Bioorganic & Medicinal Chemistry 18 (2010) 6874–6885

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

1,2,3-Triazole analogs of combretastatin A-4 as potential microtubule-binding agents

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ARTICLE INFO

Article history:
Received 4 June 2010
Revised 7 July 2010
Accepted 14 July 2010
Available online 17 July 2010

Keywords:
Combretastatin A-4
Cytotoxic
12,3-Triazoles
Molecular Modeling

ABSTRACT

A series of *cis*-restricted 1,4- and 1,5-disubstituted 1,2,3-triazole analogs of combretastatin A-4 (**1**) have been prepared. Cytotoxicity and tubulin inhibition studies showed that 2-methoxy-5-((5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)aniline (**5e**) and 2-methoxy-5-(1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazol-5-yl)aniline (**6e**) were two of the most active compounds. Molecular modeling studies revealed that the *N*-2 and *N*-3 atoms in the triazole rings in **5e** and **6e** did not form hydrogen bonds with the amino acids in the anticipated pharmacophore.

1. Introduction

Microtubule-binding agents (MBAs) are widely used in cancer chemotherapy.[1] There are two classes of MBAs: those that stabilize microtubules and promote polymerization, and those that destabilize the microtubules and promote depolymerization. Both types interfere with the mitotic spindle assembly during cell division, resulting in cell death. Studies have shown that most MBAs have antivascular effects, antiangiogenic or vascular disrupting activities, or both.[2]

One MBA that has attracted much interest as a lead compound[3] is combretastatin A-4 (CA-4, **1**) isolated from the bark of the South African tree *Combretum caffrum*.[4] This natural product binds to the colchicine binding site on tubulin and inhibits tubulin polymerization.[5] CA-4 is highly cytotoxic against a variety of human cancer cell lines and is a vascular disrupting agent.[6]

Structure–activity relationship (SAR) studies have shown that a 3,4,5-trimethoxysubstituted A ring and a 4-methoxysubstituted B ring separated by a double bond with *cis*-configuration are important for optimal cytotoxic activity (Fig. 1).[7]

The major disadvantages of CA-4 as a drug candidate are low water solubility and isomerization to the less active *trans*-form. A phosphate prodrug of CA-4 (CA-4P, **2**) has been prepared, and is currently in several clinical trials.[8] The synthesis and biological evaluation of several combretastatin analogs with a locked *cis*-type bridge between the two phenyl rings have been reported.[7a,9] However, very few analogs where a triazole moiety has replaced the *cis*-olefin have been reported.[10]

Recently, we prepared a series of 1,5-disubstituted 1,2,3-triazole analogs of CA-4 (type I).[11] The analogs were evaluated for their cytotoxic activity and their ability to inhibit tubulin polymerization.[12] One of the amino-substituted analogs (**4e**) exhibited potent cytotoxic activity and moderate activity as a tubulin inhibitor. The molecular modeling studies of **4e** revealed that the *N*-2 and *N*-3 atoms of the 1,2,3-triazole ring formed hydrogen bonds with the main chain nitrogen atoms of βA250 and βD251 in loop T7 that connects helices H7 and H8 of β-tubulin. To extend our knowledge of the 1,2,3-triazole as a suitable mimic for the *cis*-configuration present in CA-4, we have prepared three new series of analogs of CA-4 (Fig. 2). Several SAR studies have revealed that the two aryl rings of CA-4 can be separated by more than a two-atom bridge.[13] In order to investigate this we replaced the *cis*-olefin in CA-4 with a 1,5-disubstituted 1,2,3-triazole ring coupled to a methylene group (type II), thereby creating a three-atom bridge between the aryl rings. Furthermore, based on the hydrogen bonding interactions revealed in our previous study,[11] we wanted to investigate if a 1,4-disubstituted 1,2,3-triazol ring would retain some of the biological activity of the 1,5-disubstituted triazole analogs, since *trans*-resveratrol is a stilbene that has been found to be more cytotoxic than its *cis*-form.[14] To further our

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doi:10.1016/j.bmc.2010.07.032

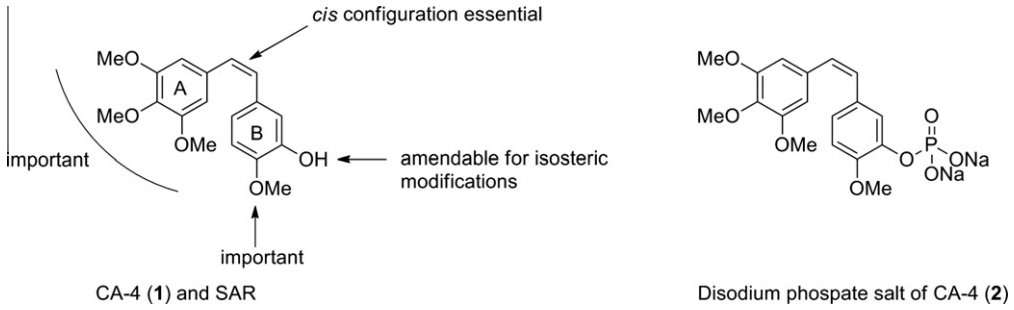


Figure 1. Combretastatin A-4 (**1**) and its prodrug (**2**).

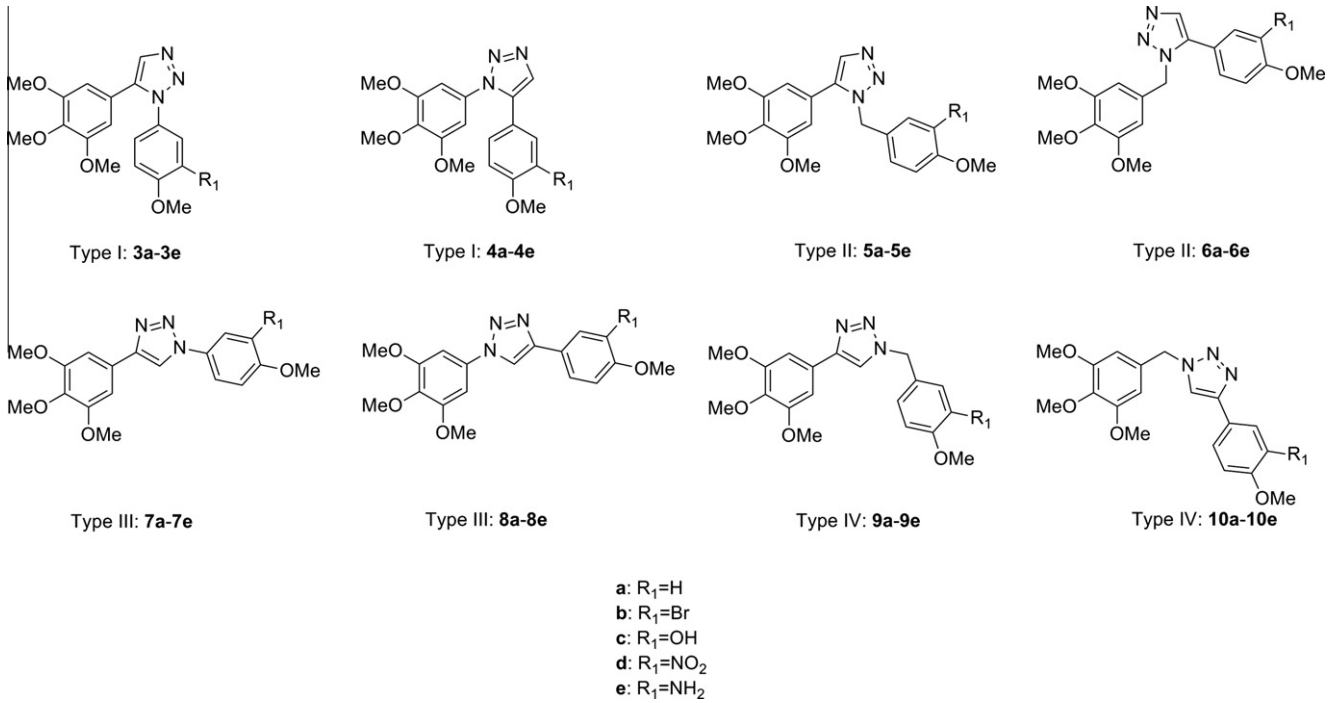


Figure 2. Structures of triazole analogs type I (1,5-disubstituted 1,2,3-triazole) **3a**–**3e** and **4a**–**4e**, type II (1,5-disubstituted 1,2,3-triazole with methylene group) **5a**–**5e** and **6a**–**6e**, type III (1,4-disubstituted 1,2,3-triazole) **7a**–**7e** and **8a**–**8e**, and type IV (1,4-disubstituted 1,2,3-triazole with methylene group) **9a**–**9e** and **10a**–**10e**.

