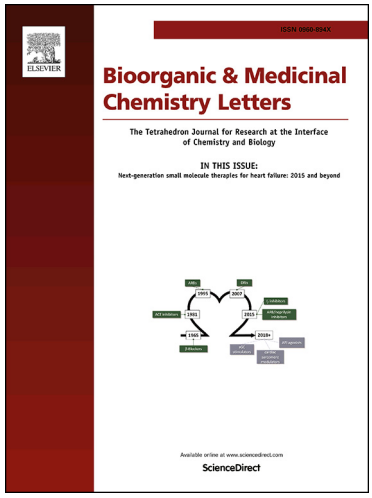
Accepted Manuscript



Discovery and optimization of 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides as potent tubulin polymerization inhibitors

Fang Yang, Cai-Ping He, Peng-Cheng Diao, Kwon Ho Hong, Jin-Jun Rao, Pei-Liang Zhao

PII: S0960-894X(18)30890-4
DOI: https://doi.org/10.1016/j.bmcl.2018.11.024
Reference: BMCL 26135

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 6 September 2018
Revised Date: 5 November 2018
Accepted Date: 12 November 2018

Please cite this article as: Yang, F., He, C-P., Diao, P-C., Ho Hong, K., Rao, J-J., Zhao, P-L., Discovery and optimization of 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides as potent tubulin polymerization inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.11.024

Discovery and optimization of 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides as potent tubulin polymerization inhibitors

Fang Yang[a,#], Cai-Ping He[a,#], Peng-Cheng, Diao[a], Kwon Ho Hong[b], Jin-Jun Rao[a,*], Pei-Liang Zhao[a,*]

[a]*Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Science, Southern Medical University, Guangzhou 510515, P.R.China*

[b]*Department of Medicinal Chemistry and Institute for Therapeutics Discovery and Development, College of Pharmacy, University of Minnesota, Minneapolis 55414, United States*

ABSTRACT: Based on our previous research, three series of new triazolylthioacetamides possessing 3,4,5-trimethoxyphenyl moiety were synthesized, and evaluated for antiproliferative activities and inhibition of tubulin polymerization. The most promising compounds **8b** and **8j** demonstrated more significant antiproliferative activities against MCF-7, HeLa, and HT-29 cell lines than our lead compound **6**. Moreover, analogues **8f**, **8j**, and **8o** manifested more potent antiproliferative activities against HeLa cell line with IC_{50} values of 0.04, 0.05 and 0.16 μM, respectively, representing 100-, 82-, and 25-fold improvements of the activity compared to compound **6**. Furthermore, the representative compound, **8j**, was found to induce significant cell cycle arrest at the G_2/M phase in HeLa cell lines via a concentration-dependent manner. Meanwhile, compound **8b** exhibited the most potent tubulin polymerization inhibitory activity with an IC_{50} value of 5.9 μM, which was almost as active as that of CA-4 (IC_{50} = 4.2 μM). Additionally, molecular docking analysis suggested that **8b** formed stable interactions in the colchicine-binding site of tubulin.

Keywords: Triazolylthioacetamides; Synthesis; Tubulin polymerization; Antiproliferative activity.

* Corresponding author. E-mail: plzhao@smu.edu.cn (P.-L. Zhao), raojj@smu.edu.cn (J.-J. Rao)

# These authors contributed equally to this work.

Microtubules have been a highly attractive therapeutic target for the development of anticancer agents due to their crucial roles in numerous cellular processes, such as maintenance of cell shape, regulation of motility, transportation of vesicles and mitochondria, and cell division.[1-3] Crucially, microtubules form highly dynamic spindle fibers required for the segregation of duplicated chromosomes during mitosis.[4,5] Microtubule-targeting agents disrupting microtubule dynamics can be divided into two classes based on their mode of action: inhibitors of tubulin polymerization and blockers/inhibitors of microtubule disassembly.[6,7] During the last few decades, many efforts have been devoted to the identification of structurally diverse tubulin inhibitors including both natural and synthetic molecules.[8-13] Among them, epothilone, paclitaxel, and vindesine have already been launched. However, they have certain limitations in their clinical utility due to P-glycoprotein-mediated drug resistance, and difficulty in synthesis and isolation.[14] Therefore, development of small moleculars as potent tubulin inhibitors with better therapeutic properties for clinical use are urgently needed.

