Bioorg. Med. Chem. Lett. 38 (2021) 127880

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis, biological evaluation, and structure-activity relationships of new tubulin polymerization inhibitors based on 5-amino-1,2,4-triazole scaffold

Fang Yang [a], Lin Chen [a], Jin-Mei Lai [a], Xie-Er Jian, Dong-Xin Lv, Li-Li Yuan, Yu-Xia Liu, Feng-Ting Liang, Xiao-Lan Zheng, Xiong-Li Li, Li-Yuan Wei, Wen-Wei You, Pei-Liang Zhao *

Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Science, Southern Medical University, Guangzhou 510515, PR China

ARTICLE INFO

Keywords:
5-Amino-1,2,4-triazoles
Synthesis
Tubulin
Antiproliferative activity

ABSTRACT

Based on our previous research, thirty new 5-amino-1*H*-1,2,4-triazoles possessing 3,4,5-trimethoxyphenyl moiety were synthesized, and evaluated for antiproliferative activities. Among them, compounds **IIa**, **IIIh**, and **IIIm** demonstrated significant antiproliferative activities against a panel of tumor cell lines, and the promising compound **IIIm** dose-dependently caused G2/M phase arrest in HeLa cells. Furthermore, analogue **IIa** exhibited the most potent tubulin polymerization inhibitory activity with an IC_{50} value of 9.4 μM, and molecular modeling studies revealed that **IIa** formed stable interactions in the colchicine-binding site of tubulin, suggesting that 5-amino-1*H*-1,2,4-triazole scaffold has potential for further investigation to develop novel tubulin polymerization inhibitors with anticancer activity.

Microtubule system of eukaryotic cells is comprised by α/β tubulin heterodimers and is a crucial element in various essential cellular processes, such as maintenance of cellular structure, organization of intracellular architecture, intracellular transport and cell division.[1–3] Due to critical roles in the above-mentioned cellular functions, microtubule has been a highly attractive therapeutic target for cancer treatment, and numerous of structurally diverse microtubule-targeting agents have been investigated.[4–7]

Combretastatin A-4 (CA-4, Fig. 1) and its derivatives are well-known tubulin inhibitors targeting colchicine-binding site which is located at the interface of α/β tubulin heterodimers. Compared with other sites, targeting the colchicine-binding site might provide a promising superiority for reducing multidrug resistance effects, and hence, these tubulin inhibitors continue to receive substantial attention in recent decades.[8–12] Extensive studies strongly demonstrated that 3,4,5-trimethoxyphenyl fragment of CA-4 analogues is indispensable,[13] and replacement of the *cis*-double bond with a heterocyclic ring has become a well-documented approach to retain or improve their remarkable activities.[14–18]

Recently, a number of conformationally restricted CA-4 analogues through incorporating the *cis*-double bond into a heterocyclic ring system have been extensively investigated. Among them, several 1,2,4-triazole derivatives,[19,20] with general structures **2**–**4** listed in Fig. 1, were reported to possess notable antiproliferative activities. In our ongoing effort to discover novel 1,2,4-triazole derivatives as tubulin inhibitors,[21–26] we found two series of 3-alkylsulfanyl-1,2,4-triazole analogues, exemplified by compounds **5**–**7** (Fig. 1), which exhibited potent antiproliferative activities toward a panel of cancer cell lines.[24–26] These results triggered us to start a pharmacophore exploration and optimization effort around the 1,2,4-triazole core. Although a great deal of 1,2,4-triazole derivatives have been reported, only a few *N*-aroyl-5-amino-1,2,4-triazoles were investigated.[19,20,27] Hence the objectives of this work aimed to design and synthesis of various substituents including acetamides, aryl ketones and benzyl groups at the *N*-1 position of the 1,2,4-triazole core to develop novel potent antiproliferative agents with general formulas **I**–**III**. All target compounds were evaluated for their antiproliferative activities, and some promising analogues were further screened for inhibition of tubulin polymerization.