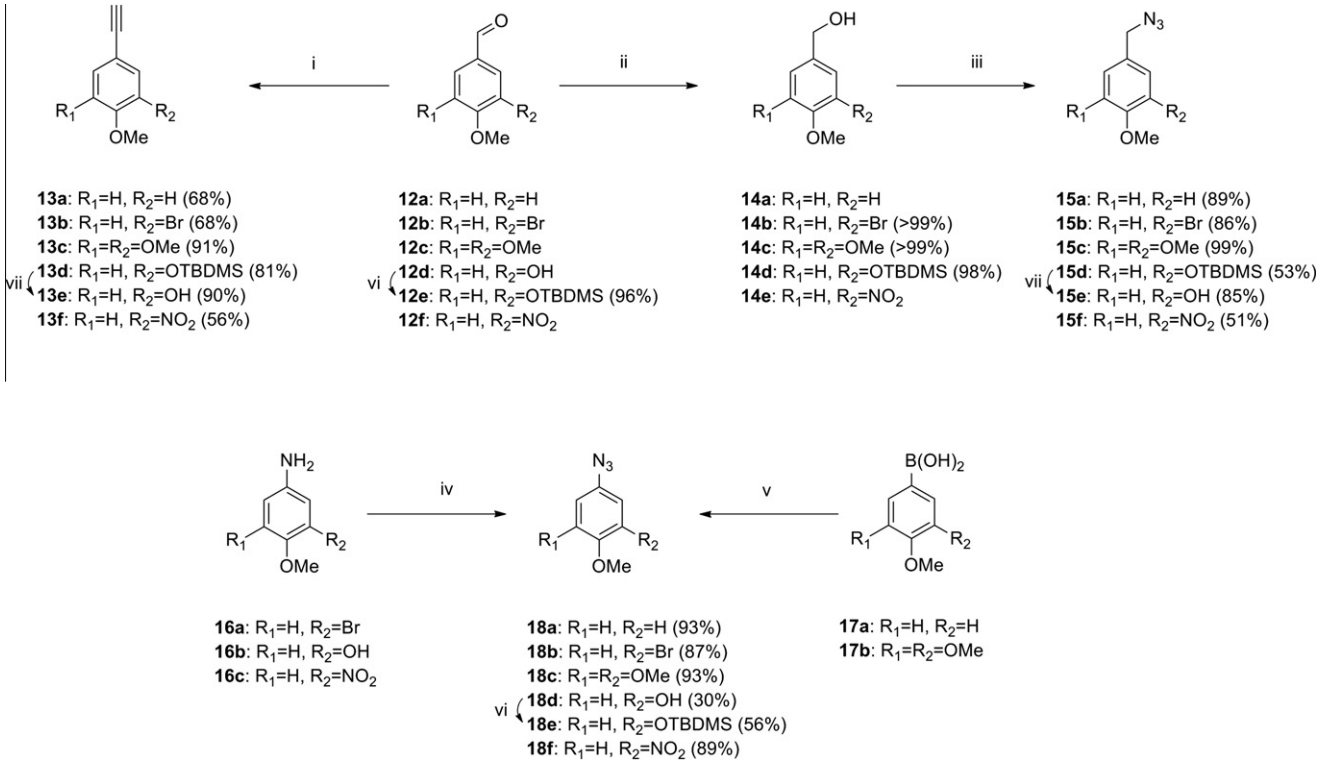
knowledge of the possibility of mimicking the olefin with a four-atom bridge, we prepared analogs where the aryl rings are separated by a 1,4-disubstituted 1,2,3-triazole ring coupled to a methylene group (type IV). Herein we report the synthesis and in vitro cytotoxic activities of several 1,4- and 1,5-disubstituted 1,2,3-triazole analogs of CA-4, as well as the inhibition data of tubulin polymerization for the most active analogs. Furthermore, molecular modeling studies of two of the prepared analogs (**5e** and **6e**) with the colchicine binding site of α,β-tubulin were performed together with a potential analog **11**.

2. Results and discussion

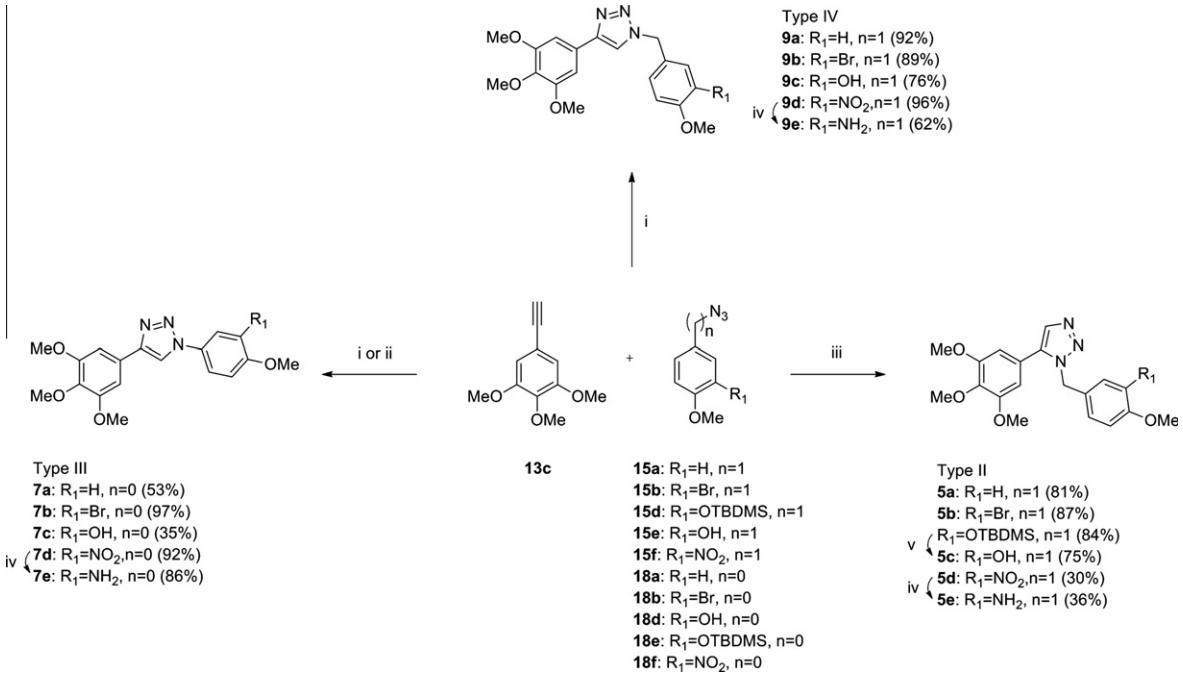
2.1. Synthesis

The syntheses of the starting materials are depicted in Scheme 1. The terminal alkynes (**13a**–**13d** and **13f**) were prepared from the corresponding benzaldehydes (**12a**–**12c** and **12e**–**12f**) using the Colvin rearrangement.[15] The benzyl azides were prepared from the same starting materials, via the benzyl alcohols. The benzyl alcohols (**14b**–**14d**) were obtained by a reduction of the aldehydes (**12b**, **12c**, and **12e**) with sodium borohydride,[16] which were subsequently converted into the benzyl azides (**15a**–**15d** and **15f**).[17] The phenyl azides (**18a**–**18d** and **18f**) were prepared either from the corresponding anilines (**16a**–**16c**) using diazotization conditions[10d] or from the corresponding boronic acids (**17a** and **17b**) after reaction with sodium azide.[18] The phenolic groups were protected as TBDMS-ethers when nedeed.[19]

The target triazoles (**5a**–**5e**, **6a**–**6e**, **7a**–**7e**, **8a**–**8e**, **9a**–**9e**, and **10a**–**10e**) were prepared from the corresponding terminal alkynes and benzyl or phenyl azides as depicted in Scheme 2 and Scheme 3. The 1,4-disubstituted 1,2,3-triazoles (**7a**–**7e**, **8a**–**8e**, **9a**–**9e**, and **10a**–**10e**) were prepared in a Cu(I)-catalyzed Huisgen cycloaddition reaction, either by the use of copper sulfate and sodium ascorbate, or copper turnings.[20] The use of copper turnings proved to be a convenient method for the synthesis of the 1,4-disubstituted 1,2,3-triazoles; however lower yields were obtained compared to using the combination of copper sulfate and sodium ascorbate. The 1,5-disubstituted 1,2,3-triazoles (**5a**–**5e** and **6a**–**6e**) were obtained either by the use of magnesium acetylides[21] or by ruthenium catalysis.[22] Removal of the TBDMS-group using TBAF afforded triazoles **5c**, **6c**, and **8c**, respectively.[23] The amino-substituted triazoles (**5e**, **6e**, **7e**, **8e**, **9e**, and **10e**) were obtained by reduction of the nitro group using $NaBH_4/CuSO_4$.[24] All new compounds exhibited spectral data in agreement with their assigned structures.