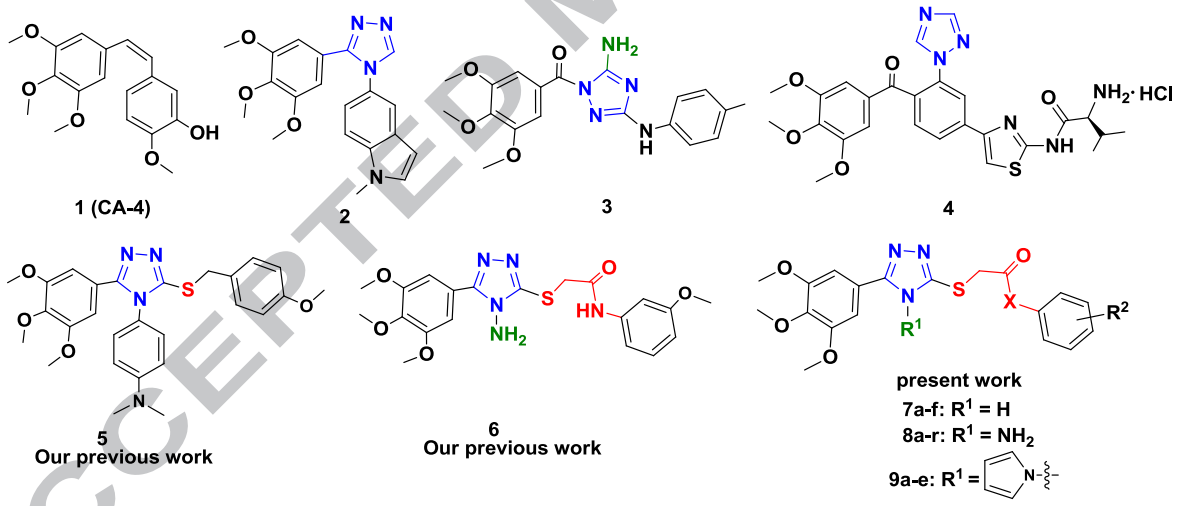
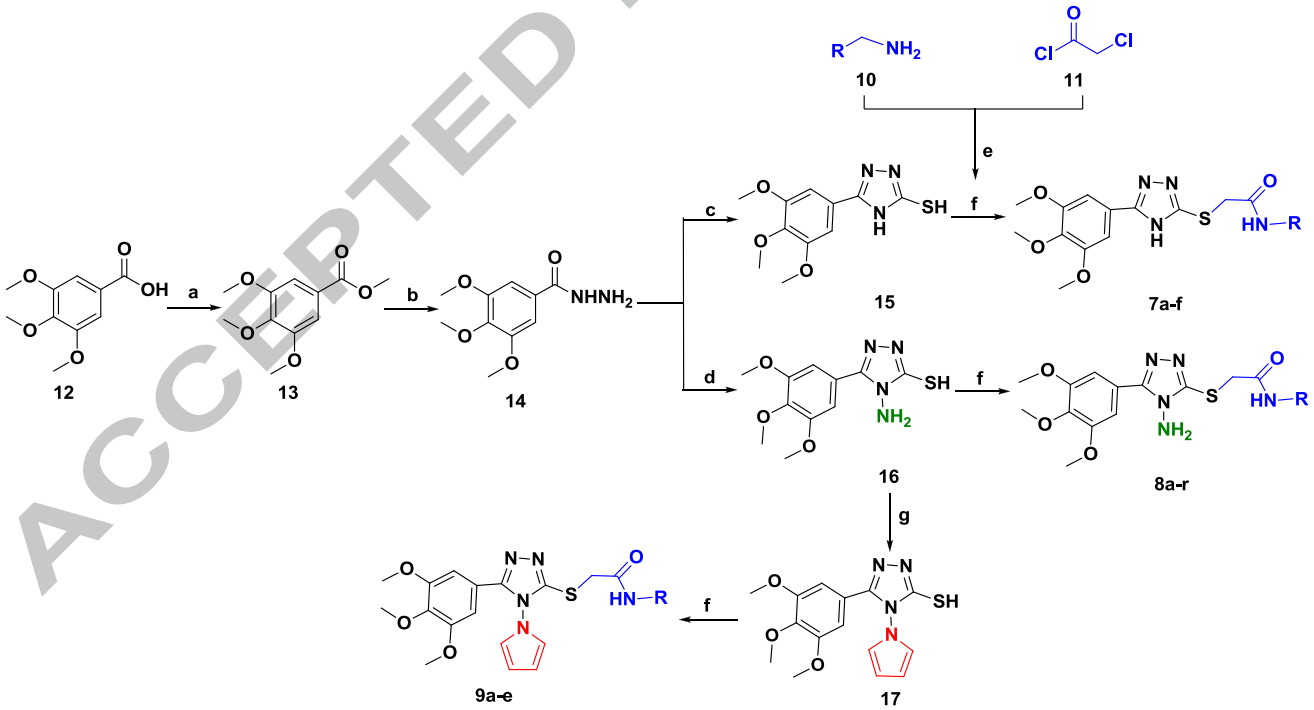


Fig. 1. Structures of selected tubulin inhibitors, including **CA-4**, triazole-based compounds **2-6**, and newly designed compounds **7-9**.

Over the past few years, one of the most active is the combretastatin A-4 (CA-4, Fig. **1**), which provides a simple structural template for the development of novel CA-4 analogues as potential anticancer agents.[15-18] To date, 1,2,4-triazole-based CA-4 analogues have attracted increasing attentions and have been identified as potent tubulin polymerization inhibitors, such as the recently reported 1*H*-indolyl-1,2,4-triazole **2**, 5-amino-1,2,4-triazole derivative **3**, and triazole-containing analogue **4** possessing submicromolar antiproliferative activities.[19-21] More recently, we described

two series of 3-alkylsulfanyl-1,2,4-triazole analogues, exemplified by compounds **5** and **6** (Fig. **1**), which exhibited remarkable antiproliferative activities against a panel of cancer cell lines.[22,23] Initial structure–activity relationship (SAR) studies indicated that both the 3,4,5-trimethoxyphenyl group of the 5-position on the 1,2,4-triazole skeleton and the N-(substituted phenyl)thioacetamide group at the 3-alkylsulfanyl moiety were essential for an optimal activity. Herein, we investigated the biological importance of the substitution of 4-position and the *N*-substitution acetamide moiety of 3-position on the 1,2,4-triazole skeleton by introducing various groups to generate 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides **7a-f**, **8a-r**, and **9a-e** (Fig. **1**). These synthesized compounds were evaluated for their antiproliferative activities, and the most promising derivatives were further evaluated for the inhibition of tubulin polymerization and the effects on the cell-cycle. To our knowledge, of the twenty-nine target analogues, compound **8b** was already reported and its growth-inhibitory effect was characterized by us.[23] Additionally, effect of these compounds on tubulin-polymerization has not been reported so far.



