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Synthesis and anti-cancer screening of novel heterocyclic-(2*H*)-1,2,3-triazoles as potential anti-cancer agents

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Abstract

trans-Cyanocombretastatin A-4 (*trans*-CA-4) analogues have been structurally modified to afford their more stable CA-4-(2*H*)-1,2,3-triazole analogues. Fifteen novel, stable 4-heteroaryl-5-aryl-(2*H*)-1,2,3-triazole CA-4 analogues (**8a–i**, **9** and **11a–e**) were evaluated for anti-cancer activity against a panel of 60 human cancer cell lines. These analogues displayed potent cytotoxic activity against both hematological and solid tumor cell lines with GI₅₀ values in the low nanomolar range. The most potent compound, **8a**, was a benzothiophen-2-yl analogue that incorporated a 3,4,5-trimethoxyphenyl moiety connected to the (2*H*)-1,2,3-triazole ring system. Compound **8a** exhibited GI₅₀ values of <10 nM against 80% of the cancer cell lines in the panel. Three triazole analogues, **8a**, **8b** and **8g**, showed particularly potent growth inhibition against the triple negative Hs578T breast cancer cell line with GI₅₀ values of 10.3 nM, 66.5 nM and 20.3 nM, respectively. Molecular docking studies suggest that these compounds bind to the same hydrophobic pocket at the interface of α - and β -tubulin that is occupied by colchicine and *cis*-CA-4, and are stabilized by Van der Waals' interactions with surrounding amino acid residues. Compound **8a** was found to inhibit tubulin polymerization *in vitro* with an IC₅₀ value of 1.7 μ M. The potent cytotoxicity of these novel compounds and their inhibition of tubulin dynamics make these triazole analogues promising candidates for development as anti-cancer drugs.

Introduction

The combretastatins (Fig. 1, **1** and **2**) were first isolated from the South African bush willow tree *Combretum caffrum*, of which *cis*-combretastatin A-4 (*cis*-CA-4, **1**) is the major component.¹ Three analogues of combretastatin *cis*-CA-4: CA-4P (**3**), Oxi4503 (**4**) and AVE8062 (**5**) (Fig. 1) are currently in clinical trials for treatment of a variety of cancers, and have improved water solubility, cytotoxicity, efficacy and a better safety profile compared to *cis*-CA-4.^{2–5} These combretastatin analogues are also known to be vascular disrupting agents (VDAs) and have proven to be advantageous cytotoxic agents, since they target the tumor vasculature causing hemorrhagic necrosis and cell death.⁶

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The CA-4 analogues are generally classified as tubulin polymerization inhibitors with an anti-mitotic mechanism of action, due to their ability to disrupt microtubule dynamics and hence mitotic spindle formation by binding to the same site as colchicine on tubulin dimers.^{7, 8} These small molecule VDAs also induce hypoxia-driven necrosis of solid tumors, and are able to induce apoptosis by cell-cycle arrest.^{9, 10}

CA-4 analogues are highly cytotoxic against human cancer cell lines. CA4-P (**3**) exhibits potent cytotoxicity against anaplastic thyroid cancer cells lines,¹¹ and Oxi-4503 (**4**) is a potent anti-leukemic compound.¹² AVE8062 (**5**) is an effective cytotoxic agent against head and neck squamous cell carcinoma,¹³ including multidrug resistant forms of this cell line.¹⁴ Tron *et al.* have reported that the *cis*-configured double bond of *cis*-CA-4, with a 3,4,5-trimethoxyphenyl ring (Ring A) on one side and a 4-methoxysubstituted phenyl ring on the other side (Ring B), is an essential structural moiety for optimal cytotoxic activity in *cis*-CA-4 analogues.^{15–17} Recently, we have investigated the anticancer activities of a series of *trans*-cyano-CA-4 analogs in which replacement of ring B with different heterocycles, such as quinoline (**6**), benzothiophene (**7a–f** and **10a–c**), benzofuran (**7g–h**) and indole (**7i** and **10d–e**), has occurred; we have determined the effect of these structural changes on anticancer activity.^{18, 19} We have also evaluated the effects of structurally modifying ring A by introducing differently substituted methoxy and hydroxy moieties into this phenyl ring.¹⁸

The isomerization of the *cis*-configured CA-4 analogues to their *trans*-forms in solution is one of the major disadvantages of these molecules. In this respect, we have shown that isomerization of *trans*-cyano CA-4 analogues to their *cis*-cyano CA-4 forms is also observed in solution.¹⁸ To restrict *cis-trans* isomerization in these CA-4 analogues, different heterocyclic ring systems, such as doxolane,²⁰ thiazole²¹ 1,5-disubstituted 1,2,3-triazoles,^{22–26} 1,2,4-triazoles^{27, 28} and imidazole,²⁹ have been incorporated into the stilbene double bond.

