30 mL) was added and the aqueous layer was extracted with dichloromethane (30 mL). The combined organic layers were washed with acetic acid (2 × 15 mL, 10%), satd $NaHCO_3$ (15 ml) and brine (30 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo. The compound was used without further purification. Yellow solid (88%); mp 45–47 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 10.25 (d, J = 0.7 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 6.58 (dd, J = 8.3, 0.7 Hz, 1H), 5.34 (s, 2H), 3.53 (s, 3H), 1.72 (s, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 188.11, 154.22, 142.31, 137.05, 124.18, 123.44, 120.09, 104.28, 97.20, 57.33, 25.82. HRMS calcd. for $C_{12}H_{14}O_5$ ($M^{\cdot+}$): 238.0841. Found 238.0844.

4.4. Syntheses of alkynes using the Colvin rearrangement: general procedure

Trimethylsilyldiazomethane (3.2 mL, 6.40 mmol, 2.0 M hexane solution) was added dropwise to a solution of lithium diisopropylamide (3.9 mL, 7.02 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 (5.11 mmol) in dry THF (10.2 mL) was added dropwise at −78 °C. The mixture was stirred at −78 °C for 2 h, then at room temperature for 4 h. The reaction was quenched with brine (5 mL), and the mixture extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (2 × 15 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo.

4.4.1. 1-Ethynyl-4-methoxy-2,3-bis(methoxymethoxy)-benzene (19)

The product was purified by chromatography (hexane/EtOAc 2:1, R_f = 0.56) affording a yellow oil (68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20 (d, J = 8.7 Hz, 1H), 6.64 (d, J = 8.7 Hz, 1H), 5.27 (s, 2H), 5.11 (s, 2H), 3.85 (s, 3H), 3.63 (s, 3H), 3.59 (s, 3H), 3.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.81. 152.58, 138.99, 129.41, 109.96, 107.88, 99.35, 98.80, 80.26, 80.00, 57.84, 57.54, 56.14. HRMS calcd. for $C_{13}H_{16}O_5$ ($M^{\cdot+}$): 252.0998. Found 252.0998.

4.4.2. 5-Ethynyl-4-(methoxymethoxy)benzo[*d*][1,3]dioxole (23a)

The product was purified by chromatography (hexane/EtOAc 6:4, R_f = 0.62) affording a pale yellow solid (57%); mp 35–37 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.01 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 8.1 Hz, 1H), 5.98 (s, 2H), 5.33 (s, 2H), 3.58 (s, 3H), 3.17 (s, 1H).^{13}C NMR (50 MHz, $CDCl_3$) δ = 149.86, 141.14, 137.66, 127.90, 109.61, 103.93, 101.61, 96.82, 79.83, 79.51, 57.01. HRMS calcd. for $C_{11}H_{10}O_4$ ($M^{\cdot+}$): 206.0579. Found 206.0588.

4.4.3. 5-Ethynyl-4-(methoxymethoxy)-2,2-dimethylbenzo-[*d*]-[1,3]dioxole (23b)

The crude product was purified by chromatography (hexane/EtOAc 8:2, R_f = 0.43) affording a pale yellow solid (59%); mp 67–68 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.96 (d, J = 8.1 Hz, 1H), 6.46 (d, J = 8.1 Hz, 1H), 5.29 (s, 2H), 3.58 (s, 3H), 3.15 (s, 1H), 1.68 (s, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 149.63, 141.03, 138.00, 127.21, 119.21, 109.10, 104.00, 96.94, 80.17, 79.19, 57.01, 25.74. HRMS calcd. for $C_{13}H_{14}O_4$ ($M^{\cdot+}$): 234.0892. Found 234.0892.

4.5. Syntheses of alkynes using the Ohira–Bestmann reaction: general procedure

Dimethyl-1-diazo-2-oxopropylphosphonate (280 mg, 1.44 mmol) was added to a suspension of the aldehyde (1.2 mmol) and K_2CO_3 (336 mg, 2.4 mmol) in MeOH (15 mL). Stirring was continued for 1.5 h. Then the reaction mixture was diluted with Et_2O (25 mL), washed with an aq solution of $NaHCO_3$ (10 mL, 5%) and dried over anhydrous magnesium sulfate. After filtration and evaporation of the solvent in vacuo the desired alkyne was obtained.

4.5.1. 1-Ethynyl-2,3-dinitro-4-methoxybenzene (27)

The product was purified by chromatography (heptane/EtOAc 3:7, R_f = 0.42) affording a yellow solid (75%); mp 162–163 °C. ^{1}H NMR (300 MHz, d_6-acetone): δ = 7.96 (d, J = 9.0 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 4.13 (s, 4H). ^{13}C NMR (75 MHz, d_6-acetone): δ = 206.23, 153.16, 138.45, 118.55, 109.22, 86.55, 76.07, 58.50. HRMS calcd. for $C_9H_6N_2O_5$ ($M^{\cdot+}$): 222.0277. Found 222.0280.