Scheme 1. General synthetic route for target compounds **7-9.** Reagents and conditions: (a) con. H_2SO_4, methanol, reflux; (b) 60% NH_2NH_2 H_2O, ethanol, reflux; (c) NH_4SCN, HCl; NaOH (d) KOH, CS_2, ethanol, rt.; 60% NH_2NH_2 H_2O, H_2O, reflux, HAc; (e) chloroacetyl chloride, Et_3N, CH_2Cl_2, r.t.; (f) K_2CO_3, acetone, 2-chloro-N-sbustituted acetamides, r.t.; (g) 2,5-Dimethoxytetrahydrofuran, HOAc, reflux.

The reaction sequence employed for the preparation of 29 triazolylthioacetamides was depicted in Scheme **1**. Briefly, the required diverse *N*-substituted 2-chloroacetamides were first

synthesized by acylation of substituted anilines (**10**) with chloroacetyl chloride (**11**).[24] 3,4,5-Trimethoxybenzohydrazide (**14**) was prepared from 3,4,5-trimethoxybenzoic acid *via* a two-step operation empolying esterification and condensation with hydrazine. The benzoylhydrazine was then treated with ammonium thiocyanate (NH_4SCN) under acidic conditions to give corresponding aroylthiourea, which was converted into the 4H-1,2,4-triazole-3-thiol (**15**) at reflux in the presence of sodium hydroxide. Subsequently, the target triazolylthioacetamides **7a-f** were generated by a nucleophilic substitution reaction under alkaline conditions. Similarly, the target compounds **8a-r** were obtained by the reaction of *N*-substituted 2-chloroacetamides with 4-amino-1,2,4-triazole-3-thiol (**16)** which was prepared according to our reported procedure.[23] Additionally, the key intermediate (**16**) was converted into the pyrrole-substituted 1,2,4-triazole (**17**) by the reaction with 2,5-dimethoxytetrahydrofuran at reflux. Stirring compound **17** with different 2-chloroacetamides at room temperature afforded the desired compounds **9a-e** in moderate to good isolated yields ranging from 74% to 81%.

The structures of the newly synthesized 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides **7a-f**, **8a-r**, and **9a-e** were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopic techniques, and the results are shown in the Experimental section. In addition, the representative compound **8p** was further confirmed by single-crystal X-ray diffraction. As shown in Fig. **2**, the crystal structure showed that **8p** had a C-shape, while the planes of the triazole and 3,4,5-trimethoxyphenyl ring were nearly parallel, with a dihedral angle of 4.83°.

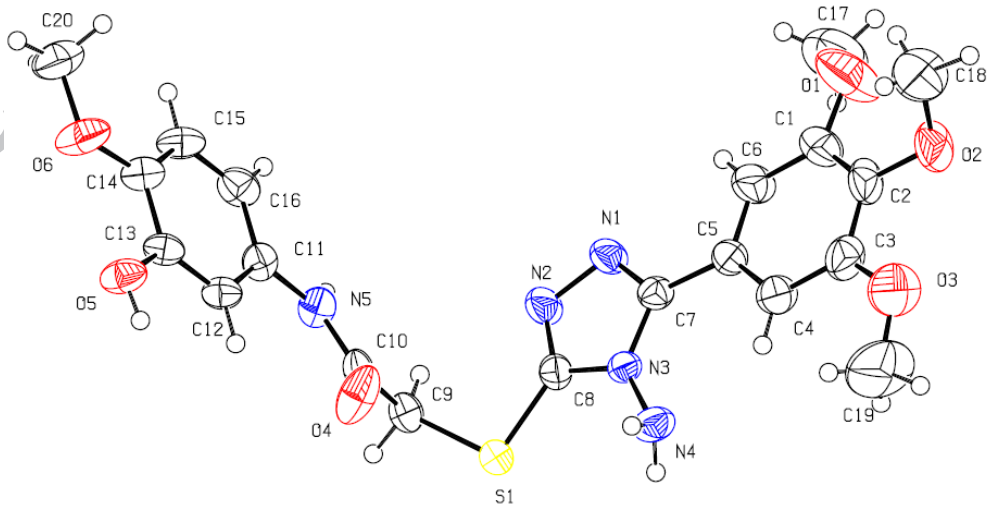
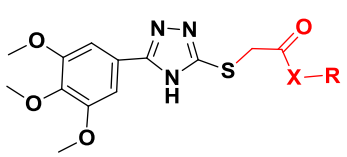


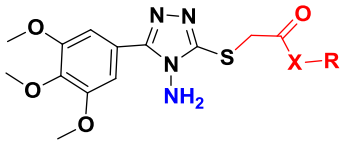
Fig. 2. Molecular structure of compound **8p** (CCDC 1847532).