In the present report, we have prepared a series of stable analogues of *cis*-cyano CA-4 by constructing a triazole ring system across the olefinic bond between aromatic rings A and B. This (2*H*)-1,2,3-triazole heterocyclic bridge unit was established by chemical modification of existing *trans*-cyano CA-4 analogues via [3+2] cycloaddition of azide ion across the double bond. This novel and simple structural modification led to an improvement in the stability and biological properties of these new CA-4 analogues. We now report on the synthesis and *in vitro* anti-cancer activities of a variety of 4-heteroaryl-5-aryl-(2*H*)-1,2,3-triazole CA-4 analogues, and their ability to inhibit tubulin polymerization *in vitro*. Some of the analogues were also tested for cytotoxicity against Hs578T breast cancer cell lines. In addition, molecular modeling studies of the most active inhibitors indicate that they interact with the colchicine binding site on α,β -tubulin, and this has been confirmed in *in vitro* tubulin polymerization inhibition studies.

Chemistry

The formation of the 1,2,3-triazole ring system is well known in the literature as a “click chemistry” product from a thermally induced Cu(I)-catalyzed (CuAAC) Huisgen [3+2]cycloaddition azide-alkyne reaction.³⁰ The click chemistry approach is widely used in

the synthesis and regioisomeric formation of 1,4-disubstituted-1,2,3-triazoles in the presence of CuAAC in excellent yields.^{31–34} The regioisomeric synthesis of 1,5-disubstituted-1,2,3-triazoles has also been reported with high selectivity utilizing different metal ions in magnesium-, cerium- and ruthenium-mediated reactions.^{35–37}

We have recently reported the synthesis of a wide variety of 4,5-disubstituted-1,2,3-(1*H*)-triazole analogues.^{38, 39} We used a two stage synthesis, i.e. the preparation of the precursor *trans*-cyano-CA-4 analogue, which utilizes our previously published procedure,¹⁸ followed by synthesis of the target (2*H*)-1,2,3-triazole CA-4 compounds **8a–8i** and **11a–11e** by reaction of the *trans*-cyano CA-4 analogue with sodium azide in DMSO at 100 °C over 1–6 hrs utilizing L-proline as a Lewis base. The desired (2*H*)-1,2,3-triazole products were obtained in 75–96% yield³⁸ (Schemes 1 and 2) (Note: the use of *cis*-cyano CA-4 analogues in the above reaction led to lower yields of the triazole product³⁹). The *N*-2 methylated analogue **9** was prepared from the reaction of **8a** with methyl iodide/K₂CO₃ in acetone (Scheme 2). Confirmation of the structure and purity of these analogues is reported elsewhere, and was obtained from single crystal X-ray diffraction studies,⁴⁰ and ¹H-NMR, ¹³C-NMR and high resolution mass spectroscopic analysis.³⁸

Biological Evaluation

A. *In vitro* growth inhibition and cytotoxicity

The above triazole analogues were evaluated for their anticancer activities against a panel of 60 human cancer cell lines, which incorporates different subpanels representing leukemia, non-small cell lung, colon, central nervous system, melanoma, ovary, renal, prostate, and breast cancer cell lines, at a concentration of 10^{–5} M utilizing the procedure described by Rubinstein et al.⁴¹ Compounds that showed 60% growth inhibition in at least eight of the 60 cell lines screened were further evaluated in a five-dose screen.

From the preliminary screen, compounds **8a**, **11b**, **11d**, **11e** and **9** were selected for five-dose studies and their GI₅₀ values determined (Table 1). GI₅₀ values represent the molar drug concentration required to cause 50% growth inhibition. In the five-dose studies, 5 different concentrations at 10-fold dilutions (10^{–4} M, 10^{–5} M, 10^{–6} M, 10^{–7} M and 10^{–8} M) were utilized and incubations were carried out over 48 h exposure to drug. Compounds **8a**, **11b**, **11d**, **11e** and **9** exhibited GI₅₀ values in the low nanomolar range against all 60 human cancer cell lines in the panel. The results are presented in Table 1.

Compound **8a** (4-(benzo[*b*]thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-2*H*-1,2,3-triazole) exhibited GI₅₀ values <0.050 μM in all the 60 human cancer cell lines in the panel, and had GI₅₀ <0.01 μM in 80% of the cancer cells in the panel (Table 1). Compound **8a** exhibited particularly potent growth inhibition properties against all six cell lines in the breast cancer subpanel, with GI₅₀ values of <0.01 μM to 0.014 μM.

Compound **9**, obtained by *N*-methylation of **8a**, exhibited GI₅₀ values ranging from 0.023 μM to 0.744 μM in 84% of the cancer cell lines screened and showed moderate growth inhibition properties in all six breast cancer cell lines, with GI₅₀ values in the range of 0.040 μM to 1.6 μM (except for the T-47D cell line). This compound also exhibited potent growth

inhibition against NCI-H522 lung cancer ($GI_{50} = 0.023 \mu M$) and MDA-MB-435 melanoma ($GI_{50} = 0.025 \mu M$) cell lines (Table 1).

Compound **11b** exhibited GI_{50} values ranging from $0.337 \mu M$ to $13.3 \mu M$ against the entire 60 cancer cell panel screened, and showed moderate growth inhibition properties against all six breast cancer cell lines, with GI_{50} values in the range of $0.63 \mu M$ to $3.21 \mu M$. This compound exhibited potent growth inhibition against MDA-MB-435 melanoma cells ($GI_{50} = 0.337 \mu M$) (Table 1).