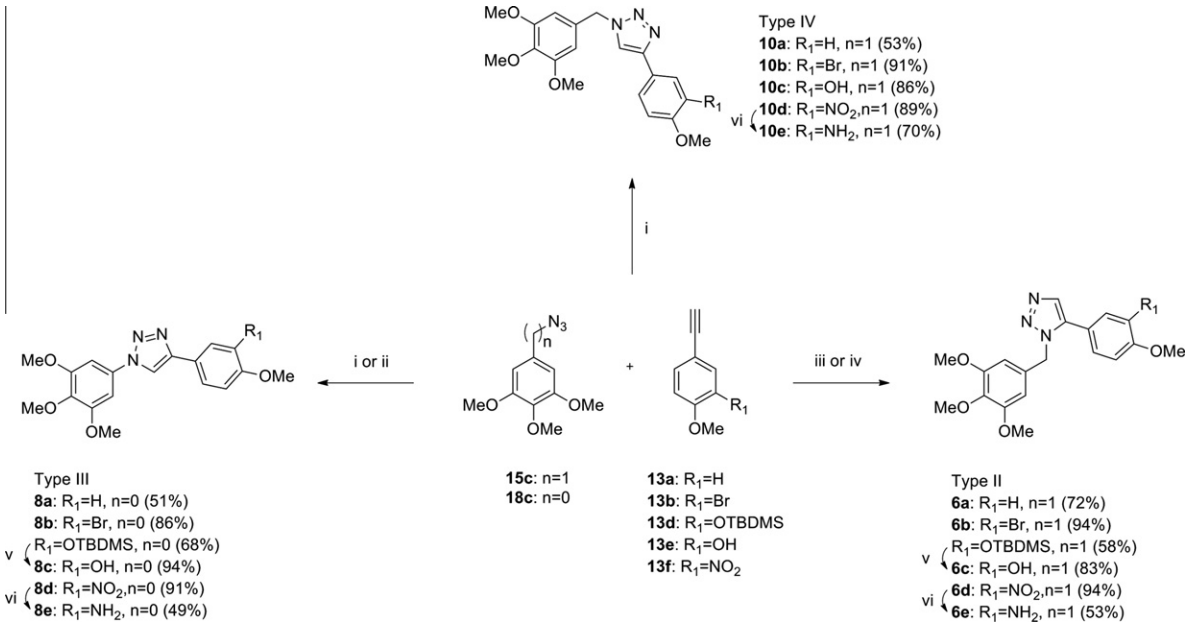
Scheme 1. Synthesis of starting materials. Reagents and conditions: (i) LDA, $TMSCHN_2$, THF, −78 °C; (ii) $NaBH_4$, EtOH, H_2O, 0 °C; (iii) $(PhO)_2PON_3$, DBU, toluene, 0 °C; (iv) HCl (aq), NaN_3, $NaNO_2$, 0 °C; (v) NaN_3, $CuSO_4$, MeOH; (vi) DIPEA, TBDMSCl, DMF; (vii) *n*-Bu_4NF, THF.



Scheme 2. Synthesis of triazoles **5a**–**5e**, **7a**–**7e**, and **9a**–**9e**. Reagents and conditions: (i) Na-ascorbate, $CuSO_4$, *t*-BuOH/H_2O (1:1); (ii) Cu-turnings, MeOH; (iii) EtMgCl, THF, Δ; (iv) $NaBH_4$, $CuSO_4$, EtOH, Δ; (v) *n*-Bu_4NF, THF.

2.2. Biological evaluation

The target triazoles (**5a**–**5e**, **6a**–**6e**, **7a**–**7e**, **8a**–**8e**, **9a**–**9e**, and **10a**–**10e**) were evaluated for their ability to inhibit the growth of K562 leukemia cancer cells using the MTT assay (Table 1).[25] These results showed that the 1,5-disubstituted 1,2,3-triazoles in general had greater cytotoxic activity than the 1,4-regioisomers, as anticipated based on the work of Tron and Genazzani.[10d] The



Scheme 3. Synthesis of triazoles **6a**–**6e**, **8a**–**8e**, and **10a**–**10e**. Reagents and conditions: (i) Na-ascorbate, $CuSO_4$, *t*-BuOH/H_2O (1:1); (ii) Cu-turnings, MeOH; (iii) EtMgCl, THF, Δ; (iv) Cp∗RuCl(COD), benzene, Δ; (v) *n*-Bu_4NF, THF; (vi) $NaBH_4$, $CuSO_4$, EtOH, Δ.

triazoles derived from the phenyl azides proved to be more cytotoxic than the triazoles derived from the benzyl azides. Hence, the cytotoxic activity decreased with increasing bridge length; 2 > 3 > 4. As in our previous study, the analogs with the A ring derived from a phenyl azide, resulted in a series of more active triazole analogs. The substituent in the 3′-position of the B-ring clearly affected the cytotoxic activity, and the most potent compounds had an amino group (**6e**, **5e**, and **8e**), a hydrogen atom (**6c**, **5c**, and **7c**) or a phenolic group (**6a** and **5a**) in the 3′-position, as shown in other studies.[10c,26]

The triazoles that exhibited cytotoxic activities were further evaluated for their ability to inhibit the polymerization of tubulin.[27] Unfortunately, none of the analogs proved to inhibit the polymerization of tubulin in any significant degree. The 1,4-disubstituted 1,2,3-triazole ring might mimic the *trans*-configuration of CA-4. Results reported herein supplement the studies reported by Tron and Genazzani.[10d] However, the 1,5-disubstituted 1,2,3-triazole ring with a methylene group between the A and B rings might be used as a mimic for the *cis*-configuration in CA-4 with regards to cytotoxicity. The cytotoxic activity is retained in a larger degree than the tubulin inhibitory activity for all triazole analogs reported herein.[11]

2.3. Molecular modeling

Molecular modeling studies were performed to investigate the difference in binding ability to the colchicine binding site of α,β-tubulin of the less active triazole analogs (**5e** and **6e**), and a potential new triazole analog (**11**) (Fig. 3). The binding modes were compared with that of the previously reported active amino-substituted triazole analog (**4e**) that was docked in our previous study.[11] Upon docking all three compounds were located in the colchicine binding site of α,β-tubulin (mostly into the β subunit). The trimethoxy aryl moiety (ring A) and the triazole ring of the analogs occupied similar regions of the binding cavity, but the analogs had different orientation for the 3-amino-4-methoxy aryl moiety (ring B) (Fig. 4). For the three analogs the free energies of binding were about 5 kcal/mol for α-tubulin, but for β-tubulin the energies were −8.5, −3.7, and −3.5 kcal/mol for **5e**, **6e**, and **11**, respectively. The corresponding values of (**4e**) in our previous study were 6 and −10 kcal/mol.

Ring A of all three triazole analogs occupied a distinct binding region at the interface of α- and β-tubulin. This moiety of **4e** was found to be buried completely inside of β-tubulin similar to what is seen in the X-ray complex of DAMA-colchicine with tubulin[28] (Fig. 4). The triazole ring in compounds **5e**, **6e**, and **11** did not form hydrogen bonds with the surrounding residues, which is contrary to **4e** and the previously described pharmacophore model.[11,29] Ring B of **5e** occupied similar Cartesian space as corresponding moieties in **4e** and DAMA-colchicine, but the corresponding moiety of **6e** and **11** occupied a completely different space close to the GTP binding region of α-tubulin (Fig. 4). The quite unexpected position of this moiety may be responsible for the relatively low binding affinities of **6e** and **11** (−3.7 and −3.5 kcal/mol, respectively) compared with **4e** and **5e**. Triazole analog **5e** binds very similar to β-tubulin as DAMA-colchicine in the X-ray complex with β-tubulin, giving a more favorable binding energy of −8.5 kcal/mol. We also assume that the low affinity of **6e** and **11** for β-tubulin reflects the weak binding affinity of **6e** and **11** for α,β-tubulin. These results suggest that the structure of **11** is not a suitable mimic for CA-4. The binding energies also support our previous assumption that β-tubulin is responsible for the strength of ligand binding.[11] The binding energies of the analogs is not in correlation with the observed cytotoxic activities, indicating that other mechanisms most likely are responsible for the cytotoxic effects.