The prepared triazolylthioacetamides were evaluated for their *in vitro* antiproliferative activities against four human cancer cell lines, including MCF-7 (mammary adenocarcinoma cells), HeLa (human cervical cancer cells), HCT116 (human colon carcinoma cells), and HT-29 (human colon carcinoma cell line) through MTT screening assay. For comparison, a potent tubulin-binding anticancer agent CA-4 and our previously reported compound **6** were employed as the positive controls, and the results expressed as IC_{50} (μM) were summarized in Table **1**. Here, the IC_{50} value represents the concentration of a compound resulting in 50% inhibition of cell growth after 48 h incubation with the compound, and is the average of three independent experiments.

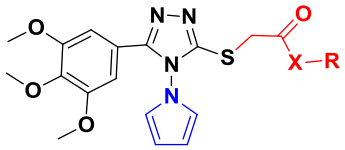
Table 1 Cytotoxic activities of compounds 7~9 against human tumor cells.



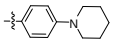
7a~f



8a~r



9a~e

Comp.	n	R	*In vitro* cytotoxicity IC_{50}(μM) [a]				
			MCF-7	**HeLa**	**HCT116**	**HT-29**	**HEK-293** [b]
7a	NH	4-CH_3Ph	>100	>100	>100	>100	NT [c]
7b	NH	4-CH_3OPh	>100	>100	>100	>100	NT
7c	NH	4-CH_3CH_2Ph	>100	33.89±1.20	>100	>100	NT
7d	NH	4-$CH_3(CH_2)_2$Ph	>100	>100	>100	>100	NT
7e	NH	3-OH,4-CH_3OPh	>100	>100	>100	>100	NT
7f	NH	3-CH_3OPh	>100	>100	>100	>100	NT
8a	NH	3,4-$(CH_3)_2$Ph	>100	0.88±0.27	>100	1.21±0.31	NT
8b	NH	4-CH_3Ph	26.83±3.96	1.61±0.38	3.45±0.47	0.82±0.23	NT
8c	NH	4-CH_3OPh	>100	2.96±0.58	61.10±6.69	15.16±2.16	NT
8d	NH	3,4-$(OCH_3)_2$Ph	>100	54.23±5.49	>100	>100	NT
8e	NH	2,4,6-$(CH_3)_3$Ph	>100	82.82±10.99	>100	>100	NT
8f	NH	2-F,4-CH_3Ph	>100	0.04±0.02	>100	0.13±0.16	61.09±6.12
8g	NH		>100	54.93±2.74	>100	75.28±11.26	NT
8h	NH		80.64±2.79	59.68±7.35	>100	>100	NT
8i	NH	3-CH_3Ph	47.1±5.94	2.96±0.58	>100	4.87±1.47	NT
8j	NH	4-CH_3CH_2Ph	25.36±4.49	0.05±0.017	71.36±6.24	0.84±0.41	>100
8k	NH	4-$(CH_3)_2$CH Ph	>100	13.73±1.22	74.77±1.63	>100	NT
8l	NH	4- $(CH_3)_3$CPh	>100	49.64±12.59	>100	>100	NT
8m	NH	4-$CH_3(CH_2)_2$Ph	41.22±4.30	18.67±0.57	41.81±3.32	50.75±8.11	NT
8n	NH	4-N$(CH_3)_2$Ph	>100	23.82±0.36	>100	43.11±3.28	NT
8o	NH	2-CH_3OPh	>100	0.16±0.05	82.85±2.11	0.13±0.17	>100
8p	NH	3-OH,4-OCH_3	>100	34.76±3.4	>100	>100	NT
8q	NCH_3	4-CH_3Ph	>100	21.32±0.37	>100	>100	NT
8r	O	4-CH_3Ph	>100	>100	>100	>100	NT
9a	NH	4-CH_3Ph	>100	>100	26.58±3.22	2.94±0.02	NT
9b	NH	4-CH_3OPh	>100	>100	>100	>100	NT

9c	NH	3,4-$(CH_3O)_2$Ph	>100	>100	>100	>100	NT
9d	/	4-CH_3Ph	>100	>100	>100	>100	NT
9e	/	4-ClPh	>100	>100	>100	>100	NT
	6		52.56±8.05	4.02±0.31[d]	0.68±0.09[d]	3.15±0.01	>100[d]
	CA4		0.041±0.0013	0.007±0.001	6.10±0.14	1.96±0.62	NT

[a] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%. [b] Normal human embryonic kidney (HEK-293) cell lines. [c] NT: not tested. [d] Data taken from ref.[23].