4.5.2. 1-Ethynyl-2,3-difluoro-4-methoxybenzene (29)

Colorless crystals that sublimated under reduced pressure in >85% yield; (heptane/EtOAc 1:1, R_f = 0.54); mp. 71–72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.17 (ddd, J = 9.4, 7.3, 2.4 Hz, 1H), 6.73–6.64 (m, 1H), 3.91 (s, 3H), 3.25 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.17 (dd, J = 252.5, 11.2 Hz), 149.95 (dd, J = 8.0, 3.3 Hz), 140.95 (dd, J = 248.3, 13.8 Hz), 127.79 (dd, J = 4.4, 1.8 Hz), 107.94 (dd, J = 3.4, 1.3 Hz), 104.34 (d, J = 13.0 Hz), 81.78 (d, J = 3.4 Hz), 75.99 (dd, J = 3.9, 0.8 Hz), 56.56 (s). HRMS calcd. for $C_9H_6F_2O$ ($M^{\cdot+}$): 168.0387. Found 168.0387.

4.6. Syntheses of 1,5-disubstituted 1,2,3-triazoles using magnesium acetylides: general procedure

The corresponding terminal alkyne (1.1 mmol) dissolved in dry THF (1.5 mL) was added dropwise to an oven dried flask containing a solution of EtMgCl in dry THF (0.5 mL, 2.0 M, 1.0 mmol) 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 (1.0 mmol) dissolved in dry THF (1.5 mL) was added dropwise. The reaction mixture was heated to 50 °C for 3 h. After quenching with aqueous NH_4Cl (6 mL), the product was extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with aqueous NH_4Cl (2 × 50 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo.

4.6.1. 1-(4-Methoxy-2,3-bis(methoxymethoxy)phenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (31)

The product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.30) affording an orange semisolid (14%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (s, 1H), 6.96 (d, J = 8.9 Hz, 1H), 6.73 (d, J = 9.0 Hz, 1H), 6.48 (s, 2H), 5.07 (s, 2H), 4.96 (s, 2H), 3.87 (s, 3H), 3.80 (s, 3H), 3.65 (s, 6H), 3.44 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.30, 153.47, 146.96, 139.73, 139.61, 138.73, 131.88, 124.77, 123.39, 122.29, 107.63, 105.38, 99.62, 98.74, 61.03, 57.55, 56.90, 56.44, 56.17. HRMS calcd. for $C_{22}H_{27}N_3O_8$ ($M^{\cdot+}$): 461.1798. Found 461.1782.

4.6.2. 5-(4-Methoxy-2,3-bis(methoxymethoxy)phenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (33)

The product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.42) affording an orange semisolid (59%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.94 (s, 1H), 6.97 (d, J = 8.7 Hz, 1H), 6.93 (d, J = 8.8 Hz, 1H) 6.67 (s, 2H), 4.98 (s, 2H), 4.89 (s, 2H), 3.81 (s, 3H), 3.67 (s, 3H), 3.62 (s, 6H), 3.35 (s, 3H), 2.91 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 154.57, 152.81, 148.59, 138.43, 137.36, 134.27, 134.24, 132.23, 125.85, 114.49, 108.67, 101.96, 98.79, 97.74, 60.08, 56.52, 56.11, 55.85. HRMS calcd. for $C_{22}H_{27}N_3O_8$ ($M^{\cdot+}$): 461.1798. Found 461.1800.

4.6.3. 5-(2,3-Difluoro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (9)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.31) affording a pale yellow solid (89%); mp

107–108 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.10 (d, J = 1.2 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 7.09 (d, J = 8.7 Hz, 1H), 6.75 (s, 2H), 3.90 (s, 3H), 3.71 (s, 3H), 3.68 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 153.05, 149.64 (dd, J = 7.5, 3.2 Hz), 147.72 (dd, J = 248.6, 11.2 Hz), 140.07 (dd, J = 246.2, 14.3 Hz), 138.00, 134.29 (d, J = 2.4 Hz), 131.61, 130.80 (d, J = 2.6 Hz), 125.47 (dd, J = 4.0, 2.6 Hz), 109.35 (d, J = 2.3 Hz), 107.94 (d, J = 11.9 Hz), 102.94, 60.18, 56.78, 56.18. HRMS calcd. for $C_{18}H_{17}F_2N_3O_4$ ($M^{\cdot+}$): 377.1187. Found 377.1188.

4.6.4. 5-(4-(Methoxymethoxy)benzo[*d*][1,3]dioxol-5-yl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (34a)

The product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.30) affording an orange solid (88%); mp 86–88 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.80 (s, 1H), 6.66 (s, 2H), 6.61 (d, J = 2.8 Hz, 2H), 6.01 (s, 2H), 5.04 (s, 2H), 3.85 (s, 3H), 3.72 (s, 6H), 3.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 153.25, 150.52, 138.22, 138.05, 137.59, 134.81, 133.81, 132.47, 124.53, 114.50, 103.98, 101.73, 101.69, 96.91, 60.92, 56.77, 56.11. HRMS calcd. for $C_{20}H_{21}N_3O_7$ ($M^{\cdot+}$): 415.1380. Found 415.1367.