The binding energies indicate that **4e** showed >10-fold higher affinity than **5e** for β-tubulin (∼1.4 kcal/mol difference in binding energy causes about 10-fold difference in affinity). The higher affinity of **4e** (free energy of binding for β-tubulin −10 kcal/mol) may be a result of favorable orientation of the trimethoxyphenyl moiety inside the cavity of β-tubulin. This moiety of **4e** overlapped with the corresponding ring in the X-ray structure of DAMA-colchicine. However, the difference in orientation of the triazole ring may also contribute to altered affinity for β-tubulin. The correct position of the trimethoxy aryl moiety has been suggested to be necessary, but not sufficient, to predict activity of potential

Table 1
Cytotoxicity and inhibition of tubulin polymerization by triazoles **5a**–**5e**, **6a**–**6e**, **7a**–**7e**, **8a**–**8e**, **9a**–**9e**, and **10a**–**10e**

Compound	K562 cell assay, IC_{50}[a] (μM) in vitro cytotoxicity	Inhibition of tubulin polymerization IC_{50}[b] (μM)
5a	8.66	n.d.
5b	>10	n.d.
5c	1.83	n.d.
5d	>10	n.d.
5e	0.73	>20[c]
6a	5.56	n.d.
6b	>10	n.d.
6c	0.53	n.d.
6d	>10	n.d.
6e	0.38	>20[c]
7a	>10	n.d.
7b	>10	n.d.
7c	3.20	>20
7d	>10	n.d.
7e	>10	n.d.
8a	>10	n.d.
8b	>10	n.d.
8c	>10	n.d.
8d	>10	n.d.
8e	1.30	>20
9a	>10	n.d.
9b	>10	n.d.
9c	>10	n.d.
9d	>10	n.d.
9e	>10	n.d.
10a	>10	n.d.
10b	>10	n.d.
10c	>10	n.d.
10e	>10	n.d.
10e	>10	n.d.
4e	0.011	4.8
1	0.010	0.6

n.d., not determined.
[a] Results of three experiments.
[b] Results of two experiments.
[c] Determined using the fluorescence based tubulin polymerization assay kit (cat. # BK011) from cytoskeleton.

compounds binding to the colchicine binding site on tubulin. Most of the reported molecular modeling studies of tubulin inhibitory analogs of CA-4 describe hydrogen bonding interactions between the methoxy group(s) of ring A and βC241.[10a,29,30] For **5e** and **6e**, the large difference in affinity for β-tubulin may be due to different orientations of ring B. From our modeling studies the orientation of this moiety seems to be very important for tubulin binding of all 1,2,3-triazole analogs of CA-4. On the other hand, the triazole analogs might be too rigid to enter the colchicine binding site, thus not exhibiting tubulin inhibitory activity despite their binding affinity to the binding site in the molecular modeling studies.

We are currently evaluating the antivascular effects of triazole analogs such as **4e**. Moreover, based on the results reported herein, other triazole mimics of the combretastatins will be prepared. These efforts will be reported in due course.

3. Conclusion

The orientation of ring B of the triazole analogs of combretastatin A-4 proved to be very important for their binding energies, as shown herein for compounds **5e** and **6e**. The present results indicate that hydrogen bonding between the nitrogen atoms in the triazole *cis*-olefin mimic may play a role for the affinity of the analogs to tubulin, but based on other molecular modeling studies it is probably less important for tubulin inhibition than the orientation of the trimethoxy substituted A ring.

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) or Versapak C18 cartridge (Supelco). NMR (^1H, ^{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 ^1H and 77.00 ppm for ^{13}C) or DMSO-d_6 (2.50 ppm for ^1H and 39.43 ppm for ^{13}C). Compounds **8d**, **8e**, **12e**, **13a**–**13d**, **13f**, and **18a**–**18f** have been described in our previous work.[11,31] Compounds **7a** and **7c**,[10d] **8a** and **8c**,[10d] **13e**,[13b] **14b**,[32] **14c**,[33] **14d**,[34] **15a**,[35] **15c**,[36] and **15d**,[37] are known compounds. Combretastatin A-4 (**1**) was prepared according to a literature procedure.[38]

4.2. Syntheses of benzyl alcohols: general procedure

The corresponding benzaldehyde (10.00 mmol) was dissolved in ethanol (10 mL) and stirred at 0 °C. A solution of sodium borohydride (0.19 g, 5.02 mmol) in ethanol/H_2O 1:1 (12 mL) was added dropwise. The resulting mixture was stirred at room temperature for 2 h. Ice was added, and the mixture was acidified using concentrated HCl. The mixture was extracted with ethyl acetate (4× 20 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo.

4.2.1. (3-Bromo-4-methoxyphenyl)methanol (14b)

Pale yellow oil (>99%). R_f = 0.88 (hexane/EtOAc 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.68 (br s, 1H), 3.89 (s, 3H), 4.60 (s, 2H), 6.88 (d, J = 8.4 Hz, 1H), 7.27 (dd, J = 8.4, 2.1 Hz, 1H), 7.56 (d, J = 2.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.30, 64.29, 111.69, 111.87, 127.30, 132.25, 134.51, 155.39. HRMS calcd. for $C_8H_9BrO_2$ (M^+): 215.9786. Found 215.9791.

4.2.2. (3,4,5-Trimethoxyphenyl)methanol (14c)

Colorless oil (>99%). R_f = 0.82 (dichloromethane/methanol 4:1). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.97 (br s, 1H), 3.83 (s, 3H), 3.86

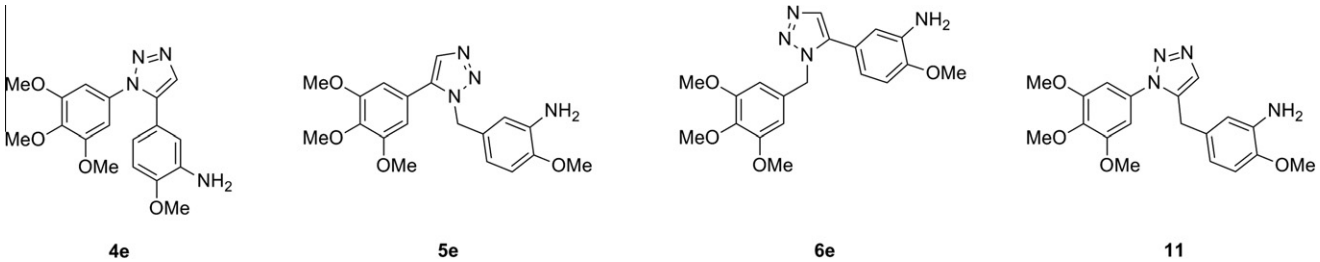


Figure 3. Structures of triazole analogs used in molecular modeling (**4e**, **5e**, **6e**, and **11**).

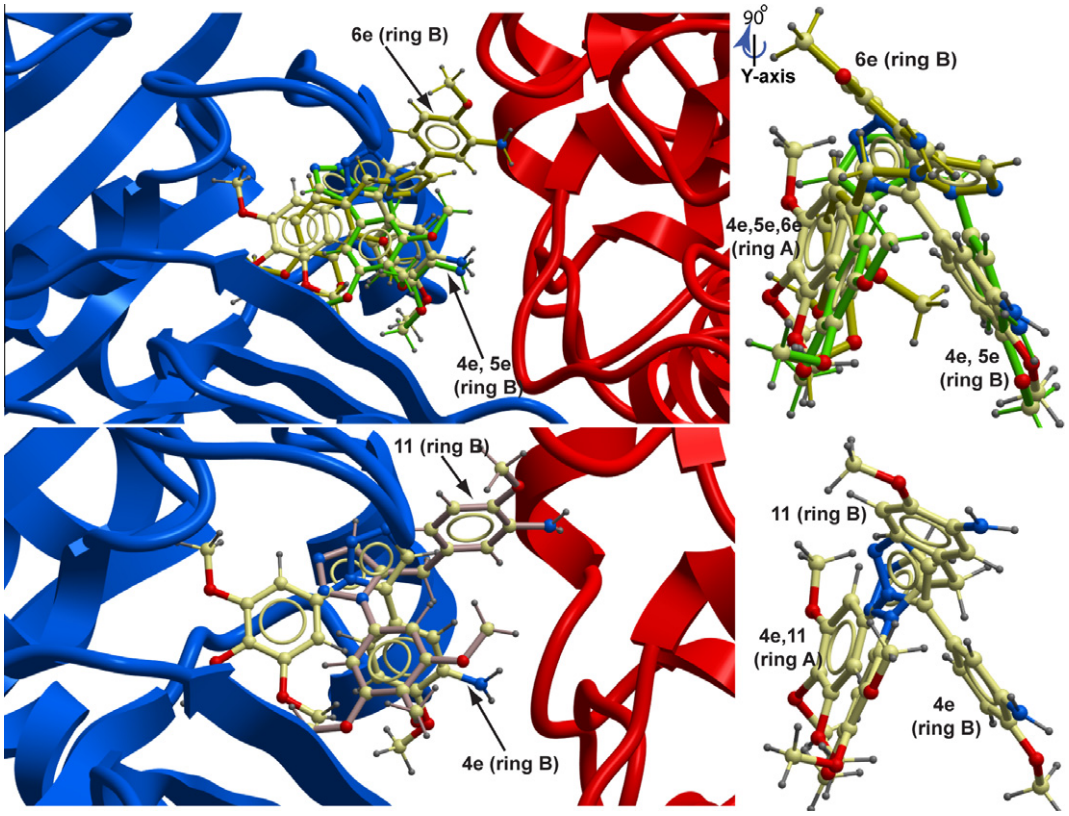


Figure 4. Binding mode of triazole analogs **4e**, **5e**, **6e**, and **11** after docking into the colchicine binding site of tubulin (PDB entry 1SA0). The figure shows the superposition of receptor–ligand complexes where the X-ray structure of tubulin is shown as ribbon representation (α-tubulin in red, and β-tubulin in blue) and stick models are used for ligands (with atom type color: carbon-white, nitrogen-blue, oxygen-red, hydrogen-gray). The orientations of the trimethoxy aryl moiety (ring A) and the 3-amino-4-methoxy aryl moiety (ring B) in the ligands are indicated for **4e**, **5e**, **6e**, and **11**. Side-views (−90° rotation relative to a and b) of superimposed ligands are also shown, indicating their relative positions of ring A and ring B.