Compound **11d** exhibited GI_{50} values ranging from $0.014 \mu M$ to $0.44 \mu M$ in 90% of the cancer cell lines screened and showed potent growth inhibition properties in all six breast cancer cell lines, with GI_{50} values in the range of $0.028 \mu M$ to $0.089 \mu M$. This compound exhibited potent growth inhibition against MDA-MB-435 melanoma cells ($GI_{50} = 0.014 \mu M$) (Table 1). The average GI_{50} value of this compound against all the cancer cell lines screened was $0.424 \mu M$.

Compound **11e** exhibited GI_{50} values ranging from $0.01 \mu M$ to $41.6 \mu M$ in 94% of the cancer cell lines screened and showed potent growth inhibition properties in all six breast cancer cell lines, with GI_{50} values in the range of $0.017 \mu M$ to $0.076 \mu M$. This compound exhibited potent growth inhibition against MDA-MB-435 melanoma cells ($GI_{50} = 0.01 \mu M$) and NCI-H522 ($GI_{50} = 0.013 \mu M$) non-small cell lung cancer cell lines (Table 1). The average GI_{50} value of this compound against all 60 cancer cell lines screened was $0.856 \mu M$.

B. Cytotoxic effects of **8a**, **8b**, and **8g** on Hs578T breast cancer cell lines

Compound **8a** and its analogues (**8b** and **8g**) were tested for cytotoxicity against the triple negative breast cancer (TNBC)-derived Hs578T cell line. The GI_{50} estimates for compounds **8a**, **8b**, and **8g** were all below 100 nM (Fig. 2). However, the potency of compound **8a** remained two- to six-fold greater than either **8b** or **8g**. Comparing the results for **8a**, **8b** and **8g** revealed that replacing the 3,4,5-trimethoxyphenyl moiety of **8a** with a 3,5-dimethoxyphenyl group to afford **8b**, results in a more pronounced effect on cytotoxicity than replacing the benzothiophen-2-yl moiety with a benzofuran-2-yl group (**8g**).

C. Molecular modeling studies

Molecular modeling studies were performed for four active (2*H*)-1,2,3-triazoles analogues (**8a**, **8g**, **11d**, and **11e**) using SYBYL-X 2.1. Binding interactions of these triazole analogues were studied by docking them at the colchicine-binding site on tubulin. 3-D coordinates for the tubulin-colchicine complex were obtained from the RSCB protein data bank (pdb id: 4O2B). For preparation of tubulin to perform the docking calculations, the geometry of the protein molecule was optimized using energy minimization techniques after the addition of hydrogen atoms. Since no structured water molecule was present in the binding pocket, all the water molecules in the protein were deleted during the preparation of structure for docking calculations. Amino acid side chain bumps were fixed and terminal amino acid were charged to mimic the biological environment. Hydrogen atoms were added to the structure. The Kollman force field was applied and the protein was minimized using the Powell method and Pullman charges. The triazole analogue structures were initially

generated in 2-D format in Chem-Draw. For generating energy-minimized structures, the 2-D structures were converted to 3-D using ChemDraw3D and all the structures were saved in Mol2 format. The structures were imported to Sybyl in the Mol2 format. In SYBYL, TRIPOS force field was applied and 3-D structure coordinates were taken through a series of minimization processes. Firstly, all the structures were minimized using the BFGS method. Then, MOPAC charges were calculated for each molecule followed by another minimization step. Each molecule was then checked for charges, bond angle, torsion angles and geometry. All the molecules were saved in one directory and prepared for docking via the ligand preparation tool. For the docking analysis using the Surflex program, a protomol was generated at the colchicine-binding site in tubulin. This protomol is the representation of the binding site that simulates the binding environment experienced by the ligand. The docking analysis is performed using the Surflex Dock-Gemox module which lists the C-Scores (consolidated scores) for each molecule. The C-Score is a measure of the goodness of fit. The C-Score function combines the binding score obtained from five different scoring algorithms; namely: Total Score, G Score, PMF Score, D Score and Chem Score. In these docking calculations, the flexibilities of the ligands are accounted for by considering 20 different conformational states and scoring each of them.

Docking analysis at the colchicine binding site determined the binding modes of the four triazole analogues. Compound **8a** exhibited the strongest binding interactions at the colchicine binding site in tubulin compared to the other three analogues. **8a** participates in strong hydrogen bonding with ASN 258 and THR 353 (Fig. 3) and makes a weak hydrogen bond with ASN 258. Out of 20 different tested conformations of **8a**, four conformations showed the highest C-Score of 4, which was higher than any of the C-Scores for the other three triazole analogues. Compound **8g** participates in hydrogen bonding with ASN 258 and ALA 317, with one conformation exhibiting the highest C-Score of 4. Compounds **11d** and **11e** each participate in a hydrogen bonding interaction with ASN 258, while **11d** participates in another hydrogen bonding interaction with THR 356, and **11e** makes hydrogen bond with ALA 317 (Fig. 3). ASN 258 hydrogen bond interactions are common in all four docked triazoles.