4.6.5. 5-(4-(Methoxymethoxy)-2,2-dimethylbenzo[*d*][1,3]-dioxol-5-yl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (34b)

The product was purified by chromatography (hexane/EtOAc 6:4, R_f = 0.23) affording an orange solid (90%); mp 122–124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.79 (s, 1H), 6.67 (s, 2H), 6.54 (d, J = 8.1 Hz, 1H), 6.48 (d, J = 8.1 Hz, 1H), 5.00 (s, 2H), 3.83 (s, 3H), 3.70 (s, 6H), 3.08 (s, 3H), 1.68 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 153.19, 150.20, 138.12, 137.94, 137.92, 134.86, 134.00, 132.52, 123.78, 119.46, 114.19, 104.13, 101.60, 97.02, 60.91, 56.67, 56.02, 25.67. HRMS calcd. for $C_{22}H_{25}N_3O_7$ ($M^{\cdot+}$): 443.1693. Found 443.1683.

4.7. Thermal [3+2] Huisgen cycloaddition reaction

Alkyne **27** (100 mg, 0.42 mmol) and azide **32** (92 mg, 0.42 mmol) was dissolved in toluene (2.5 mL) and H_2O (2.5 mL) was added. The reaction mixture was heated to reflux for 3 days. Additional amounts of toluene (2.5 mL each time, total of four times) was added when it had evaporated from the reaction mixture. The solvent was removed in vacuo and the products were dissolved in THF and absorbed on celite for purification by flash chromatography (CH_2Cl_2/EtOAc, 9:1–1:1).

4.7.1. 5-(4-Methoxy-2,3-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7)

Chromatography (CH_2Cl_2/EtOAc 8:2, R_f = 0.34) affording a orange solid (43%); mp 162–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (s, 1H), 7.35 (d, J = 8.9 Hz, 1H), 7.25 (d, J = 8.9 Hz, 1H), 6.55 (s, 2H), 3.99 (s, 3H), 3.81 (s, 3H), 3.72 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.59, 152.71, 143.08, 138.88, 134.66, 134.27, 134.08, 130.60, 130.54, 116.18, 113.46, 102.46, 77.00, 60.85, 57.52, 56.21. HRMS calcd. for $C_{18}H_{17}N_5O_8$ ($M^{\cdot+}$): 431.1077. Found 431.1064.

4.7.2. 4-(4-Methoxy-2,3-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (12)

Chromatography (CH_2Cl_2/EtOAc 8:2, R_f = 0.54) affording a white solid (45%); mp 221–222 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.31 (s, 1H), 8.13 (d, J = 9.0 Hz, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.25 (s, 2H), 4.08 (s, 3H), 3.90 (s, 6H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 153.56, 151.32, 140.92, 140.43, 137.74, 133.76, 133.33, 132.04, 122.49, 118.22, 115.46, 98.41, 80.02, 80.00, 60.23, 58.11, 56.33. HRMS calcd. for $C_{18}H_{17}N_5O_8$ ($M^{\cdot+}$): 431.1077. Found 431.1066.

4.8. Reduction of nitro groups: general procedure

The triazole (0.08 mmol) was suspended in MeOH/H_2O (10 mL, 2:1) and saturated $CuSO_4{\cdot}5H_2O$ solution (200 μL) was added and stirred at ambient temperature. $NaBH_4$ was added (15 mg, 0.40 mmol) and an exothermic reaction occurred. The reaction mixture turned black. Additional portions of $NaBH_4$ were added until the color of the solution changed from brown to black/green. RP-18 TLC (CH_3CN/H_2O, 6:4) and normal phase TLC analysis using a (EtOAc) was used to monitor the reaction. When complete conversion of the starting material was observed, the reaction was diluted with HCl (10 mL, 0.5 M) and EtOAc (5 mL). The organic phase was extracted a second time with HCl (5 mL, 0.5 M). The combined aqueous extracts was made basic with NaOH (1 M) and extracted with EtOAc (4 × 10 mL). The combined EtOAc fractions were washed with aqueous ammonia (2 × 5 mL, 5%) before dried over anhydrous magnesium sulfate and then concentrated in vacuo.

4.8.1. 3-Methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)benzene-1,2-diamine (8)

The product was purified by chromatography (CH_2Cl_2/EtOAc, 2:8–1:9) affording a brown solid (28%); mp 69–70. R_f = 0.58 (CH_3CN/H_2O 6:4 °C). ^{1}H NMR (300 MHz, acetonitrile-d_3): δ = 7.74 (s, 1H), 6.75 (s, 2H), 6.44 (d, J = 8.5 Hz, 1H), 6.38 (d, J = 8.5 Hz, 1H), 3.80 (s, 3H), 3.73 (br s, 4 H, 2 × NH_2), 3.71 (s, 3H), 3.66 (s, 6H). ^{13}C NMR (75 MHz, acetonitrile-d_3): δ = 154.25, 149.71, 138.96, 136.57, 135.57, 135.10, 133.48, 124.34, 121.42, 107.15, 103.09, 102.54, 60.94, 56.80, 56.44. HRMS calcd. for $C_{18}H_{21}N_5O_4$ ($M^{\cdot+}$): 371.1594. Found 371.1586.