It was interesting to note that almost all of the *N*-unsubstituted and *N*-pyrrole triazolylthioacetamides **7a-f** and **9a-e** were inactive (IC_{50} >100 μM) against four tested cell lines, while most *N*-amino substituted analogues **8a-r** exhibited moderate to strong antiproliferative activities against the tested cell lines. Particularly, analogues **8b** and **8j** displayed higher antiproliferative activities against MCF-7, HeLa and HT-29 cell lines than our previously reported compound **6**. Moreover, compounds **8f**, **8j** and **8o** showed 100-, 82-, and 25-fold improvement compared to lead compound **6** in inhibiting HeLa cell proliferation with IC_{50} values of 0.04, 0.05, and 0.16 μM, respectively. These results clearly demonstrate that *N*-amino analogues **8a-r** manifest better antitumor activities against tested cell lines than *N*-unsubstituted **7a-f** and *N*-pyrrole derivatives **9a-e**. More interestingly, analogues **8f**, **8j** and **8o** exhibited weak inhibitory effect on the normal human embryonic kidney cells, HEK-293, suggesting that the compound may be selective against cancer cells.

Within the series of *N*-amino analogues **8a-r**, further analysis clearly revealed that different antiproliferative activities were observed when various R groups were introduced. Our previous SAR studies[23] indicated that introduction of electron-donating groups on the phenyl group (R) could improve antiproliferative activities, and the newly synthesized analogue **8b**, with 4-methyl substituent on the phenyl ring, displayed strong antiproliferative activity against four tested cell lines. However, introduction of more electron-donating groups on the phenyl group results in significant decrease of antiproliferative activities (**8b** vs **8d**, **8e**, **8k, 8l**, **8m**, 8**n**). It is worth noting that replacement of 4-methyl with 4-ethyl group leads to obvious enhancement in antiproliferative activities against MCF-7, HT-29 and HeLa cell lines (**8b** vs **8j**). Most interestingly, compound **8q** with N-CH_3 as a linker, and compound **8r** bearing an oxygen atom as a linker almost did not show any antiproliferative activities, indicating that the NH linker (X) might play a crucial role in modulating the antitumor activity.

In order to investigate the mode of action of these compounds on cancer cells, one selected analogue **8j** was examined for the influence on the cell cycle progression. In this study, HeLa cells were treated with compound **8j** at given concentrations (0.25, 0.5 μM) for 48h. As shown in Fig. **3** and Table **2**, the cell cycle analysis results revealed that the G2/M peak significantly increased from 12.91% to 27.30% (0.25 μM), and 54.77% (0.5 μM) after 48 h of treatment. These data suggest that compound **8j** induced a significant cell cycle arrest at the G_2/M phase in a concentration-dependent manner, compared to untreated cells.

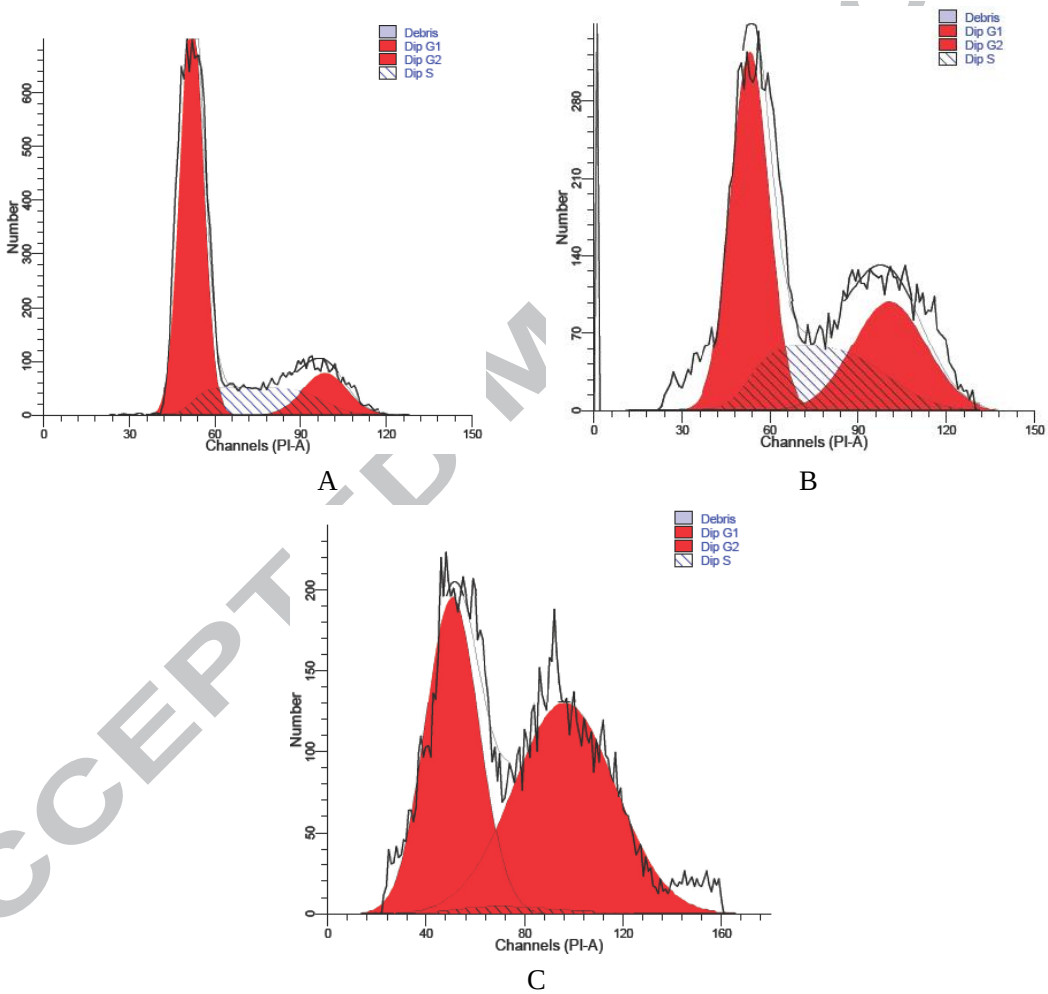


Fig. 3. Effect of compound **8j** on cell cycle and apoptosis in HeLa cells. Flow cytometry analysis of HeLa cells stained with propidium iodide and treated with **8j** for 48 h. (A) Control; (B) **8j**, 0.25 μM; (C) **8j**, 0.5 μM.