In summary, **8a** exhibited the strongest binding interactions with tubulin at the colchicine-binding site, which is consistent with the anticancer screening data (Table 1). Thus, the above molecular modeling data predicts that stable, more drug-like triazolo derivatives of the *cis*-cyano CA-4 analogues formed by constructing a heterocyclic ring system across the olefinic bond between aromatic rings A and B will retain the tubulin targeting properties of the parent compounds.

D. Inhibition of tubulin polymerization

The ability of compounds **8a**, **11d**, **11e** and **9** to inhibit tubulin polymerization was determined using a commercially available *in vitro* assay (Cytoskeleton Inc., Denver, CO). We examined the ability of three of the most active CA-4-(2*H*)-1,2,3-triazoles, **8a**, **11d**, and **11e**, to inhibit tubulin polymerization using an *in vitro* assay. Compound **8a**, which exhibited the greatest overall potency against the NCI 60 human cancer cell line panel, was found to inhibit tubulin polymerization with an IC₅₀ value of 1.7 (± 0.4) μM (Fig. 4), where the

reported values represent the mean $\pm$ s.d. ($n=3$). Compounds **11d** and **11e** inhibited tubulin polymerization with IC₅₀ values of 18.5 ($\pm$ 9.8) μ M and 13.5 ($\pm$ 3.5) μ M, respectively, in this assay. The *N*-methylated analogue **9** failed to inhibit tubulin polymerization even at concentrations as high as 200 μ M (data not shown). These results are consistent with the idea that compound **8a** exerts some of its cytotoxic effects on cells through inhibition of tubulin polymerization. The less potent inhibition of tubulin polymerization by compounds **11d** and **11e** is consistent with the general trend towards decreasing potency (but still significant), as determined from GI₅₀ values from the cancer cell screens, when compared to compound **8a**, and is also consistent with the molecular docking studies. However, based on the *in vitro* assay results, it would appear that the cytotoxic effects of compound **9** observed in cell culture are not mediated by inhibition of tubulin polymerization, but are instead the result of an unknown mechanism of action.

Conclusions

We have evaluated a small library of novel 4-heteroaryl-5-aryl-(2*H*)-1,2,3-triazoles that are structurally related to *trans*-cyano CA-4 analogues that inhibit tubulin polymerization. These compounds have been evaluated in a panel of 60 human cancer cell lines for their anti-cancer activities, and in assays to determine their ability to inhibit tubulin polymerization. The most potent compound, **8a**, and two closely related analogues, **8b** and **8g**, were also tested for cytotoxicity against triple negative Hs578T breast cancer cell lines. Compound **8a**, a benzothiophen-2-yl derivative was shown to be a more potent anti-cancer agent than its isosteric indol-2-yl (**8i**) and benzofuran-2-yl (**8g**) congeners. Compound **8a** was also found to be the most effective inhibitor of tubulin polymerization. However, the benzothiophen-3-yl isostere of **8a**, compound **11a**, did not possess any significant anti-cancer activity, although indol-3-yl analogues that incorporate a 3,4,5-trimethoxyphenyl (**11d**) or a 3,5-dimethoxyphenyl (**11e**) moiety into their structure were potent anti-cancer agents. Interestingly, a significant decrease in anti-cancer activity was observed with the *N*-2-methylated analogue **9** when compared to the parent compound **8a**.

In summary, the anti-cancer screening data, inhibition of tubulin polymerization, and molecular docking studies are all promising indicators that compound **8a** warrants further study as a potential anti-cancer agent.

EXPERIMENTAL PROCEDURES

General synthetic procedure for the synthesis of 4-heteroaryl-5-(substituted phenyl)-2*H*-1,2,3-triazoles (**8a–i** and **11a–e**):²⁵

Sodium azide (0.01 mol) in DMSO (1 ml) was added to the appropriate heteroaryl cyanostilbene (0.01 mol) followed by addition of L-proline (0.002 mol). The resulting reaction mixture was heated to 100 °C and stirred for 1–6 h. After completion of the reaction, the reaction mixture was cooled to 20 °C, water (10 ml) was added, and the resulting slurry was extracted with ethyl acetate (2 $\times$ 10 ml). The organic layers were separated and combined, dried over anhydrous sodium sulphate, concentrated on a rotovaporator and purified by silica gel column chromatography (ethylacetate:hexanes 1:4) to afford the pure product.

Synthetic procedure for the synthesis of 4-(benzo[*b*] thiophen-2-yl)-2-methyl-5-(3,4,5-trimethoxyphenyl)-2*H*-1,2,3-triazole (**9**)²⁵

A mixture of 4-(benzo[*b*]thiophen-2-yl)-5-(3,4,5-trimethoxy phenyl)-2*H*-1,2,3-triazole (**6a**) (1 mmol), K₂CO₃ (10 mmol) and MeI (2 mmol) in 10 volumes of acetone was refluxed for 8 h and the reaction monitored by TLC analysis. After completion, the reaction mixture was filtered to remove the K₂CO₃, washed with acetone and the filtrate evaporated to dryness on a rotovaporator. The resulting residue was submitted to ethyl acetate/hexane flash column chromatography to yield 4-(benzo[*b*]thiophen-2-yl)-2-methyl-5-(3,4,5-trimethoxyphenyl)-2*H*-1,2,3-triazole (**9**) as a light yellow solid.