4.8.2. 3-Methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)benzene-1,2-diamine (13)

The product was purified by chromatography (R_f = 0.50, EtOAc) affording a brown oil (19%). ^{1}H NMR (300 MHz, acetonitrile-d_3): δ = 8.50 (s, 1H), 7.16 (s, 2H), 7.03 (d, J = 8.6 Hz, 1H), 6.48 (d, J = 8.6 Hz, 1H), 5.31 (s, 2H, NH_2), 3.92 (s, 6H), 3.85 (s, 3H), 3.79 (s, 3H), 3.67 (s, 2H, NH_2). ^{13}C NMR (75 MHz, acetonitrile-d_3) δ = 155.02, 149.95, 148.69, 139.07, 135.58, 133.98, 124.54, 119.98, 118.68, 109.15, 102.52, 100.99, 99.41, 61.05, 57.13, 56.45. HRMS calcd. for $C_{18}H_{21}N_5O_4$ ($M^{\cdot+}$): 371.1594. Found 371.1590.

4.9. Deprotection of methoxymethyl groups: general procedure

The corresponding MOM-protected triazole (0.24 mmol) was dissolved in 3 M HCl/THF (1:1) (10 mL), and the mixture was stirred at room temperature or heated to reflux (compounds **10** and **11**) for 5 h. Water (20 mL) was added, and the reaction mixture was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent removed in vacuo.

4.9.1. 3-Methoxy-6-(5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzene-1,2-diol (5)

The product was purified by recrystallization in hexane/diethyl ether (1:1) affording a yellow solid (86%); mp 127–129 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.32 (s, OH), 9.08 (s, OH), 8.12 (s, 1H), 6.79 (d, J = 8.8 Hz, 1H), 6.65 (s, 2H), 6.63 (d, J = 8.9 Hz, 1H), 3.84 (s, 3H), 3.64 (s, 3H), 3.61 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 152.75, 149.71, 142.55, 138.52, 137.72, 134.69, 131.52, 122.10, 118.79, 118.27, 104.80, 102.69, 60.01, 56.07, 55.65. HRMS calcd. for $C_{18}H_{19}N_3O_6$ ($M^{\cdot+}$): 373.1274. Found 373.1276.

4.9.2. 3-Methoxy-6-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)benzene-1,2-diol (6)

The product was purified by recrystallization in hexane/diethyl ether (1:1) affording a pale beige solid (77%); mp 214–216 °C. ^{1}H

NMR (300 MHz, DMSO-d_6): δ = 8.97 (s, OH), 8.82 (s, OH), 7.83 (s, 1H), 6.72 (s, 2H), 6.57 (d, J = 8.6 Hz, 1H), 6.52 (d, J = 8.6 Hz, 1H), 3.78 (s, 3H), 3.68 (s, 3H), 3.65 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 152.74, 149.49, 144.64, 137.33, 135.02, 134.01, 133.92, 132.52, 120.53, 107.74, 103.21, 101.91, 60.11, 55.92, 55.84. HRMS calcd. for $C_{18}H_{19}N_3O_6$ ($M^{\cdot+}$): 373.1274. Found 373.1269.

4.9.3. 5-(1-(3,4,5-Trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)benzo[*d*][1,3]dioxol-4-ol (10)

The product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.33) affording a white solid (54%); mp 216–217 °C. ^{1}H NMR (300 MHz, methanol-d_4) δ = 7.91 (s, 1H), 7.82 (s, 2H), 6.74 (s, 3H), 6.71 (d, J = 8.1 Hz, 2H), 6.48 (d, J = 8.1 Hz, 2H), 5.97 (s, 3H), 3.79 (s, 5H), 3.73 (s, 10H). ^{13}C NMR (75 MHz, methanol-d_4) δ = 154.81, 151.79, 140.86, 139.68, 136.79, 136.54, 135.26, 134.29, 125.70, 111.52, 103.40, 103.14, 102.01, 61.33, 56.85. HRMS calcd. for $C_{18}H_{17}N_3O_6$ ($M^{\cdot+}$): 371.1117. Found 371.1123.

4.9.4. 2,2-Dimethyl-5-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-5-yl)benzo[*d*][1,3]dioxol-4-ol (11)

The product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.40) affording a white solid (65%); mp 204–206 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.69 (s, 1H), 6.65 (s, 2H), 6.53 (d, J = 8.1 Hz, 1H), 6.37 (d, J = 8.1 Hz, 1H), 3.81 (s, 3H), 3.68 (s, 6H), 1.68 (s, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ = 153.15, 149.75, 138.61, 138.00, 135.35, 134.13, 134.05, 132.40, 123.48, 119.68, 109.05, 101.79, 101.76, 60.97, 56.06, 25.82. HRMS calcd. for $C_{20}H_{21}N_3O_6$ ($M^{\cdot+}$): 399.1430. Found 399.1431.

4.10. Synthesis of 4-(4-methoxy-2,3-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (12) using $CuSO_4$ and sodium ascorbate

To a suspension of 1-ethynyl-4-methoxy-2,3-dinitrobenzene (**27**) (22 mg, 0.1 mmol) and 5-azido-1,2,3-trimethoxybenzene (**32**) (21 mg, 0.1 mmol) in *t*-BuOH (1 mL), a solution of $CuSO_4{\cdot}5H_2O$ (25 mg, 0.1 mmol in 0.5 mL H_2O) and sodium ascorbate (10 mg, 0.05 mmol) were added. The reaction mixture was stirred overnight at 45 °C where after the reaction was complete as observed by TLC and H_2O (10 mL) was added. The aqueous phase was extracted with EtOAc (3 × 5 mL) and the combined organic phases was washed with aqueous ammonia (2 × 5 mL, 5%) and brine before dried with $MgSO_4$ and concentrated in vacuo. Purified by flash chromatography (CH_2Cl_2/EtOAc 8:2, R_f = 0.54) affording a white solid (67%). Physical and spectral data (^{1}H- and ^{13}C-NMR, HRMS) were in accordance for structure of **12** with those data obtained by the thermal Huisgen cycloaddition.