Table 2 Effect of compound 8j on cell cycle distribution in HeLa **cells.**

Concentration	G_0/G_1(%)	S(%)	G_2/M(%)
0μM	66.77	20.31	12.91
0.25μM	47.35	25.35	27.30
0.50μM	43.23	2.00	54.77

Cell cycle analysis results revealed a concentration dependent increase in HeLa cells arrested at the G2/M phase, which is in accord with the typical propensity of tubulin polymerization inhibitors. To evaluate whether the newly synthesized triazolylthioacetamides could potentially interact with tubulin, ten representative analogues were selected for the evaluation of their *in vitro* inhibitory activities against tubulin polymerization at 10 μM concentration and CA-4 was also used as the reference. The results were summarized in Table **3**. To some extent, there is correlation with respect to antitubulin activity and antiproliferative activity of the tested compounds. For example, two triazolylthioacetamides **8b** and **8j** were found to be potent anti-cancer agents against all the tested cell lines, and also exhibited much higher than 35% inhibition of the tubulin polymerization at a concentration of 10 μM. Notably, compound **8b** displayed anti-tubulin activity with an IC_{50} value of 5.9 μM, which was almost as active as that of CA-4 (IC_{50} = 4.2 μM).

Table 3 Tubulin polymerization inhibitory activities of representative selected compounds

Comp.	X	R	Tubulin polymerization	
			% inhibition [a]	IC_{50}(μM)
7c	NH	4-CH_3CH_2Ph	11	-[b]
8a	NH	3,4-$(CH_3)_2$Ph	18	-
8b	NH	4-CH_3Ph	55	5.9
8c	NH	4-CH_3OPh	24	-
8i	NH	3-CH_3Ph	25	-
8j	NH	4-CH_3CH_2Ph	38	-
8m	NH	4-$CH_3(CH_2)_2$Ph	19	-
8o	NH	2-CH_3OPh	25	-
8p	NH	3-OH,4-OCH_3	21	-
9a	NH	4-CH_3Ph	17	-
		CA-4	80	4.22

[a] Compounds were tested at a final concentration of 10μM. [b] -: not tested.

The possible binding mode for the most active compound **8b** with tubulin was investigated by docking simulations, using Glide and the pose with the best emodel score (docking score -7.0, glide emodel -71.1 kcal/mol) was used for MM-GBSA relative binding free energy calculation using Prime.[25,26] As shown in Fig. **4**, docking studies suggest various hydrogen bonding and hydrophobic interactions that appear to play a key role in binding. NH of the 4-methylphenylamine moiety of compound **8b** forms a hydrogen bonding interaction with Thr179 (3.0 Å) of α-subunit. Meanwhile, the carbonyl of the thioacetamide group establishes hydrogen bonds with Asn258 (2.9 Å) of

β-subunit. In addition, the 3,4,5-trimethoxyphenyl triazole moiety of compound **8b** occupies the hydrophobic pocket formed by Tyr202, Val238, Cys241, Leu242, Ala250, Leu252, Leu255, Ala316, Ala317 and Ile378.