NCI-60 cell line anti-cancer screening assay

All the synthesized molecules were evaluated for their anti-cancer activity in a preliminary screen against a panel of 60 human cancer cell lines (NCI-60 panel) at a concentration of 10⁻⁵ M utilizing the procedure described by Rubinstein et al.⁴¹ In this cellular assay the growth inhibition of the test compounds is measured by determining percentage cell growth (PG) inhibition. Optical density (OD) measurements of sulforhodamine B (SRB)-derived colour, just before exposing the cells to the test compound (OD_{t_{zero}}), and after 48 h exposure to the test compound (OD_{test}) or the control vehicle (OD_{ctrl}) is recorded. The growth percentage compared to control is calculated utilizing the reported protocols.⁴² From the preliminary screening potent compounds were further evaluated for five dose studies designed to determine GI₅₀ values, which represent the molar drug concentration required for 50% cell growth inhibition. The compounds were dissolved in dimethyl sulfoxide (DMSO)/H₂O and evaluated using five different concentrations at 10-fold dilutions (10⁻⁴ M, 10⁻⁵ M, 10⁻⁶ M, 10⁻⁷ M and 10⁻⁸ M) following 48 hr of incubation.

Screening against breast cancer cell lines (Hs578T) assay

Hs578T cells were seeded at 3,000 cells per well into 96-well plates and incubated overnight at 37 °C in DMEM containing 10% (v/v) FBS medium. The following day, various concentrations of triazole CA-4 analogues, or vehicle, were added to each plate and the plates were then incubated for an additional 48 hr. Cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS, pH 7.4) and incubated with 2 μM Calcein-AM for 30 min at 25 °C. Fluorescence was measured using a 490 nm excitation wavelength and monitoring emission at 520 nm. The fluorescence intensity is proportional to the number of viable cells. The fluorescence intensity of control (untreated) cells was taken as 100% viability, and the relative cell viability compared to control was calculated.

Inhibition of tubulin polymerization assay

The tubulin polymerization was performed in 80 mM PIPES (pH 6.9) buffer containing 2 mM MgCl₂, 0.5 mM EGTA, 100 mM GTP, and 10% (v/v) glycerol. The assay buffer was prepared on ice immediately prior to performing the tubulin polymerization experiments in order to minimize GTP hydrolysis. Tubulin (4 mg) was reconstituted in 1 mL of cold assay buffer for a final concentration of 4 mg/mL. The reconstituted tubulin was placed on ice for a minimum of 3 minutes prior to starting the experiment. Compounds were dissolved in 50% (v/v) DMSO/dH₂O solution. The 96-well plate was pre-warmed to 37 °C for 30 minutes in a

Synergy4 plate reader (BioTek, Winooski, VT). Compound (10× of final concentration) or DMSO control (4 μ L) was added to the plate and incubated for 2 minutes at 37 °C. Cold tubulin (36 μ L of 4 mg/mL) was added to each well and polymerization was initiated by placing the plate at 37 °C. Polymerization was measured over time by monitoring the change in absorbance at 340 nm. The initial portion of the reaction was fit to a linear equation and the slope was used to estimate the rate of tubulin polymerization. The rate of polymerization in the presence of triazole analogue was normalized against the control reaction and percent activity was plotted against the log of the inhibitor concentration. The IC₅₀ values for test compounds were calculated using a variable slope dose-response curve.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Pettit GR, Cragg GM, Herald DL, Schmidt JM, Lobavanijaya P. *Can. J. Chem.* 1982; 60:1374–1376.
2. Beale TM, Bond PJ, Brenton JD, Charnock-Jones DS, Ley SV, Myers RM. *Bioorg. Med. Chem.* 2012; 20:1749–1759. [PubMed: 22304851]
3. Nathan P, Zweifel M, Padhani AR, Koh DM, Ng M, Collins DJ, Harris A, Carden C, Smythe J, Fisher N, Taylor NJ, Stirling JJ, Lu SP, Leach MO, Rustin GJ, Judson I. *Clin. Cancer Res.* 2012; 18:3428–3439. [PubMed: 22645052]
4. Zimmerman EI, Turner DC, Buaboonnam J, Hu S, Orwick S, Roberts MS, Janke LJ, Ramachandran A, Stewart CF, Inaba H, Baker SD. *Blood.* 2013; 122:3607–3615. [PubMed: 24046014]
5. Pawel JV, Gorbounova V, Reck M, Kowalski DM, Allard A, Chadja M, Rey A, Bennouna J, Grossi F. D. Investigators. *Lung Cancer.* 2014; 85:224–229. [PubMed: 24888230]
6. Kremmidiotis G, Leske AF, Lavranos TC, Beaumont D, Gasic J, Hall A, O'Callaghan M, Matthews CA, Flynn B. *Mol. Cancer Ther.* 2010; 9:1562–1573. [PubMed: 20515948]
7. D'Amato RJ, Lin CM, Flynn E, Folkman J, Hamel E. *Proc. Natl. Acad. Sci. U. S. A.* 1994; 91:3964–3968. [PubMed: 8171020]
8. Kuznetsov G, TenDyke K, Towle MJ, Cheng H, Liu J, Marsh JP, Schiller SE, Spyvee MR, Yang H, Seletsky BM, Shaffer CJ, Marceau V, Yao Y, Suh EM, Campagna S, Fang FG, Kowalczyk JJ, Littlefield BA. *Mol. Cancer Ther.* 2009; 8:2852–2860. [PubMed: 19825803]
9. Romagnoli R, Baraldi PG, Carrion MD, Lopez Cara C, Preti D, Fruttarolo F, Pavani MG, Tabrizi MA, Tolomeo M, Grimaudo S, Di Cristina A, Balzarini J, Hadfield JA, Brancale A, Hamel E. *J. Med. Chem.* 2007; 50:2273–2277. [PubMed: 17419607]
10. Tozer GM, Kanthou C, Baguley BC. *Nat. Rev. Cancer.* 2005; 5:423–435. [PubMed: 15928673]
11. Sosa JA, Balkissoon J, Lu SP, Langecker P, Elisei R, Jarzab B, Bal CS, Marur S, Gramza A, Ondrey F. *Surgery.* 2012; 152:1078–1087. [PubMed: 23158178]
12. Madlambayan GJ, Meacham AM, Hosaka K, Mir S, Jorgensen M, Scott EW, Siemann DW, Cogle CR. *Blood.* 2010; 116:1539–1547. [PubMed: 20472832]
13. Clemenson C, Jouannot E, Merino-Trigo A, Rubin-Carrez C, Deutsch E. *Invest. New Drugs.* 2013; 31:273–284. [PubMed: 22810221]
14. Nakamura H, Kuroda H, Saito H, Suzuki R, Yamori T, Maruyama K, Haga T. *Chem. Med. Chem.* 2006; 1:729–740. [PubMed: 16902927]