4.11. Biological Assays

4.11.1. Cancer Cell Growth Inhibition

To assess cell viability, the alamarBlue® (AB) assay (dye purchased from Biosource International, Nivelles, Belgium) was used as previously described.[31,32] This involved aspirating medium at the end of each treatment period and adding 100 μl of fresh medium containing 10% v/v AB to control and treated wells. Plates were incubated at 37 °C for six hours prior to measuring the absorbance at 540 nm and at 595 nm wavelengths using a spectrophotometric plate reader (DYNEX Technologies, USA). Experimental data were normalized to control values.

4.11.2. Inhibition of Angiogenesis

Endothelial cell tube formation assay was modified from a method previously described.[33] Matrigel (12.5 mg/ml) was thawed at 4 °C, and 50 Al were quickly added to each well of a 96-well plate and allowed to solidify for 10 min at 37 °C. Once solid, the wells were incubated for 30 min with ECs (30,000 cells/well). After adhesion of the cells, the medium was removed and replaced by fresh medium supplemented with triazole analogs with five different concentrations ranging from 10 μM to 0.001 μM and incubated at 37 °C for 18 h. The tubes of growth were visualized with an inverted ZEISS microscope at a magnification of 10. The length of the capillary network was quantified with a map scale calculator (KURABO Angiogenesis Image Analysis Software).

4.11.3. Inhibition of Tubulin Assembly

The method applied was that described by Lawrence et al.[34] Tubulin was isolated from porcine brain and stored at −78 °C. Samples were prepared directly in a 96-well microtitre 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 (10 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 ambient 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.

4.12. Molecular modeling

4.12.1. General

The compounds were drawn in Maestro (v. 9.8) and with the Macromodel package the structures were energy minimized using the OPLS2005 force field. All molecular modeling calculations were performed using the software Glide (v. 5.6) running on Linux x86_64 workstation. The conformation of colchicine was taken from the tubulin-ligand complex filed in the Brookhaven Protein Data Bank (pdb entry code 1SA0). The protein complex was prepared for docking with Maestro/Macromodel (v. 9.1.207) Protein Preparation Wizard where bond orders were assigned, hydrogens added, water molecules removed, hydrogen bond optimized and finally the protein energy minimized (converge threshold 0.30 kcal/mol). The native ligand was redocked into the processed protein to verify that the program in use could identify the correct binding mode of the ligand. Figure 2 was prepared with Pymol (v. 1.3) in Windows 7.

4.12.2. Docking with Glide

Standard parameters were used for the with Glide grid generation. A mesh of 0.375 Å and 56 × 60 × 50 number of points were used for the grid size. The grid was centered on the mass center of the DAMA-colchicine coordinates. Docking in Glide was performed with standard parameters in standard precision mode. Ten poses per ligand were generated. As a standard of comparison both CA-1 (**1**) and CA-4 (**2**) were docked together with the set of analogues **5**–**13**.

4.12.3. Energy minimization

The result complexes from the Glide docking were energy minimized with Macromodel after the reinsertion of GDP and GTP that were removed with the protein preparation wizard. Minimization was performed with Maestro/Macromodel using OPLS2005 as force field in implicit water phase. Minimization method was PRCG with maximum iterations 30000, converge on gradient and convergence threshold 0.01.