(s, 6H), 4.62 (s, 2H), 6.59 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.04, 60.81, 65.45, 103.76, 136.63, 137.27, 153.32. HRMS calcd. for $C_{10}H_{14}O_4$ (M^+): 198.0892. Found 198.0887.

4.2.3. (3-(*tert*-Butyldimethylsilyloxy)-4-methoxyphenyl)-methanol (14d)

Colorless oil (98%). R_f = 0.51 (petroleum ether/EtOAc 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.16 (s, 6H), 1.00 (s, 9H), 1.69 (br s, 1H), 3.80 (s, 3H), 4.56 (s, 2H), 6.82 (d, J = 8.1 Hz, 1H), 6.86–6.93 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −4.64, 18.42, 25.70, 55.52, 65.05, 111.99, 120.07, 120.45, 133.66, 145.06, 150.55. HRMS calcd. for $C_{14}H_{24}O_3Si$ (M^+): 268.1495. Found 268.1490.

4.3. Syntheses of benzyl azides: general procedure

A mixture of the corresponding benzyl alcohol (7.67 mmol) and diphenylphosphorazidate (1.9 mL, 8.82 mmol) was dissolved in dry toluene (15 mL) under argon. The mixture was cooled to 0 °C, and neat 1,8-diazabicyclo[5.4.0]undec-7-ene (1.4 mL, 9.37 mmol) was added dropwise. The reaction was stirred for 2 h at 0 °C and then at room temperature for 19 h. The mixture was washed with water (2× 20 mL) and aqueous 5% HCl (2× 20 mL). The organic layer was dried over anhydrous magnesium sulfate and the solvent was removed in vacuo.

4.3.1. 1-(Azidomethyl)-4-methoxybenzene (15a)

The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.66) affording a colorless oil (89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.81 (s, 3H), 4.27 (s, 2H), 6.91 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 54.39, 55.28, 114.18, 127.38, 129.72, 159.61. HRMS calcd. for $C_8H_9N_3O$ (M^+): 163.0746. Found 163.0745.

4.3.2. 4-(Azidomethyl)-2-bromo-1-methoxybenzene (15b)

The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.71) affording a colorless oil (86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.90 (s, 3H), 4.25 (s, 2H), 6.89 (d, J = 8.4 Hz, 1H), 7.23 (dd, J = 8.4, 2.2 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 53.64, 56.25, 111.90, 128.45, 128.86, 133.21, 155.84. HRMS calcd. for $C_8H_8BrN_3O$ (M^+): 240.9851. Found 240.9861.

4.3.3. 5-(Azidomethyl)-1,2,3-trimethoxybenzene (15c)

The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.39) affording a colorless oil (99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.85 (s, 3H), 3.88 (s, 6H), 4.28 (s, 2H), 6.53 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.12, 56.13, 60.83, 105.12, 130.97, 137.90, 153.46. HRMS calcd. for $C_{10}H_{13}N_3O_3$ (M^+): 223.0957. Found 223.0954.

4.3.4. (5-(Azidomethyl)-2-methoxyphenoxy)(*tert*-butyl)dimethylsilane (15d)

The crude product was purified by chromatography (hexane/EtOAc 9:1, R_f = 0.56) affording a colorless oil (67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.16 (s, 6H), 1.00 (s, 9H), 3.81 (s, 3H), 4.21 (s, 2H), 6.81–6.89 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −4.64, 18.44, 25.70, 54.41, 55.48, 112.02, 121.15, 121.84, 127.75, 145.20, 151.11. HRMS calcd. for $C_{14}H_{23}N_3O_2Si$ ([M+Na]$^+$): 316.1451. Found 316.1447.

4.3.5. 4-(Azidomethyl)-1-methoxy-2-nitrobenzene (15f)

The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.37) affording a yellow oil (51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.98 (s, 3H), 4.36 (s, 2H), 7.11 (d, J = 8.6 Hz, 1H), 7.51 (dd, J = 8.6, 2.3 Hz, 1H), 7.82 (d, J = 2.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 53.30, 56.65, 113.94, 125.39, 127.89, 133.74, 139.54, 152.82. HRMS calcd. for $C_8H_8N_4O_3$ (M^+): 208.0596. Found 208.0599.

4.4. Syntheses of phenyl azides: general procedure

Sodium azide (78 mg, 1.2 mmol) and copper sulfate (25 mg, 0.1 mmol) were placed in a flask. Methanol (3 mL) and the corresponding boronic acid (1.0 mmol) were added subsequently. The reaction mixture was stirred vigorously at room temperature for 6 h. Water (10 mL) was added, followed by an extraction with hexane (3× 15 mL). The combined organic layers were washed with aqueous 5% NH_3 (2× 10 mL), dried over anhydrous magnesium sulfate and the solvent was removed in vacuo.

4.4.1. 1-Azido-4-methoxybenzene (18a)

Yellow oil (93%). R_f = 0.76 (hexane/EtOAc 2:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.74 (s, 3H), 6.95–6.99 (m, 2H), 7.03–7.07 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.32, 115.25, 120.09, 131.33, 156.70; MS (EI): 150.0 (M+1).

4.4.2. 5-Azido-1,2,3-trimethoxybenzene (18c)

Pale orange solid (93%). Mp 42–45 °C. R_f = 0.68 (hexane/EtOAc 2:1). ^{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, 134.77, 134.87, 153.66. HRMS calcd. for $C_9H_{11}N_3O_3$ (M^+): 209.0800. Found 209.0795.

4.5. Syntheses of 1,4-disubstituted 1,2,3-triazoles using copper sulfate: general procedure

A mixture of the corresponding azide (4.21 mmol) and the corresponding alkyne (4.21 mmol) was dissolved in t-BuOH/H_2O 1:1 (20 mL). Sodium ascorbate (167.0 mg, 20 mol %) and copper sulfate (52.6 mg, 5 mol %) were added. After stirring overnight water/ice (40 mL) was added. The product was either worked up by filtration, followed by rinsing with aqueous 5% NH_3 (×3) and cold ether (×2), or by extraction with dichloromethane (4× 100 mL). The combined organic layers were washed with aqueous 5% NH_3 (3× 100 mL) and brine (100 mL), and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo.

4.5.1. 1-(3-Bromo-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7b)

Pale brown solid (97%). Mp 154–156 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.71 (s, 3H), 3.87 (s, 6H), 3.94 (s, 3H), 7.24 (s, 2H), 7.36 (d, J = 9.0 Hz, 1H), 7.95 (dd, J = 8.9, 2.6 Hz, 1H), 8.17 (d, J = 2.6 Hz, 1H), 9.26 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.84, 56.63, 60.00, 102.52, 111.10, 113.21, 119.48, 120.48, 124.22, 125.65, 130.36, 137.39, 147.20, 153.28, 155.39. HRMS calcd. for $C_{18}H_{18}BrN_3O_4$ (M^+): 419.0481. Found 419.0475.

4.5.2. 1-(4-Methoxy-3-nitrophenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7d)

Yellow solid (92%). Mp 257–258 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.71 (s, 3H), 3.88 (s, 6H), 4.03 (s, 3H), 7.24 (s, 2H), 7.65 (d, J = 9.2 Hz, 1H), 8.25 (dd, J = 9.1, 2.7 Hz, 1H), 8.48 (d, J = 2.7 Hz, 1H), 9.34 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.87, 57.18, 60.03, 102.58, 115.78, 116.58, 119.69, 125.58, 129.06, 137.49, 139.03, 143.51, 147.40, 151.67, 153.31. HRMS calcd. for $C_{18}H_{18}N_4O_6$ (M^+): 386.1226. Found 386.1232.

4.5.3. 4-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (8d)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.37) affording a yellow solid (91%). Mp 229–230 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 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): δ = 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.

4.5.4. 1-(4-Methoxybenzyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (9a)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.28) affording a blank semisolid (92%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.67 (s, 3H), 3.74 (s, 3H), 3.83 (s, 6H), 5.55 (s, 2H), 6.95 (d, J = 8.7 Hz, 2H), 7.14 (s, 2H), 7.32 (d, J = 8.7 Hz, 2H), 8.57 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 52.51, 55.05, 55.81, 59.96, 102.38, 114.08, 121.06, 126.21, 127.78, 129.42, 137.08, 146.63, 153.17, 159.07. HRMS calcd. for $C_{19}H_{21}N_3O_4$ (M^+): 355.1532. Found 355.1529.