Synthesis of the target 5-amino-3-(3,4,5-trimethoxyphenyl)-1,2,4-triazoles **I**–**III** was described in Scheme 1. The key intermediate 3-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazol-5-amine **11** was accomplished using a four-step procedure from 3,4,5-trimethoxybenzoic acid according to reported procedure.[20] While required intermediates chloro acetamides were prepared *via* a biphasic acylation of appropriate aniline with chloroacetyl chloride in the presence of triethylamine and dichloromethane with excellent yields, using our recently reported

* Corresponding author.
E-mail address: plzhao@smu.edu.cn (P.-L. Zhao).
[a] These authors contributed equally to this work.

https://doi.org/10.1016/j.bmcl.2021.127880
Received 17 November 2020; Received in revised form 6 February 2021; Accepted 10 February 2021
Available online 24 February 2021

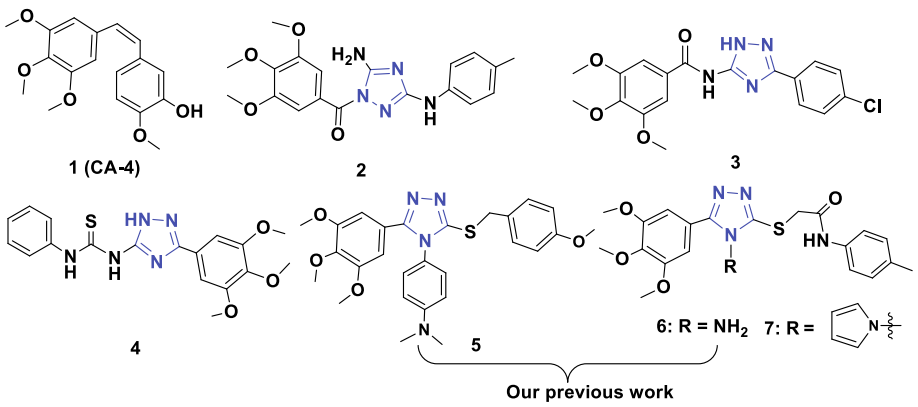
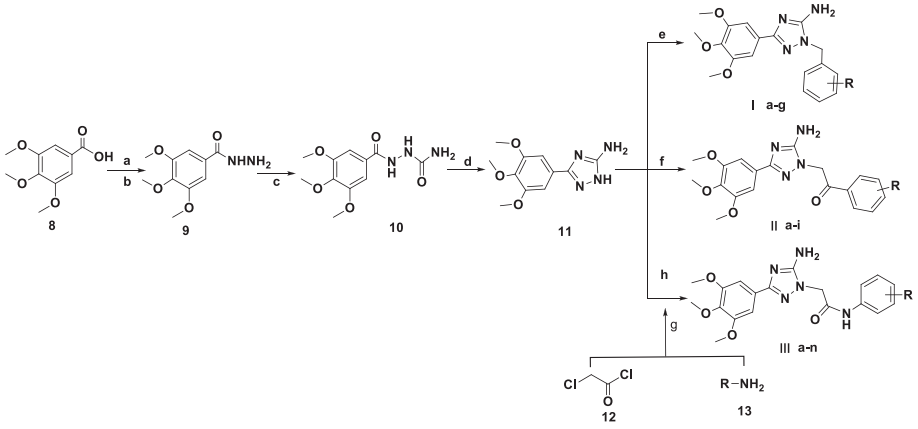


Fig. 1. Structures of selected tubulin inhibitors, including **CA-4**, and 1,2,4-triazole-based compounds **2**–**7**.



Scheme 1. Regents and conditions: (a) conc. H_2SO_4, ethanol, reflux; (b) $NH_2NH_2{\cdot}H_2O$, ethanol, reflux; (c) S-methylisothiourea sulfate; (d) 5% NaOH, 36 h, reflux; (e) substituted benzyl chloride, K_2CO_3, DMF, r.t.; (f) substituted phenacyl bromides, K_2CO_3, DMF, r.t.; (g) chloroacetyl chloride, Et_3N, CH_2Cl_2, r.t.; (h) K_2CO_3, DMF, 80 °C.