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References and notes

1. Cragg, G. R.; Kingston, D. G. I.; Newman, D. J. In *Anticancer Agents from Natural Products*; Cragg, G. R., Kingston, D. G. I., Newman, D. J., Eds.; CRC Press: Taylor & Francis, Boca Raton FL, 2005; pp 1–3.
2. (a) Stanton, R. A.; Gernert, K. M.; Nettles, J. H.; Aneja, R. *Med. Res. Rev.* **2011**, *31*, 443; (b) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012.
3. (a) Boulikas, T.; Alevizopoulos, N.; Ladopoulou, A.; Belimezi, M.; Pantos, A.; Christofis, P.; Roberts, M. In *The Cancer Clock*; Missailidis, S., Ed.; John Wiley & Sons Ltd: West Sussex, 2007; pp 173–218; (b) Mahboobi, S.; Sellmer, A.; Beckers, T. In *Bioactive Natural Products (Part M)*; Rahman, A.-U., Ed.; Elsevier: Amsterdam, 2006; Vol. 33, pp 719–750; (c) Desai, A.; Mitchison, T. J. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 83; (d) Jordan, M. A.; Wilson, L. In *The Role of Microtubules in Cell Biology*; Fojo, T., Ed.; Neurobiology and Oncology: Humana Press, Totowa, 2008; pp 47–81.
4. (a) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984; (b) Chaplin, D. J.; Horsman, M. R.; Siemann, D. W. *Curr. Opin. Invest. Drugs* **2006**, *7*, 522; (c) Tozer, G. M.; Kanthou, C.; Lewis, G.; Prise, V. E.; Vojnovic, B.; Hill, S. A. *Br. J. Radiol.* **2008**, *81*, S12; (d) Giavazzi, R.; Bonezzi, K.; Taraboletti, G. In *The Role of Microtubules in Cell Biology, Neurobiology, and Oncology*; Fojo, T., Ed.; Humana Press: Totowa, 2008; pp 519–530; (e) Hadfield, J. A.; Ducki, S.; Hirst, N.; McGown, A. T. In *Progress in Cell Cycle Research*; Meijer, L., Jézéquel, A., Roberge, M., Eds.; Life in Progress Editions: Roscoff, 2003; Vol. 5, pp 309–325; (f) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259; (g) Cai, S. X. *Recent Patents Anticancer Drug Discov.* **2007**, *2*, 79; (h) Kiselyov, A.; Balakin, K. V.; Tkachenko, S. E.; Savchuk, N.; Ivachtchenko, A. V. *Anticancer Agents Med. Chem.* **2007**, *7*, 189; (i) Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253; (j) Hill, S. A.; Tozer, G. M.; Pettit, G. R. *Anticancer Res.* **2002**, *22*, 1453.
5. (a) Shan, Y.; Zhang, J.; Liu, Z.; Wang, M.; Dong, Y. *Curr. Med. Chem.* **2011**, *18*, 523; (b) Kanthou, C.; Tozer, G. M. *Int. J. Exp. Pathol.* **2009**, *90*, 284; (c) Pasquier, E.; André, N.; Braguer, D. *Curr. Cancer Drug Targets* **2007**, *7*, 566; (d) Young, S. L.; Chaplin, D. J. *Expert Opin. Investig. Drugs* **2004**, *13*, 1171; (e) Cirla, A.; Mann, J. *Nat. Prod. Rep.* **2003**, *20*, 558.
6. (a) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119; (b) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
7. (a Pinney, K. G.; Jelinek, C.; Edvardsen, K.; Chaplin, D. J.; Pettit, G. R. In *Anticancer Agents from Natural Products*; Cragg, G. R., Kingston, D. G. I., Newman, D. J., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, 2005; pp 23–46; (b) Patterson, D. M.; Rustin, G. J. S. *Drugs Future* **2007**, *32*, 1025; (c) Patterson, D. M.; Ross, P.; Koetz, B.; Saleem, A.; Stratford, M.; Stirling, J.; Padhani, A.; Asselin, M.; Price, P.; Rustin, G. J. *J. Clin. Oncol. (Meet. Abstr.)* **2007**, *25*, 14146; (d) Akerley, W. L.; Schabel, M.; Morrell, G.; Horvath, E.; Yu, M.; Johnsson, B.; Arbogast, K. *J. Clin. Oncol. (Meet. Abstr.)* **2007**, *25*, 14060; (e) Nathan, P. D.; Judson, I.; Padhani, A.; Harris, A.; Carden, C. P.; Smythe, J.; Collins, D.; Leach, M.; Walicke, P.; Rustin, G. J. *J. Clin. Oncol. (Meet. Abstr.)* **2008**, *26*, 3550; For a recent review of the clinical trials of combretastatin A-4 (3) and its prodrug 4, see: (f) Siemann, D. W.; Chaplin, D. J.; Walicke, P. A. *Expert Opin. Inv. Drug.* **2009**, *18*, 189; (g) www.oxigene.com, (accessed Aug 15, 2011).
8. (a) Pettit, G. R.; Lippert, J. W., III *Anti-Cancer Drug Des.* **2000**, *15*, 203; (b) Salmon, H. W.; Siemann, D. W. *Clin. Cancer Res.* **2006**, *12*, 4090.