4.5.5. 1-(3-Bromo-4-methoxybenzyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (9b)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.20) affording a pale yellow solid (89%). Mp 113–114 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.68 (s, 3H), 3.83 (s, 6H), 3.84 (s, 3H), 5.58 (s, 2H), 7.12–7.15 (m, 3H), 7.37 (dd, J = 8.5, 2.2 Hz, 1H), 7.63 (d, J = 2.1 Hz, 1H), 8.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.73, 55.81, 56.22, 59.97, 102.42, 110.53, 112.80, 121.19, 126.12, 128.90, 129.42, 132.54, 137.13, 146.68, 153.18, 155.22. HRMS calcd. for $C_{19}H_{20}BrN_3O_4$ (M^+): 433.0637. Found 433.0642.

4.5.6. 2-Methoxy-5-((4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)phenol (9c)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.15) affording a white solid (76%). Mp 142–143 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.67 (s, 3H), 3.75 (s, 3H), 3.83 (s, 6H), 5.47 (s, 2H), 6.74–6.79 (m, 2H), 6.92 (d, J = 8.1 Hz, 1H), 7.15 (s, 2H), 8.55 (s, 1H), 9.09 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 52.72, 55.55, 55.81, 59.97, 102.37, 112.20, 115.03, 118.82, 121.12, 126.23, 128.28, 137.06, 146.60, 147.56, 153.18. HRMS calcd. for $C_{19}H_{21}N_3O_5$ (M^+): 371.1481. Found 371.1477.

4.5.7. 1-(4-Methoxy-3-nitrobenzyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (9d)

Pale yellow solid (96%). Mp 159–160 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.68 (s, 3H), 3.83 (s, 6H), 3.92 (s, 3H), 5.67 (s, 2H), 7.15 (s, 2H), 7.40 (d, J = 8.8 Hz, 1H), 7.66 (dd, J = 8.7, 2.3 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 8.63 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.44, 55.82, 56.73, 59.97, 102.44, 114.81, 121.32, 124.73, 126.07, 128.11, 134.27, 137.17, 138.84, 146.73, 151.84, 153.19. HRMS calcd. for $C_{19}H_{20}N_4O_6$ (M^+): 400.1383. Found 400.1377.

4.5.8. 4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (10a)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.27) affording a white solid (53%). Mp 157–158 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.64 (s, 3H), 3.76 (s, 6H), 3.78 (s, 3H), 5.51 (s, 2H), 6.74 (s, 2H), 7.00 (d, J = 8.9 Hz, 2H), 7.77 (d, J = 8.9 Hz, 2H), 8.50 (s, 1H). ^{13}C NMR

(75 MHz, DMSO-d_6): δ = 53.17, 55.04, 55.83, 59.87, 105.67, 114.17, 120.26, 123.20, 126.42, 131.20, 137.26, 146.44, 152.94, 158.89. HRMS calcd. for $C_{19}H_{21}N_3O_4$ (M^+): 355.1532. Found 355.1531.

4.5.9. 4-(3-Bromo-4-methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (10b)

White solid (91%). Mp 132–133 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.64 (s, 3H), 3.77 (s, 6H), 3.87 (s, 3H), 5.52 (s, 2H), 6.74 (s, 2H), 7.18 (d, J = 8.7 Hz, 1H), 7.84 (dd, J = 8.6, 2.1 Hz, 1H), 8.05 (d, J = 2.1 Hz, 1H), 8.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 53.28, 55.84, 56.21, 59.87, 105.74, 110.95, 112.93, 120.88, 124.67, 125.63, 129.37, 131.00, 137.29, 145.11, 152.95, 154.90. HRMS calcd. for $C_{19}H_{20}BrN_3O_4$ (M^+): 433.0637. Found 433.0638.

4.5.10. 2-Methoxy-5-(1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl)phenol (10c)

The crude product was purified by chromatography (chloroform/EtOAc 1:1, R_f = 0.15) affording a white solid (86%). Mp 156–157 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.64 (s, 3H), 3.76 (s, 6H), 3.78 (s, 3H), 5.50 (s, 2H), 6.74 (s, 2H), 6.96 (d, J = 8.4 Hz, 1H), 7.22 (dd, J = 8.3, 2.1 Hz, 1H), 7.29 (d, J = 2.1 Hz, 1H), 8.45 (s, 1H), 9.09 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 53.15, 55.53, 55.83, 59.88, 105.68, 112.37, 112.45, 116.25, 120.27, 123.57, 131.25, 137.25, 146.63, 147.45, 152.93. HRMS calcd. for $C_{19}H_{21}N_3O_5$ (M^+): 371.1481. Found 371.1476.

4.5.11. 4-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (10d)

The crude product was purified by chromatography (chloroform/EtOAc 1:1, R_f = 0.23) affording a yellow solid (89%). Mp 154–155 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.64 (s, 3H), 3.77 (s, 6H), 3.96 (s, 3H), 5.55 (s, 2H), 6.75 (s, 2H), 7.45 (d, J = 8.9 Hz, 1H), 8.14 (dd, J = 8.8, 2.2 Hz, 1H), 8.32 (d, J = 2.2 Hz, 1H), 8.68 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 53.35, 55.85, 56.72, 59.87, 105.78, 114.88, 121.22, 121.44, 123.31, 130.70, 130.89, 137.33, 139.41, 144.50, 151.33, 152.97. HRMS calcd. for $C_{19}H_{20}N_4O_6$ (M^+): 400.1383. Found 400.1388.

4.6. Syntheses of 1,4-disubstituted 1,2,3-triazoles using copper turnings: general procedure

A mixture of the corresponding azide (2 mmol) and the corresponding alkyne (2 mmol) was dissolved in methanol (5 mL). A copper turning was added and the mixture was stirred violently for 48 h. The solvent was removed in vacuo.

4.6.1. 1-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (7a)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.56) affording a pale pink solid (53%). Mp 169–170 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.71 (s, 3H), 3.85 (s, 3H), 3.87 (s, 6H), 7.18 (d, J = 9.1 Hz, 2H), 7.25 (s, 2H), 7.84 (d, J = 9.0 Hz, 2H), 9.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.49, 55.86, 60.02, 102.56, 114.83, 119.41, 121.43, 125.84, 129.96, 137.34, 147.09, 153.27, 159.19. HRMS calcd. for $C_{18}H_{19}N_3O_4$ (M^+): 341.1376. Found 341.1377.

4.6.2. 2-Methoxy-5-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenol (7c)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.17) affording a pale yellow solid (35%). Mp 184–185 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.70 (s, 3H), 3.85 (s, 3H), 3.87 (s, 6H), 7.13 (d, J = 8.8 Hz, 1H), 7.25 (s, 2H), 7.29 (dd, J = 8.7, 2.6 Hz, 1H), 7.37 (d, J = 2.6 Hz, 1H), 9.17 (s, 1H), 9.64 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.83, 55.87, 60.01, 102.55, 107.56, 110.39, 112.42, 119.28, 125.85, 130.06, 137.31, 147.01, 147.25, 147.88, 153.26. HRMS calcd. for $C_{18}H_{19}N_3O_5$ (M^+): 357.1325. Found 357.1323.

4.6.3. 4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (8a)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.60) affording a pale yellow solid (51%). Mp 181–182 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.73 (s, 3H), 3.81 (s, 3H), 3.90 (s, 6H), 7.07 (d, J = 8.8 Hz, 2H), 7.25 (s, 2H), 7.86 (d, J = 8.8 Hz, 2H), 9.17 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.11, 56.20, 60.14, 97.79, 114.33, 118.74, 122.72, 126.57, 132.47, 137.21, 147.00, 153.44, 159.19. HRMS calcd. for $C_{18}H_{19}N_3O_4$ (M^+): 341.1376. Found 341.1366.

4.6.4. 4-(3-Bromo-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (8b)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.56) affording a pale yellow solid (86%). Mp 164–165 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.72 (s, 3H), 3.89 (s, 6H), 3.90 (s, 3H), 7.24–7.27 (m, 3H), 7.92 (dd, J = 8.6, 2.0 Hz, 1H), 8.10 (d, J = 2.1 Hz, 1H), 9.25 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.21, 56.30, 60.16, 97.73, 111.11, 113.09, 119.33, 124.20, 125.74, 129.50, 132.37, 137.29, 145.66, 153.48, 155.22. HRMS calcd. for $C_{18}H_{18}BrN_3O_4$ ([M+H]$^+$): 420.0553. Found 420.0562.

4.6.5. 4-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (8c-protected)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.55) affording a pale yellow solid (68%). Mp 117–118 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.17 (s, 6H), 0.99 (s, 9H), 3.73 (s, 3H), 3.82 (s, 3H), 3.90 (s, 6H), 7.11 (d, J = 8.5 Hz, 1H), 7.25 (s, 2H), 7.39 (d, J = 2.1 Hz, 1H), 7.50 (dd, J = 8.4, 2.1 Hz, 1H), 9.15 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = −4.74, 18.10, 25.50, 55.32, 56.23, 60.12, 97.91, 112.67, 117.38, 118.88, 119.06, 123.08, 132.48, 137.26, 144.45, 146.81, 150.48, 153.42. HRMS calcd. for $C_{24}H_{33}N_3O_5Si$ (M^+): 471.2189. Found 471.2185.