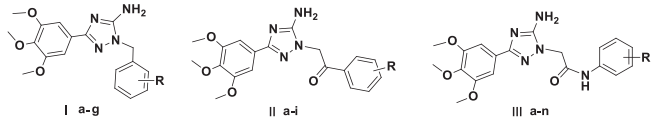
methodology.[22] Subsequently, the 5-amino-3-(3,4,5-trimethoxyphenyl)-1,2,4-triazole derivatives **Ia**–**g** and **IIa**–**i** were acquired through a nucleophilic substitution reaction of appropriate benzyl chloride and phenacyl bromide, with the key intermediate **11**, using solid K_2CO_3 as a base in anhydrous *N*,*N*-dimethylformamide at room temperature. Meanwhile, increase of the temperature to 80 °C, the reaction of triazole **11** with diverse *N*-substituted 2-chloroacetamides provided desired 1,2,4-triazoles **IIIa**–**n** in moderate to good isolated yields ranging from 65% to 85%. The chemical structures of the final compounds were fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS spectroscopic techniques, and the spectral data agree with the proposed structures.

The newly prepared compounds **Ia**–**g**, **IIa**–**i**, and **IIIa**–**n** were evaluated for their *in vitro* antiproliferative activities at single concentration of 100 μM toward five human cancer cell lines taken from different tissues, including MCF-7 (huma mammary adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung adenocarcinoma cells), PC-3 (human prostate cancer cell lines), and Panc-1 (human pancreatic carcinoma cell line) through MTT screening assay. The results for each compound were reported as the percent growth of treated cells compared to untreated control cells. Unfortunately, as shown in Table 1, nearly all of the 5-amino-1,2,4-triazole derivatives were ineffective toward A549 and PC-3 with growth inhibition (GI) percent lower than 50%. Interestingly, most compounds displayed substantial antiproliferative activities against MCF-7, HeLa, and Panc-1 cell lines. For example, the growth inhibition values of compounds **Ia**, **Ie**, **Ig**, **IIa**, **IIi**, **IIIh**, **IIIm** ranged from 51 to 95% on the three tested cell lines. Especially, derivative **IIIm** demonstrated the most powerful cell growth inhibition activity against a broad-spectrum of tumor cell types with GI values of 95, 93, 93, 93, and 70% on HeLa, MCF-7, A549, Panc-1, and PC-3, respectively.

According to the above data, some promising compounds were then selected for further evaluation for their antiproliferative activities toward MCF-7, HeLa, Panc-1, and K562 cell lines. As summarized in Table 2, **IIIm** exhibited the most promising antiproliferative activities on K562 and HeLa cell lines with IC_{50} values of 9.23 and 18.94 μM, respectively. It was interesting to note that compounds **I**–**III** with electron-donating groups always exerted more potent cytotoxic activity than the corresponding derivatives having electron withdrawing group (**Ia** vs. **Ie**, **Ia** vs. **Ig**, **IIa** vs. **IIi**, **IIIh** vs. **IIIn**, **IIIm** vs. **IIIn**). Moreover, acetamide derivatives **IIIh**, **IIIm** having bulky groups (*n*-propyl and *t*-butyl), displayed much higher antiproliferative activities than the corresponding derivatives with small substituents.

To explore the mode of action of these compounds on cancer cells, effect of compound **IIIm** on cell division in HeLa cell line was investigated by determining distribution of the cells in different phases of the cell cycle using flow cytometric technique. In this study, HeLa cells were treated with compound **IIIm** at indicated concentrations (10, 20, 40 μM). As depicted in Fig. 2, cell cycle analysis suggested that the

Table 1
Single concentration growth inhibition of compounds **Ia**–**g**, **IIa**–**i**, and **IIIa**–**n** toward five human tumor cell lines.