15. Tron GC, Pirali T, Sorba G, Pagliai F, Busacca S, Genazzani AA. *J. Med. Chem.* 2006; 49:3033–3044. [PubMed: 16722619]
16. Hsieh HP, Liou JP, Mahindroo N. *Curr. Pharm. Des.* 2005; 11:1655–1677. [PubMed: 15892667]
17. Cirila A, Mann J. *Nat. Prod. Rep.* 2003; 20:558–564. [PubMed: 14700199]
18. Penthala NR, Sonar VN, Horn J, Leggas M, Yadlapalli JS, Crooks PA. *Med. Chem. Comm.* 2013; 4:1073–1078.
19. Penthala NR, Janganati V, Bommagani S, Crooks PA. *Med. Chem. Comm.* 2014; 5:886–890.
20. Shirai R, Takayama H, Nishikawa A, Koiso Y, Hashimoto Y. *Bioorg. Med. Chem. Lett.* 1998; 8:1997–2000. [PubMed: 9873473]
21. Ohsumi K, Hatanaka T, Fujita K, Nakagawa R, Fukuda Y, Nihei Y, Suga Y, Morinaga Y, Akiyama Y, Tsuji T. *Bioorg. Med. Chem. Lett.* 1998; 8:3153–3158. [PubMed: 9873694]
22. Odlo K, Hentzen J, dit Chabert JF, Ducki S, Gani OA, Sylte I, Skrede M, Florenes VA, Hansen TV. *Bioorg. Med. Chem.* 2008; 16:4829–4838. [PubMed: 18396050]
23. Vilanova C, Torijano-Gutierrez S, Diaz-Oltra S, Murga J, Falomir E, Carda M, Alberto Marco J. *Eur. J. Med. Chem.* 2014; 87:125–130. [PubMed: 25240870]
24. Akselsen OW, Odlo K, Cheng JJ, Maccari G, Botta M, Hansen TV. *Bioorg. Med. Chem.* 2012; 20:234–242. [PubMed: 22137934]
25. Odlo K, Fournier-Dit-Chabert J, Ducki S, Gani OA, Sylte I, Hansen TV. *Bioorg. Med. Chem.* 2010; 18:6874–6885. [PubMed: 20708408]
26. Demchuk DV, Samet AV, Chernysheva NB, Ushkarov VI, Stashina GA, Konyushkin LD, Raihstat MM, Firgang SI, Philchenkov AA, Zavelevich MP, Kuiava LM, Chekhun VF, Blokhin DY, Kiselyov AS, Semenova MN, Semenov VV. *Bioorg. Med. Chem.* 2014; 22:738–755. [PubMed: 24387982]
27. Zhang Q, Peng Y, Wang XI, Keenan SM, Arora S, Welsh WJ. *J. Med. Chem.* 2007; 50:749–754. [PubMed: 17249649]
28. Romagnoli R, Baraldi PG, Cruz-Lopez O, Lopez Cara C, Carrion MD, Brancale A, Hamel E, Chen L, Bortolozzi R, Basso G, Viola G. *J. Med. Chem.* 2010; 53:4248–4258. [PubMed: 20420439]
29. Wang L, Woods KW, Li Q, Barr KJ, McCroskey RW, Hannick SM, Gherke L, Credo RB, Hui YH, Marsh K, Warner R, Lee JY, Zielinski-Mozng N, Frost D, Rosenberg SH, Sham HL. *J. Med. Chem.* 2002; 45:1697–1711. [PubMed: 11931625]
30. Huisgen R, Szeimies G, Möbius L. *Chem. Ber.* 1967; 100:2494–2507.
31. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. *Angew. Chem. Int. Ed.* 2002; 41:2596–2599.
32. Tornøe CW, Christensen C, Meldal M. *J. Org. Chem.* 2002; 67:3057–3064. [PubMed: 11975567]
33. Feldman AK, Colasson B, Fokin VV. *Org. Lett.* 2004; 6:3897–3899. [PubMed: 15496058]
34. Ramachary DB, Shashank AB, Karthik S. *Angew. Chem. Int. Ed. Engl.* 2014; 53:10420–10424. [PubMed: 25079606]
35. Krasinski A, Fokin VV, Sharpless KB. *Org. Lett.* 2004; 6:1237–1240. [PubMed: 15070306]
36. Zhang L, Chen X, Xue P, Sun HH, Williams ID, Sharpless KB, Fokin VV, Jia G. *J. Am. Chem. Soc.* 2005; 127:15998–15999. [PubMed: 16287266]
37. Wang YC, Xie YY, Qu HE, Wang HS, Pan YM, Huang FP. *J. Org. Chem.* 2014; 79:4463–4469. [PubMed: 24742349]
38. Penthala NR, Madadi NR, Janganati V, Crooks PA. *Tetrahedron Lett.* 2014; 55:5562–5565. [PubMed: 25267862]
39. Madadi NR, Penthala NR, Song L, Hendrickson HP, Crooks PA. *Tetrahedron Lett.* 2014; 55:4207–4211.
40. Penthala NR, Madadi NR, Bommagani S, Parkin S, Crooks PA. *Acta Crystallogr. Sect. E: Struct. Rep. Online.* 2014; 70:392–395.
41. Rubinstein LV, Shoemaker RH, Paull KD, Simon RM, Tosini S, Skehan P, Scudiero DA, Monks A, Boyd MR. *J. Natl. Canc. Inst.* 1990; 82:1113–1118.
42. Madadi NR, Penthala NR, Janganati V, Crooks PA. *Bioorg. Med. Chem. Lett.* 2014; 24:601–603. [PubMed: 24361000]