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

The corresponding terminal alkyne (3.3 mmol) dissolved in dry THF (3 mL) was added dropwise to an oven dried flask containing a solution of EtMgCl in dry THF (1.5 mL, 2.0 M, 3.0 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 (3.0 mmol) dissolved in dry THF (3 mL) was added dropwise. The reaction mixture was heated to 50 °C for 1.5 h. After quenching with aqueous NH_4Cl (6 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 was removed in vacuo.

4.7.1. 1-(4-Methoxybenzyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (5a)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.21) affording a pale yellow solid (81%). Mp 97–98 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.69 (s, 3H), 3.70 (s, 3H), 3.71 (s, 6H), 5.61 (s, 2H), 6.66 (s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 7.92 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 50.73, 55.02, 55.88, 59.99, 106.01, 113.97, 121.79, 128.03, 128.20, 132.97, 137.37, 138.05, 153.01, 158.71. HRMS calcd. for $C_{19}H_{21}N_3O_4$ (M^+): 355.1532. Found 355.1528.

4.7.2. 1-(3-Bromo-4-methoxybenzyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (5b)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.20) affording a pale yellow solid (87%). Mp 165–166 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.70 (s, 3H), 3.74 (s, 6H), 3.80 (s, 3H), 5.63 (s, 2H), 6.66 (s, 2H), 7.05 (s, 1H), 7.06 (s, 1H), 7.29 (s, 1H), 7.92 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 50.04, 55.91, 56.18, 60.01, 106.08, 110.41, 112.69, 121.67, 127.87, 129.54, 131.74, 133.05, 137.41, 138.13, 153.05, 154.89. HRMS calcd. for $C_{19}H_{20}BrN_3O_4$ (M^+): 433.0637. Found 433.0646.

4.7.3. 1-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxybenzyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (5c-protected)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.35) affording a pale yellow solid (84%). Mp 98–100 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.09 (s, 6H), 0.94 (s, 9H), 3.73 (s, 6H), 3.76 (s, 3H), 3.87 (s, 3H), 5.45 (s, 2H), 6.42 (s, 2H), 6.62–6.67 (m, 2H), 6.76 (d, J = 8.2 Hz, 1H), 7.72 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −4.67, 18.39, 25.62, 51.44, 55.51, 56.13, 60.93, 106.23, 112.09, 119.63, 120.29, 121.97, 128.28, 132.80, 138.99, 145.38, 150.87, 153.47. HRMS calcd. for $C_{25}H_{35}N_3O_5Si$ ([M+H]$^+$): 486.2418. Found 486.2433.

4.7.4. 1-(4-Methoxy-3-nitrobenzyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (5d)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.17) affording a pale orange solid (30%). Mp 184–185 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.68 (s, 3H), 3.73 (s, 6H), 3.87 (s, 3H), 5.70 (s, 2H), 6.67 (s, 2H), 7.31 (d, J = 8.8 Hz, 1H), 7.37 (dd, J = 8.7, 2.1 Hz, 1H), 7.58 (d, J = 2.0 Hz, 1H), 7.91 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 49.86, 55.91, 56.69, 59.96, 106.15, 114.67, 121.58, 123.90, 128.20, 133.10, 133.38, 137.55, 138.16, 138.67, 151.51, 153.07. HRMS calcd. for $C_{19}H_{20}N_4O_6$ (M^+): 400.1383. Found 400.1380.

4.7.5. 5-(4-Methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (6a)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.16) affording a pale yellow solid (72%). Mp 115–116 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.60 (s, 3H), 3.62 (s, 6H), 3.80 (s, 3H), 5.56 (s, 2H), 6.28 (s, 2H), 7.06 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 7.85 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.14, 55.23, 55.61, 59.86, 104.41, 114.42, 118.70, 130.01, 131.33, 132.67, 136.81, 137.31, 152.78, 159.91. HRMS calcd. for $C_{19}H_{21}N_3O_4$ (M^+): 355.1532. Found 355.1531.

4.7.6. 5-(3-(*tert*-Butyldimethylsilyloxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (6c-protected)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.38) affording a yellow solid (58%). Mp 108–110 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.05 (s, 6H), 0.90 (s, 9H), 3.61 (s, 3H), 3.63 (s, 6H), 3.80 (s, 3H), 5.56 (s, 2H), 6.26 (s, 2H), 6.84 (d, J = 1.6 Hz, 1H), 7.10–7.11 (m, 2H), 7.89 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = −4.97, 17.95, 25.34, 51.00, 55.43, 55.60, 59.83, 103.87, 112.73, 118.82, 120.24, 122.65, 131.58, 132.64, 136.82, 137.21, 144.28, 151.38, 152.91. HRMS calcd. for $C_{25}H_{35}N_3O_5Si$ ([M+H]$^+$): 486.2418. Found 486.2425.

4.8. Syntheses of 1,5-disubstituted 1,2,3-triazoles using ruthenium catalysis: general procedure

A mixture of the corresponding azide (1 mmol), the corresponding alkyne (1.1 mmol) and Cp∗RuCl(COD) (3.8 mg, 10 mol %) was refluxed in dry benzene (10 mL) in a Schlenk flask under argon for 3 h. The solvent was removed in vacuo.

4.8.1. 5-(3-Bromo-4-methoxyphenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (6b)

The crude product was purified by chromatography (hexane/EtOAc 1:1, R_f = 0.17) affording a white solid (94%). Mp 105–106 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.60 (s, 3H), 3.64 (s, 6H), 3.89 (s, 3H), 5.58 (s, 2H), 6.32 (s, 2H), 7.22 (d, J = 8.6 Hz, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.66 (d, J = 2.2 Hz, 1H), 7.91 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.36, 55.62, 56.40, 59.88, 104.44, 110.89, 112.83, 120.12, 129.48, 131.22, 132.82, 133.11, 135.96, 136.87, 152.84, 156.01. HRMS calcd. for $C_{19}H_{20}BrN_3O_4$ (M^+): 433.0637. Found 433.0638.

4.8.2. 5-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (6d)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.14) affording a pale orange solid (94%). Mp 151–152 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.59 (s, 3H), 3.63 (s, 6H), 3.96 (s, 3H), 5.63 (s, 2H), 6.32 (s, 2H), 7.47 (d, J = 8.8 Hz, 1H), 7.75 (dd, J = 8.8, 2.3 Hz, 1H), 7.98–7.99 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.47, 55.59, 56.93, 59.85, 104.49, 114.88, 118.76, 124.85, 130.98, 133.50, 134.44, 135.27, 136.90, 139.23, 152.19, 152.86. HRMS calcd. for $C_{19}H_{20}N_4O_6$ (M^+): 400.1383. Found 400.1384.

4.9. Deprotection of TBDMS-ethers: general procedure

Tetra-*n*-butyl ammonium fluoride in THF (1 M, 7.5 mL, 7.5 mmol) was added dropwise to a solution of the corresponding TBDMS-ether (5.72 mmol) in dry THF (11.5 mL) under argon. The reaction mixture was stirred at room temperature for 1.5 h and then treated with water (11.5 mL). The mixture was extracted with chloroform (3× 40 mL). The combined organic layers were washed with water (40 mL) and brine (40 mL), and dried over anhydrous magnesium sulfate and the solvent was removed in vacuo.

4.9.1. 2-Methoxy-5-((5-(3,4,5-trimethoxyphenyl)-1*H*-*1,2,3-triazol-1-yl)methyl)phenol (*5c*)*

The crude product was purified by chromatography (hexane/EtOAc 1:5, R_f = 0.47) affording a white solid (75%). Mp 171–172 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.69 (s, 3H), 3.70 (s, 9H), 5.53 (s, 2H), 6.47 (dd, J = 8.2, 2.1 Hz, 1H), 6.52 (d, J = 2.1 Hz, 1H), 6.66 (s, 2H), 6.84 (d, J = 8.3 Hz, 1H), 7.93 (s, 1H), 9.07 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 50.85, 55.56, 55.85, 59.99, 105.95, 112.23, 113.97, 117.48, 121.76, 128.67, 132.95, 137.37, 138.04, 146.62, 147.16, 152.99. HRMS calcd. for $C_{19}H_{21}N_3O_5$ (M^+): 371.1481. Found 371.1490.

4.9.2. 2-Methoxy-5-(1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazol-5-yl)phenol (6c)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.19) affording a pale yellow solid (83%). Mp 194–195 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.60 (s, 3H), 3.63 (s, 6H), 3.81 (s, 3H), 5.54 (s, 2H), 6.30 (s, 2H), 6.87–6.90 (m, 2H), 7.04 (d, J = 8.9 Hz, 1H), 7.81 (s, 1H), 9.32 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.07, 55.60, 59.87, 104.42, 112.38, 115.69, 118.94, 119.75, 131.41, 132.46, 136.81, 137.56, 146.70, 148.55, 152.78. HRMS calcd. for $C_{19}H_{21}N_3O_5$ (M^+): 371.1481. Found 371.1478.