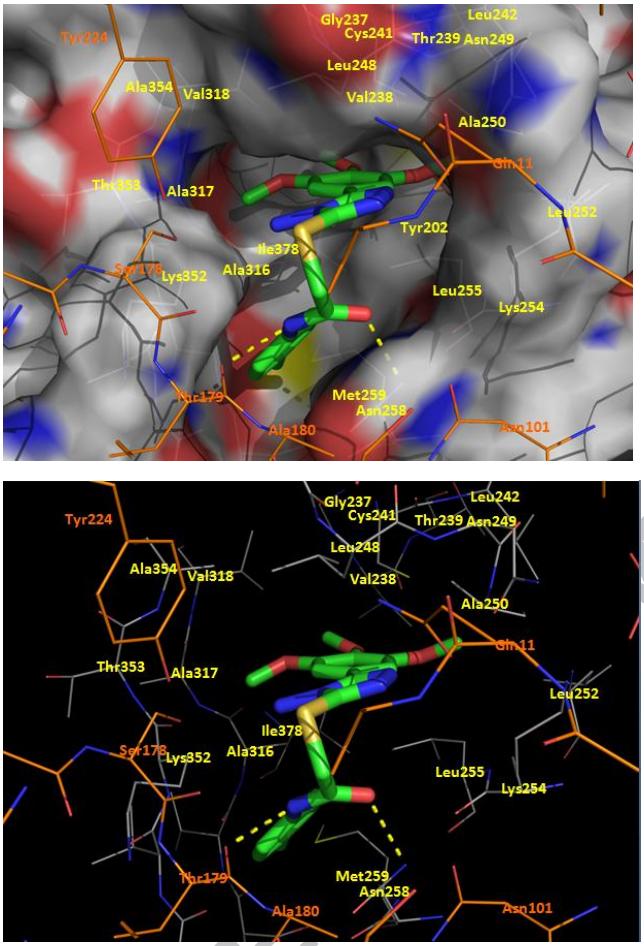


Fig.4. Docking pose of compound **8b** in tubulin with (A) and without (B) the surface of the pocket (PDP ID: 1SA0). Amino acid residues of tubulin beta chain are labeled in yellow and those in tubulin alpha in orange. MM-GBSA ΔG is -114.0 kcal/mol.

In conclusion, based on our previously reported antiproliferative compound **6**, three series of novel triazolylthioacetamide derivatives bearing 3,4,5-trimethoxyphenyl were synthesized and evaluated for their antiproliferative activities. The most promising compounds **8b** and **8j** displayed higher antiproliferative activities against MCF-7, HeLa and HT-29 cell lines than lead compound **6**. Moreover, compounds **8f**, **8j**, and **8o** showed more potent antiproliferative activities against HeLa cell proliferation with IC_{50} values of 0.04, 0.05 and 0.16 μM, respectively, representing 100-, 82-, and 25-fold improvement compared to lead compound **6**. Additionally, in mechanistic studies, the

representative compound **8j** was found to induce significant cell cycle arrest at the G_2/M phase in HeLa cell lines in a concentration-dependent manner. Notably, the compound **8b** exhibited the most potent antitubulin activity with an IC_{50} value of 5.9 μM, almost as active as that of CA-4 (IC_{50} = 4.2 μM). Furthermore, docking studies revealed that **8b**, as a typical potent tubulin polymerization inhibitor, formed stable interactions in the colchicine binding site in the tubulin. These preliminary results encourage further investigation on triazolylthioacetamides aiming to develop new potential tubulin polymerization inhibitors with anticancer activity.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 21372113), and the Science and Technology Program of Guangzhou, China (No. 201707010198).

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.xx.xxx.

References and Notes

1. Amos LA. What tubulin drugs tell us about microtubule structure and dynamics. *Semin. Cell Dev. Biol*. 2011; 22: 916–926.
2. Muroyama A, Lechler T. Microtubule organization, dynamics and functions in differentiated cells. *Development*. 2017; 144: 3012–3021.
3. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov*. 2010; 9: 790–803.
4. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nature reviews Cancer.* 2004; 4: 253–265.
5. Kline-Smith SL, Walczak CE. Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics. *Mol Cell*. 2004; 15: 317–327.
6. Brancale A, Silvestri R. Indole, a core nucleus for potent inhibitors of tubulin polymerization. *Med Res Rev.* 2007; 27: 209–238.

7. Aguayo-Ortiz R, Cano-González L, et al. Structure-based approaches for the design of benzimidazole-2-carbamate derivatives as tubulin polymerization inhibitors. *Chem Biol Drug Des*. 2017; 90: 40–51.
8. Hu MJ, Zhang B, Yang HK, et al. Design, synthesis and molecular docking studies of novel indole-pyrimidine hybrids as tubulin polymerization inhibitors, *Chem. Biol. Drug Des.* 2015; 86: 1491–1500.
9. Siddiqui-Jain A, Hoj JP, Cescon DW, et al. Pharmacology and in vivo efficacy of pyridine-pyrimidine amides that inhibit microtubule polymerization. *Bioorg Med Chem Lett*. 2018; 28: 934–941.
10. Banerjee S, Arnst KE, Wang, Y. et al. Heterocyclic-fused pyrimidines as novel tubulin polymerization inhibitors targeting the colchicine binding site: structural basis and antitumor efficacy. *J Med Chem*. 2018; 61: 1704–1718.
11. Diao PC, Li Q, Hu MJ, et al. Synthesis and biological evaluation of novel indole-pyrimidine hybrids bearing morpholine and thiomorpholine moieties. *Eur J Med Chem.* 2017; 134: 110–118.
12. Zhang B, Li YH, Liu Y, et al. Design, synthesis and biological evaluation of novel 1,2,4-triazolo [3,4-b][1,3,4] thiadiazines bearing furan and thiophene nucleus, *Eur. J. Med. Chem.* 2015; 103: 335–342.
13. Dohle W, Jourdan FL, Menchon G, et al. Quinazolinone-based anticancer agents: synthesis, antiproliferative SAR, antitubulin activity, and tubulin co-crystal structure. *J Med Chem*. 2018; 61: 1031–1044.
14. Kamal A, Reddy NV, Nayak VL, et al. Synthesis and biological evaluation of benzo[b]furans as inhibitors of tubulin polymerization andinducers of apoptosis, *Chem Med Chem.* 2014; 9: 117–128.
15. Karacivi M, Sumer Bolu B, Sanyal R. Targeting to the bone: alendronate-directed Combretastatin A-4 bearing antiangiogenic polymer-drug conjugates. *Mol Pharm*. 2017; 14: 1373–1383.
16. Horký P, Voráčová M, Konečná K, et al. Nontoxic combretafuranone analogues with high in vitro antibacterial activity. *Eur J Med Chem.* 2018; 143: 843–853.
17. Liu CT, Bi KJ, Chang WL, et al. Synthesis of a tubulin assembly inhibitor OXi8006. *Chin. J.*

Org. Chem. 2015; 35: 484–489.