Comp.	R	Growth inhibition percent % at 100 μM IC_{50}(μM)[a]				
		MCF-7	HeLa	A549	PC-3	Panc-1
Ia	4-CH_3	55	76	34	39	53
Ib	4-F	34	28	42	41	7
Ic	2-F	33	33	24	35	5
Id	4-Cl	49	55	38	48	39
Ie	4-NO_2	52	58	13	21	53
If	3-CN	43	28	30	/a	6
Ig	3.5-$(CF_3O)_2$	55	73	6	36	71
IIa	4-CH_3O	73	76	21	31	57
IIb	4-CH_3	53	70	/	35	17
IIc	3-CH_3O	30	27	20	37	/
IId	3,4-F_2	48	31	14	28	52
IIe	4-F	27	9	13	18	/
IIf	4-CI	/	38	26	26	6
IIg	4-Br	27	41	12	38	6
IIh	H	30	42	15	/	4
IIi	3-Br	59	66	35	19	51
IIIa	4-CH_3	/	35	/	31	5
IIIb	3-CH_3O	34	39	17	6	/
IIIc	4-CH_3O	50	30	9	33	/
IIId	2-F, 4-CH_3	21	32	12	25	22
IIIe	4-CH_2CH_3	31	12	27	40	/
IIIf	3.4-$(CH_3O)_2$	12	41	22	42	14
IIIg	3-CH_3	23	18	34	50	10
IIIh	4-$CH_2CH_2CH_3$	70	75	37	61	91
IIIi	2.4.6-$(CH_3)_3$	34	49	16	50	32
IIIj	3.4-$(CH_3)_2$	32	20	29	/	32
IIIk	4-$N(CH_3)_2$	14	29	7	35	/
IIIl	4-$CH(CH_3)_2$	8	45	/	37	22
IIIm	4-$C(CH_3)_3$	93	95	93	70	93
IIIn	4-CI	37	30	22	14	12

/[a]: not detectable under the employed experimental conditions.

Table 2
In vitro antiproliferative evaluation of representative compounds toward four cancer cell lines.

Compd.	IC_{50} (μM)[a]			
	MCF-7	HeLa	Panc-1	K562
Ia	73.76 ± 3.35	46.90 ± 1.33	61.52 ± 3.80	17.1 ± 0.63
Ie	82.10 ± 4.21	57.19 ± 2.31	>100	>100
Ig	>100	53.14 ± 3.24	73.01 ± 1.02	31.53 ± 1.06
IIa	40.64 ± 0.72	52.17 ± 3.66	59.82 ± 5.95	65.99 ± 4.12
IIb	53.02 ± 4.11	54.04 ± 0.83	>100	33.04 ± 2.17
IIi	68.41 ± 2.19	58.24 ± 4.75	79.61 ± 3.92	>100
IIIh	44.71 ± 1.68	56.12 ± 2.72	20.03 ± 5.04	41.1 ± 0.97
IIIm	35.56 ± 0.23	18.94 ± 1.98	35.15 ± 6.50	9.23 ± 1.81
CA-4(nM)	170 ± 7.0	4.7 ± 0.2	ND[b]	610 ± 20

[a] IC_{50} is the concentration of compound required to decrease cellular growth by 50% and data are indicated as the mean ± SD from three independent experiments.
[b] ND: not determined.

percentage of cell in G2/M phase reached from 14.45% to 28.74% after treatment of 48 h. Hence, the significant increase revealed that **IIIm** induced a significant cell cycle arrest at the G_2/M phase in a concentration-dependent manner, compared to untreated cells.

Six representative compounds were further evaluated for *in vitro* inhibitory effects on tubulin polymerization at 10 μM concentration to examine whether these analogues could potentially interact with tubulin, and the typical microtubule depolymerization agent CA-4 was employed as a positive reference. The results showed that most tested compounds exhibited potent inhibitory activity (Table 3**)**. Particularly, compounds **IIa** and **IIIm** with greatest antiproliferative efficacy, also significantly inhibited tubulin polymerization by 52 and 48%, respectively. Notably, it was found in Fig. 3, analogue **IIa** inhibited tubulin polymerization in a similar way to CA-4, and demonstrated potent antitubulin activities with an IC_{50} value of 9.4 μM. These results indicated that the mechanism of **IIa** was consistent with previously reported CA-4, suggesting that compound **IIa** is a novel tubulin polymerization inhibitor.