9. (a) Pettit, G. R.; Thornhill, A. J.; Moser, B. R.; Hogan, F. *J. Nat. Prod.* **2008**, *71*, 1561; (b) Folkes, L. K.; Christlieb, M.; Madej, E.; Stratford, M. R. L.; Wardman, P. *Chem. Res. Toxicol.* **2007**, *20*, 1885; (c) Rice, L.; Pampo, C.; Lepler, S.; Rojiani, A. M.; Siemann, D. W. *Microvasc. Res.* **2011**, *81*, 44.
10. (a) Holwell, S. E.; Cooper, P. A.; Grosios, K.; Lippert, J. W., III; Pettit, G. R.; Snyder, S. D.; Bibby, M. C. *Anticancer Res.* **2002**, *22*, 707; (b) Holwell, S. E.; Cooper, P. A.; Thompson, M. J.; Pettit, G. R.; Lippert, J. W., III; Martin, S. W.; Bibby, M. C. *Anticancer Res.* **2002**, *22*, 3933; (c) Hua, J.; Sheng, Y.; Pinney, K. G.; Garner, C. M.; Kane, R. R.; Prezioso, J. A.; Pettit, G. R.; Chaplin, D. J.; Edvardsen, K. *Anticancer Res.* **2003**, *23*, 1433; (d) Salmon, H. W.; Mladinich, C.; Siemann, D. W. *Eur. J. Cancer* **2006**, *42*, 3073.
11. Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W., III; Shnyder, S. D.; Cooper, P. A.; Bibby, M. C. *Clin. Cancer Res.* **2004**, *10*, 1446.
12. (a) Chaudhary, A.; Pandeya, S. N.; Kumar, P.; Sharma, P. P.; Gupta, S.; Soni, N.; Verma, K. K.; Bhardwaj, G. *Mini-Rev. Med. Chem.* **2007**, *7*, 1186; (b) Hsieh, H. P.; Liou, J. P.; Mahindroo, N. *Curr. Pharm. Des.* **2005**, *11*, 1655; (c) Nam, N.-H. *Curr. Med. Chem.* **2003**, *10*, 1697; (d) Singh, R.; Kaur, H. *Synthesis* **2009**, 2471; (e) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033.
13. See Ref. 5a, and the following selected references: (a) Peifer, C.; Stoiber, T.; Unger, E.; Totzke, F.; Schächtele, C.; Marmé, D.; Brenk, R.; Klebe, G.; Schollmeyer, D.; Dannhardt, G. *J. Med. Chem.* **2006**, *49*, 1271; (b) Pirali, T.; Busacca, S.; Beltrami, L.; Imovilli, D.; Pagliai, F.; Miglio, G.; Massarotti, A.; Verotta, L.; Tron, G. C.; Sorba, G.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 5372; (c) Quintin, J.; Roullier, C.; Thoret, S.; Lewin, G. *Tetrahedron* **2006**, *62*, 4038; (d) Sun, C.-M.; Lin, L.-G.; Yu, H.-J.; Cheng, C.-Y.; Tsai, Y.-C.; Chu, C.-W.; Din, Y.-H.; Chau, Y.-P.; Don, M.-J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1078; (e) Tsyganov, D. V.; Yakubov, A. P.; Konyushkin, L. D.; Firgang, S. I.; Semenov, V. V. *Russ. Chem. Bull.* **2007**, *56*, 2460; (f) Xue, N.; Yang, X.; Wu, R.; Chen, J.; He, Q.; Yang, B.; Lu, X.; Hu, Y. *Bioorg. Med. Chem.* **2008**, *16*, 2550.
14. (a) Odlo, K.; Hentzen, J.; Fournier dit Chabert, J.; Ducki, S.; Gani, O. A. B. S. M.; Sylte, I.; Skrede, M.; Florenes, V. A.; Hansen, T. V. *Bioorg. Med. Chem.* **2008**, *16*, 4829; (b) Odlo, K.; Fournier dit Chabert, J.; Ducki, S.; Gani, O. A. B. S. M.; Sylte, I.; Hansen, T. V. *Bioorg. Med. Chem.* **2010**, *18*, 6874; (c) Cafici, L.; Pirali, T.; Condorelli, F.; Del Grosso, E.; Massarotti, A.; Sorba, G.; Canonico, P. L.; Tron, G. C.; Genazzani, A. A. *J. Comb. Chem.* **2008**, *10*, 732; (d) Romagnoli, R.; Baraldi, P. G.; Cruz-Lopez, O.; Lopez Cara, C.; Carrion, M. D.; Brancale, A.; Hamel, E.; Chen, L.; Bortolozzi, R.; Basso, G.; Viola, G. *J. Med. Chem.* **2010**, *53*, 4248; (e) Zhang, Q.; Peng, Y.; Wang, X. I.; Keenan, S. M.; Arora, S.; Welsh, W. J. *J. Med. Chem.* **2007**, *50*, 749; (f) Pati, H. N.; Wicks, M.; Holt, H. K. H., Jr.; LeBlanc, R.; Weisbruch, P.; Forrest, L.; Lee, M. *Heterocycl. Commun.* **2005**, *11*, 117.
15. (a) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579; (b) Lawrence, N. J.; Hepworth, L. A.; Rennison, D.; McGown, A. T.; Hadfield, J. A. *J. Fluorine Chem.* **2003**, *123*, 101; (c) Chang, J.-Y.; Yang, M.-F.; Chang, C.-Y.; Chen, C.-M.; Kuo, C.-C.; Liou, J.-P. *J. Med. Chem.* **2006**, *49*, 6412; (d) Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackley, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. *Bioorg. Med. Chem.* **2006**, *14*, 3231; (e) Siles, R.; Ackley, J. F.; Hadimani, M. B.; Hall, J. J.; Mugabe, B. E.; Guddneppanavar, R.; Monk, K. A.; Chapuis, J.-C.; Pettit, G. R.; Chaplin, D. J.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Pinney, K. G. *J. Nat. Prod.* **2008**, *71*, 313; (f) Pettit, G. R.; Thornhill, A.; Melody, N.; Knight, J. C. *J. Nat. Prod.