Fig 1.
Chemical structures of *cis*-CA-4 (**1**) and its derivatives (**2–6**).

Fig. 2.

Hs578T TNBC cell viability is sensitive to compounds **8a**, **8b**, and **8g**. Dose-response curves for Hs578T cell viability in the presence of varying concentrations of compounds **8a** (closed circles), **8b** (closed squares), and **8g** (open circles) are shown. The values shown represent the mean $\pm$ s.d. (n=3). Fitting the data to a variable slope log (inhibitor) vs. response equation yields the following GI₅₀ estimates (with 95% confidence intervals): **8a**: GI₅₀ = 10 (9.8–11) nM, **8b**: GI₅₀ = 65 (52–82) nM, **8g**: GI₅₀ = 20 (16–26) nM.

Fig. 3.
Binding modes of compounds **8a**, **8g**, **11d** and **11e** at the colchicine binding site on tubulin

Fig. 4. Compound **8a** inhibits tubulin polymerization. (A) An *in vitro* assay was used to measure inhibition of tubulin polymerization by compound **8a**. (B) A dose-response curve for tubulin polymerization in the presence of varying concentrations of compound **8a**. The values shown represent the mean $\pm$ s.d. (n=3).

Entry	X	R ¹	R ²	R ³
8a	S	OCH ₃	OCH ₃	OCH ₃
8b	S	OCH ₃	H	OCH ₃
8c	S	H	OCH ₃	H
8d	S	OH	OCH ₃	H
8e	S	3,4-methylenedioxy		H
8f	S	H	SCH ₃	H
8g	O	OCH ₃	OCH ₃	OCH ₃
8h	O	OCH ₃	H	OCH ₃
8i	NH	OCH ₃	OCH ₃	OCH ₃

Scheme 1.

Synthesis of substituted 4-heteroaryl-2-yl-5-phenyl-2*H*-1,2,3-triazoles (**8a-i**).

Entry	X	R ¹	R ²	R ³
11a	S	OCH ₃	OCH ₃	OCH ₃
11b	S	OCH ₃	OCH ₃	H
11c	S	H	OCH ₃	H
11d	NH	OCH ₃	OCH ₃	OCH ₃
11e	NH	OCH ₃	H	OCH ₃

Scheme 2.

Synthesis of substituted 4-heteroaryl-3-yl-5-phenyl-2H-1,2,3-triazoles (**11a-e**).

Scheme 3.

Synthesis of 4-(benzo [b]thiophen-2-yl)-2-methyl-5-(3,4,5-trimethoxyphenyl)-2*H*-1,2,3-(2*H*)-triazole (**9**).