4.9.3. 2-Methoxy-5-(1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)phenol (8c)

The crude product was purified by chromatography (hexane/EtOAc 1:2, R_f = 0.38) affording a white solid (94%). Mp 211–212 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.72

(s, 3H), 3.82 (s, 3H), 3.90 (s, 6H), 7.03 (d, J = 8.4 Hz, 1H), 7.26 (s, 2H), 7.32 (dd, J = 8.3, 2.0 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 9.12 (s, 1H), 9.17 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.57, 56.20, 60.11, 97.73, 112.42, 112.59, 116.41, 118.69, 123.08, 132.48, 137.16, 146.76, 147.23, 147.75, 153.43. HRMS calcd. for $C_{18}H_{19}N_3O_5$ ([M+H]$^+$): 358.1397. Found 358.1413.

4.9.4. 5-Ethynyl-2-methoxyphenol (13e)

The crude product was purified by chromatography (hexane/EtOAc 9:1, R_f = 0.15) affording a pale yellow solid (90%). Mp 61–62 °C (hexane/ether 9:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.97 (s, 1H), 3.89 (s, 3H), 5.61 (s, 1H), 6.78 (d, J = 8.1 Hz, 1H), 7.01–7.06 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.91, 75.60, 83.54, 110.36, 114.86, 118.01, 124.80, 145.23, 147.30. HRMS calcd. for $C_9H_8O_2$ (M$^+$): 148.0524. Found 148.0526.

4.9.5. 5-(Azidomethyl)-2-methoxyphenol (15e)

The crude product was purified by chromatography (hexane/EtOAc 8:3, R_f = 0.40) affording a pale yellow oil (85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.90 (s, 3H), 4.23 (s, 2H), 5.68 (br s, 1H), 6.81 (dd, J = 8.2, 1.9 Hz, 1H), 6.85 (d, J = 8.1 Hz, 1H), 6.90 (d, J = 1.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 54.50, 55.96, 110.62, 114.60, 120.13, 128.48, 145.82, 146.58. HRMS calcd. for $C_8H_9N_3O_2$ (M$^+$): 179.0695. Found 179.0693.

4.10. Syntheses of amino-substituted triazoles: general procedure

Saturated copper sulfate solution (0.8 mL) was added to the corresponding nitro-substituted triazole (2.01 mmol) dissolved in ethanol (10 mL). The mixture was cooled to 0 °C, and a solution of sodium borohydride (380 mg, 10.05 mmol) in ethanol/H_2O 1:1 (6 mL) was added dropwise to the reaction mixture. The mixture was refluxed for 4 h. After cooling, saturated copper sulfate solution (0.8 mL) and a solution of sodium borohydride (380 mg, 10.05 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 was removed in vacuo.

4.10.1. 2-Methoxy-5-((5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)aniline (5e)

Recrystallization in methanol and ether afforded a pale yellow solid (36%). Mp 69–71 °C (methanol/ether 3:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.69 (s, 3H), 3.70 (s, 6H), 3.71 (s, 3H), 4.83 (br s, 2H), 5.48 (s, 2H), 6.23 (dd, J = 8.2, 2.0 Hz, 1H), 6.40 (d, J = 2.0 Hz, 1H), 6.68 (s, 2H), 6.71 (d, J = 8.2 Hz, 1H), 7.93 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 51.17, 55.26, 55.85, 59.98, 105.94, 110.35, 111.69, 114.24, 121.84, 128.58, 132.89, 137.37, 137.81, 138.00, 145.78, 152.98. HRMS calcd. for $C_{19}H_{22}N_4O_4$ (M$^+$): 370.1641. Found 370.1650.

4.10.2. 2-Methoxy-5-(1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazol-5-yl)aniline (6e)

The crude product was purified by reversed phase chromatography (MeOH/H_2O 2:1, R_f = 0.43) and recrystallized in methanol and ether affording a yellow solid (53%). Mp 148–150 °C (methanol/ether 3:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.60 (s, 3H), 3.63 (s, 6H), 3.80 (s, 3H), 4.95 (br s, 2H), 5.54 (s, 2H), 6.31 (s, 2H), 6.64 (dd, J = 8.2, 2.0 Hz, 1H), 6.74 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 8.2 Hz, 1H), 7.76 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 50.98, 55.33, 55.60, 59.86, 104.49, 110.63, 113.42, 116.35, 118.95, 131.46, 132.19, 136.79, 138.12, 138.17, 146.99, 152.75. HRMS calcd. for $C_{19}H_{22}N_4O_4$ (M$^+$): 370.1641. Found 370.1638.

4.10.3. 2-Methoxy-5-(4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzenamine (7e)

Recrystallization in methanol and ether afforded a pale pink solid (86%). Mp 166–167 °C (methanol/ether 3:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.70 (s, 3H), 3.84 (s, 3H), 3.87 (s, 6H), 5.20 (br s, 2H), 6.97–6.98 (m, 2H), 7.21 (d, J = 1.6 Hz, 1H), 7.25 (s, 2H), 9.10 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.56, 55.88, 60.02, 102.54, 105.01, 106.96, 110.54, 119.22, 125.97, 130.44, 137.26, 138.83, 146.24, 146.88, 153.26. HRMS calcd. for $C_{18}H_{20}N_4O_4$ (M$^+$): 356.1485. Found 356.1477.

4.10.4. 2-Methoxy-5-((4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl)methyl)aniline (9e)

The crude product was purified by reversed phase chromatography (MeOH/H_2O 2:1 (1% TFA), R_f = 0.38) affording a pale yellow solid (62%). Mp 114–115 °C (methanol/ether 3:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.67 (s, 3H), 3.74 (s, 3H), 3.83 (s, 6H), 4.81 (br s, 2H), 5.41 (s, 2H), 6.53–6.56 (m, 2H), 6.78 (d, J = 8.2 Hz, 1H), 7.15 (s, 2H), 8.51 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 53.11, 55.25, 55.81, 59.96, 102.37, 110.34, 112.79, 115.64, 121.04, 126.28, 128.10, 137.04, 137.87, 146.15, 146.56, 153.17. HRMS calcd. for $C_{19}H_{22}N_4O_4$ (M$^+$): 370.1641. Found 370.1638.

4.10.5. 2-Methoxy-5-(1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl)aniline (10e)

Pale brown solid (70%). Mp 126–127 °C (methanol/ether 3:1). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.64 (s, 3H), 3.76 (s, 6H), 3.78 (s, 3H), 4.80 (br s, 2H), 5.49 (s, 2H), 6.73 (s, 2H), 6.82 (d, J = 8.3 Hz, 1H), 6.97 (dd, J = 8.2, 2.1 Hz, 1H), 7.16 (d, J = 2.1 Hz, 1H), 8.35 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 53.08, 55.23, 55.83, 59.87, 105.67, 110.55, 113.37, 119.94, 123.38, 131.31, 137.23, 137.73, 146.16, 147.17, 152.91. HRMS calcd. for $C_{19}H_{22}N_4O_4$ (M$^+$): 370.1641. Found 370.1642.

4.11. Biological assays

4.11.1. Cancer cell growth inhibition on K562 human leukemia cell line

The method applied was previously described by Edmondson and coworkers.[25a] 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 microtitre 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.

4.11.2. Inhibition of tubulin assembly

The method applied was that described by Lawrence and coworkers.[27] 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 $MgCl_2$, and 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 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

Three triazoles, **5e**, **6e**, and **11**, were modeled by the Internal Coordinate Mechanics (ICM[39,40]) v.3.5-ln program package. The ligands were geometry-optimized and assigned partial atomic charges according to the MMFF94 force field.[41] The X-ray crystallographic structures of α- and β-tubulin (PDB entry 1SA0;[28] chain A and chain B, respectively) in complex with guanosine-5′-triphosphate (GTP) were loaded into ICM, and hydrogen atoms were added. ECEPP/3[42] atom charges were assigned, followed by energy minimization to relieve atomic clashes. A grid map was calculated that included the ligand binding site of 1SA0. The grid size was kept similar to our previous docking of triazole analog **4e**.[11] The triazole analogs were docked as flexible-ligands on the rigid α,β-tubulin conformation. The docked ligand conformation that exhibited the most favorable docking energy was further refined using a flexible-ligand, flexible-receptor method in ICM.[43] The free binding energy of the refined tubulin-ligand complex was calculated to predict the contributions of different subunits in binding the different triazole analogs.

Acknowledgments

Financial support to K.O. from the School of Pharmacy, University of Oslo, is gratefully appreciated. Financial support to J.F. from the Conseil Régional d'Auvergne, FEDER and Oséo is greatly appreciated.

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