18. Tangutur AD, Kumar D, Krishna KV, et al. Microtubule targeting agents as cancer chemotherapeutics: an overview of molecular hybrids as stabilizing and destabilizing agents. *Curr Top Med Chem.* 2017; 1: 2523–2537.
19. Zhang Q, Peng Y, Wang XI, et al. Highly potent triazole-based tubulin polymerization inhibitors. *J Med Chem.* 2007; 50: 749–754.
20. Romagnoli R, Baraldi PG, Salvador MK, et al. Synthesis, antimitotic and antivascular activity of 1-(3′, 4′, 5′-Trimethoxybenzoyl)-3-arylamino-5-amino-1, 2, 4-triazoles. *J Med Chem*. 2014; 57: 6795–6808.
21. Oh DY, Kim TM, Han SW, et al. Phase I study of CKD-516, a novel vascular disrupting agent, in patients with advanced solid tumors. Cancer Research and Treatment, 2016; 48: 28–36.
22. Li YH, Zhang B, Yang HK, et al. Design, synthesis, and biological evaluation of novel alkylsulfanyl-1,2,4-triazoles as cis-restricted combretastatin A-4 analogues. *Eur J Med Chem.* 2017; 125: 1098–1106.
23. Zhao PL, Chen P, Li Q, et al. Design, synthesis and biological evaluation of novel 3-alkylsulfanyl-4-amino-1,2,4-triazole derivatives. *Bioorg Med Chem Lett*. 2016; 26: 3679–3683.
24. Li Q, Chen P, Yang HK, et al. 3,4,5-Trimethoxyphenyl moiety Synthesis, antiproliferative evaluation, and structure–activity relationships of novel triazole–isoindoline hybrids bearing. *Chem Paper* 2018; 72: 651–659.
25. Glide, version 6.9, Schrodinger, LLC, New York, NY, 2015.

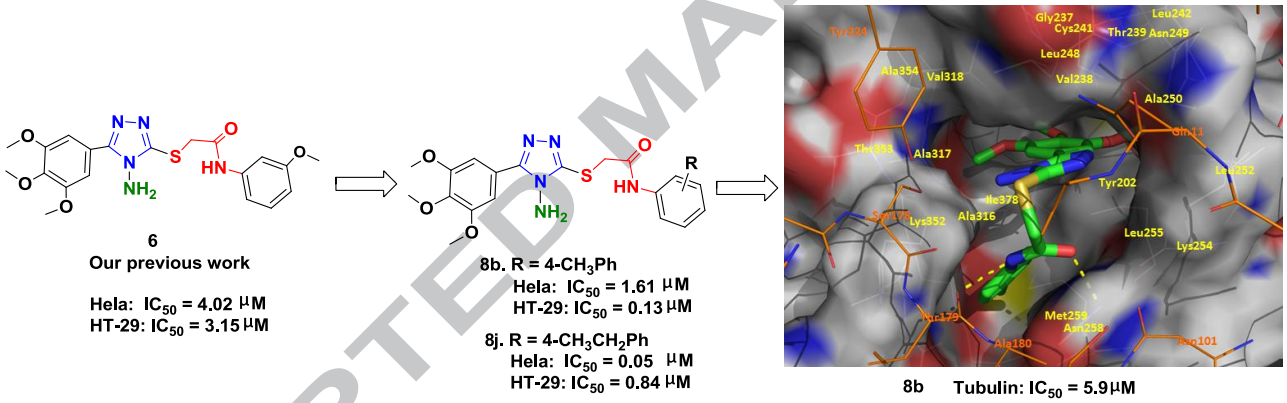
    Prime, version 4.2, Schrodinger, LLC, New York, NY, 2015.
26. Olsson MHM, Søndergard CR, Rostkowski M, et al. PROPKA3: consistent treatment of internal and surface residues in empirical pKa predictions. *J Chem Theor Comput.* 2011; 7: 525–537.
27. LigPrep, version 3.6, Schrodinger, LLC, New York, NY, 2015.

Discovery and optimization of 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides as potent tubulin polymerization inhibitors

Fang Yang[a,#], Cai-Ping He[a,#], Peng-Cheng, Diao[a], Kwon Ho Hong[b], Jin-Jun Rao[a,*], Pei-Liang Zhao[a,*]

[a]*Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Science, Southern Medical University, Guangzhou 510515, P.R.China*

[b]*Department of Medicinal Chemistry and Institute for Therapeutics Discovery and Development, College of Pharmacy, University of Minnesota, Minneapolis 55414, United States*



Highlights

► Based on our previous work, 29 novel triazolylthioacetamides possessing 3,4,5-trimethoxylphenyl groups were synthesized. ► **8f** and **8j** showed potent antiproliferative activities against Hela with IC_{50} values of 0.04 and 0.05 μM, respectively. ► **8b** exhibited potent antitubulin activity with IC_{50} values of 5.9 μM, which was proximate to CA-4 (IC_{50} = 4.2 μM).