Table 1
Antitumor activity (GI₅₀ in μ M)^a data for compounds **8a**, **9**, **11b**, **11d**, **11e** in the NCI 60-cell line screen

Panel/cell line	8a	9	11b	11d	11e
	GI ₅₀ (μ M)	GI ₅₀ (μ M)	GI ₅₀ (μ M)	GI ₅₀ (μ M)	GI ₅₀ (μ M)
<u>Leukemia</u>					
CCRF-CEM	0.028	0.22	3.29	0.048	0.037
HL-60(TB)	<0.01	0.048	1.70	0.046	0.033
K-562	<0.01	0.042	0.516	0.053	0.031
MOLT-4	0.042	0.333	3.05	0.16	0.063
RPMI-8226	<0.01	0.362	3.64	0.09	0.053
SR	<0.01	nd	nd	0.09	0.047
<u>Lung Cancer</u>					
A549/ATCC	0.023	0.167	4.52	0.09	0.088
EKVX	0.010	0.466	3.42	na	na
HOP-62	0.015	0.553	2.18	na	na
HOP-92	<0.01	0.080	0.921	nd	0.029
NCI-H226	nd	>100	13.2	0.39	0.075
NCI-H23	<0.01	0.392	3.94	0.27	0.124
NCI-H322M	<0.01	1.26	7.79	nd	40.7
NCI-H460	<0.01	0.069	3.56	0.042	0.038
NCI-H522	<0.01	0.023	0.601	0.021	0.013
<u>Colon Cancer</u>					
COLO 205	<0.01	0.082	2.82	0.034	0.035
HCC-2998	0.036	0.407	4.21	0.44	0.251
HCT-116	<0.01	0.073	2.84	0.041	0.031
HCT-15	<0.01	0.058	1.67	0.035	0.031
HT29	0.020	0.055	2.78	0.033	0.031
KM12	0.022	0.054	2.28	0.045	0.045
SW-620	<0.01	0.063	3.09	0.043	0.035
<u>CNS Cancer</u>					
SF-268	<0.01	0.63	3.69	0.071	0.067

Panel/cell line	8a	9	11b	11d	11e
	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)
SF-295	<0.01	0.036	2.33	0.032	0.026
SF-539	<0.01	0.149	1.84	0.031	0.024
SNB-19	<0.01	0.254	4.67	0.061	0.056
SNB-75	<0.01	nd	nd	0.030	0.025
U251	<0.01	0.078	2.19	0.059	0.054
<u>Melanoma</u>					
LOX IMVI	<0.01	0.168	3.04	0.071	0.055
MALME-3M	nd	16.9	13.3	nd	nd
MI4	<0.01	0.076	1.95	0.036	0.031
MDA-MB-435	<0.01	0.025	0.337	0.014	0.010
SK-MEL-2	<0.01	0.059	1.44	0.030	0.031
SK-MEL-28	<0.01	13.0	4.63	0.112	Nd
SK-MEL-5	<0.01	0.225	2.83	0.043	0.034
UACC-257	<0.01	51.6	10.9	13.9	>100.0
UACC-62	<0.01	0.053	2.77	0.043	0.034
<u>Ovarian Cancer</u>					
IGROV1	<0.01	0.707	4.21	na	na
OVCAR-3	<0.01	0.354	1.94	0.033	0.028
OVCAR-4	0.042	nd	6.33	1.16	2.67
OVCAR-5	0.042	2.36	7.91	0.381	0.416
OVCAR-8	<0.01	0.352	4.24	1.16	0.070
NCI/ADR-RES	<0.01	0.103	2.39	0.034	0.030
SK-OV-3	<0.01	0.311	2.37	0.042	0.034
<u>Renal Cancer</u>					
786-0	<0.01	0.312	3.26	0.035	0.030
A498	<0.01	0.041	2.96	0.035	0.033
ACHN	<0.01	0.294	3.94	0.043	0.045
CAKI-1	<0.01	3.77	3.69	0.044	0.038
RXF 393	nd	0.286	2.11	0.164	0.052
SN12C	0.048	nd	8.80	1.39	0.069

Panel/cell line	8a	9	11b	11d	11e
	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)
TK-10	nd	0.578	2.98	1.39	0.083
UO-31	<0.01	0.744	2.78	0.087	0.062
<u>Prostate Cancer</u>					
PC-3	0.034	0.077	3.08	0.044	0.038
DU-145	<0.01	0.263	3.77	0.090	0.075
<u>Breast Cancer</u>					
MCF7	<0.01	0.040	1.45	0.029	0.029
MDA-MB-231/ATCC	0.014	1.60	3.21	0.089	0.076
HS 578T	<0.01	0.270	2.25	0.042	0.031
BT-549	<0.01	0.422	2.17	0.028	0.017
T-47D	nd	>100	2.83	0.048	0.044
MDA-MB-468	<0.01	0.082	0.63	0.042	0.037

na: not analyzed; nd: not determined;

^a GI₅₀: 50% growth inhibition, concentration of drug resulting in a 50% reduction in net cell growth as compared to cell numbers on day 0.