* **2009**, *72*, 380; (g) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200; (h) Pettit, G.; Lippert, J.; Boyd, M.; Verdier-Pinard, P.; Hamel, E. *Anti-Cancer Drug Des.* **2000**, *15*, 361; (i) Pettit, G. R.; Lippert, J. W.; Herald, D. L.; Hamel, E.; Pettit, R. K. *J. Nat. Prod.* **2000**, *63*, 969; (j) Nam, N.-H.; Kim, Y.; You, Y.-J.; Hong, D.-H.; Kim, H.-M.; Ann, B.-Z. *Arch. Pharm. Res* **2002**, *25*, 600; (k) Harrowven, D. C.; Guy, I. L.; Howell, M.; Packham, G. *Synlett* **2006**, 2977; (l) Sriram, M.; Hall, J. J.; Grohmann, N. C.; Strecker, T. E.; Wootton, T.; Franken, A.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2008**, *16*, 8161; (m) Carr, M.; Greene, L. M.; Knox, A. J. S.; Lloyd, D. G.; Zisterer, D. M.; Meegan, M. J. *Eur. J. Med. Chem.* **2010**, *45*, 5752; (n) Duan, J.-X.; Cai, X.; Meng, F.; Lan, L.; Hart, C.; Matteucci, M. *J. Med. Chem.* **2007**, *50*, 1001.
16. Botta, M.; Forli, S.; Magnani, M.; Manetti, F. In *Topics in Current Chemistry*; Carlomagno, T., Ed.; Springer-Verlag: Berline Heidelberg, 2009; Vol. 286, pp 279–328.
17. Odlo, K.; Klaveness, J.; Rongved, P.; Hansen, T. V. *Tetrahedron Lett.* **2006**, *47*, 1101.
18. Artacho, J.; Nilsson, P.; Bergquist, K.-E.; Wendt, O. F.; Wärnmark, K. *Chem. Eur. J.* **2006**, *12*, 2692.
19. Kaisalo, L.; Latvala, A.; Hase, T. *Synth. Commun.* **1986**, *16*, 645.
20. Stork, G.; Takahashi, T. *J. Am. Chem. Soc.* **1977**, *99*, 1275.
21. (a) Colvin, E. W.; Hamill, B. J. *J. Chem. Soc., Chem. Commun.* **1973**, 151; (b) Miwa, K.; Aoyama, T.; Shioiri, T. *Synlett* **1994**, 107.
22. Jones, G. H.; Venuti, M. C.; Young, J. M. USA Patent 4840965, 1989.
23. (a) Hofsløkken, N. U.; Skattebøl, L. *Acta Chem. Scand.* **1999**, *53*, 258; (b) Hansen, T. V.; Skattebøl, L. *Org. Synth.* **2005**, *82*, 64.
24. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *13*, 3769.
25. (a) Ohira, S. *Synth. Commun.* **1986**, *19*, 561; (b) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521.
26. (a) Krasiński, A.; Fokin, V. V.; Sharpless, K. B. *Org. Lett.* **2004**, *6*, 1237; (b) Akimova, G. S.; Chistokletov, V. N.; Petrov, A. A. *Zh. Org. Khim.* **1967**, *3*, 968; (c) Akimova, G. S.; Chistokletov, V. N.; Petrov, A. A. *Zh. Org. Khim.* **1967**, *3*, 2241; (d) Akimova, G. S.; Chistokletov, V. N.; Petrov, A. A. *Zh. Org. Khim.* **1968**, *4*, 389.
27. Yardley, J. P.; Fletcher, H., III *Synthesis* **1976**, 244.
28. (a) Huisgen, R.; Szeimies, G.; Möbius, L. *Chem. Ber.* **1967**, *100*, 2494; (b) Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984; pp 1–176; (c) Lwowski, W. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984; pp 559–651.
29. Yoo, S.-E.; Lee, S.-H. *Synlett* **1990**, 419.
30. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
31. Fatokoun, A. A.; Stone, T. W.; Smith, R. A. *Bone* **2006**, *39*, 542.
32. (a) Chen, S. C.; Mei-Kuang, L.; Cheng, J.-J.; Wang, D. L. *FEMS Microb. Lett.* **2005**, *249*, 247; (b) Pi, X.; Yan, C.; Berk, B. C. *Circ. Res.* **2004**, *94*, 362.
33. Jones, M. K.; Wang, H.; Peskar, B. M.; Levin, E.; Itani, R. M.; Sarfeh, I. J.; Tarnawski, A. S. *Nat. Med.* **1999**, *5*, 1418.
34. Lawrence, N. J.; McGown, A. T.; Ducki, S.; Hadfield, J. A. *Anti-Cancer Drug Des.* **2000**, *15*, 135.
35. Ravelli, R. B. G.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198.
36. (a) Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. *Nat. Rev. Drug Disc.* **2004**, *3*, 935; (b) O'Boyle, N. M.; Liebeschuetz, J. W.; Cole, J. C. *J. Chem. Inf. Model.* **2009**, *49*, 1871.
37. Nguyen, T. L.; McGrath, C.; Hermone, A. R.; Burnett, J. C.; Zaharevitz, D. W.; Day, B. W.; Wipf, P.; Hamel, E.; Gussio, R. *J. Med. Chem.* **2005**, *48*, 6107.
38. Anwar, H. F.; Hansen, T. V. *Synlett* **2008**, 2681.
39. Hahn, E.; Rupprecht, S.; Kramp, W.; Neumeier, R. CA2048899 (A1), 1992, 61.
40. Akselsen, Ø. W.; Skattebøl, L.; Hansen, T. V. *Tetrahedon Lett.* **2009**, *50*, 6339.
41. Poisson, T.; Gembus, V.; Dalla, V.; Oudeyer, S.; Levacher, V. *J. Org. Chem.* **2010**, *75*, 7704.
42. (a) Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135; (b) Odlo, K. Ph.D. Thesis, University of Oslo, Oslo, 2010.