Molecular modeling studies were performed to further confirm the capability of the promising compound **IIa** for binding to the colchicine-binding site in tubulin. Binding pose shown in Fig. 4 indicates that **IIa** binds to the β-tubulin subunit, at its interface with α-tubulin, similar to that of colchicine and CA-4. Oxygen atom of carbonyl moiety in **IIa** forms a powerful hydrogen bonding with amine group of Val181 (1.9 Å) of α-subunit. Meanwhile, there is a hydrogen bonding interaction between the free amine of 1,2,4-triazole nucleus and thiol group of Met259 (3.5 Å) of β-subunit. In addition, nitrogen atom of *N*-4 position in triazole ring also establishes a weak hydrogen bonding with Lys352 (3.6 Å) of β-subunit.

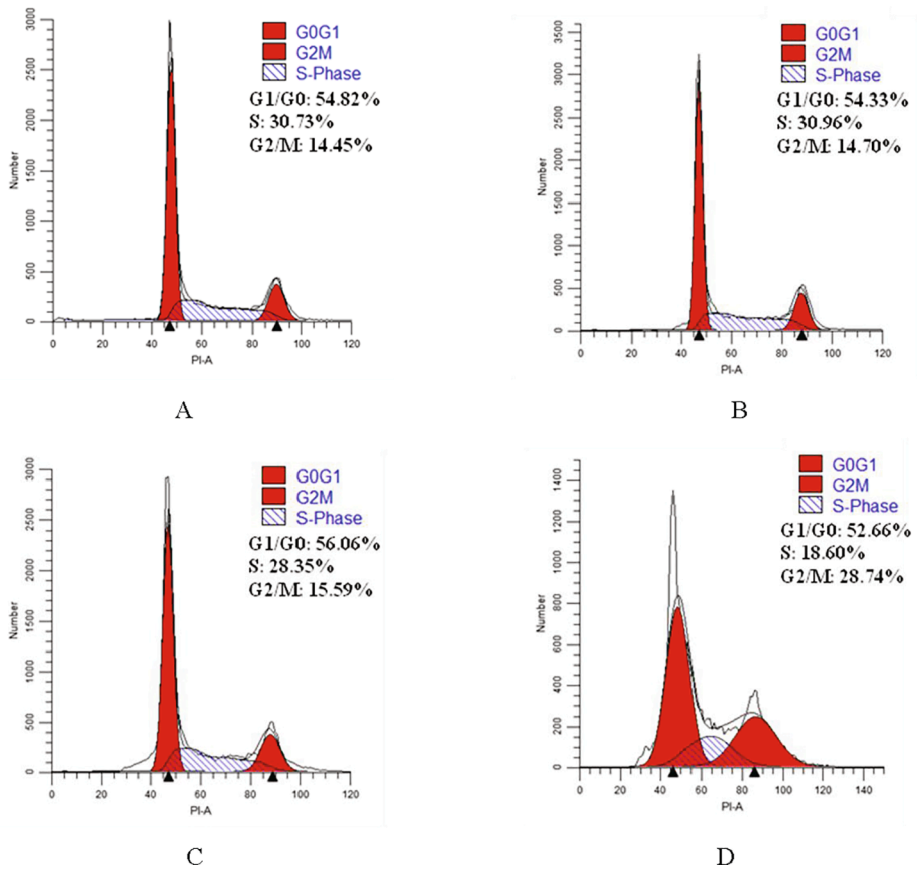


Fig. 2. Effect of compound **IIIm** on cell cycle in HeLa cells. Flow cytometry analysis of HeLa cells stained with propidium iodide and treated with **IIIm** for 48 h. (A) Control; (B) **IIIm**, 10 μM; (C) **IIIm**, 20 μM; (C) **IIIm**, 40 μM.

Table 3
Tubulin polymerization inhibitory activities of representative selected compounds.

Comp.	Tubulin polymerization	
	% inhibition[a]	IC_{50}(μM)[b]
Ia	25	–[c]
IIa	52	9.4 ± 0.3
IIb	42	–
IIIh	14	–
IIIm	48	–
CA-4	79	4.22 ± 0.15

[a] Compounds were tested at a final concentration of 10 μM.
[b] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates.
[c] NT: Not test.

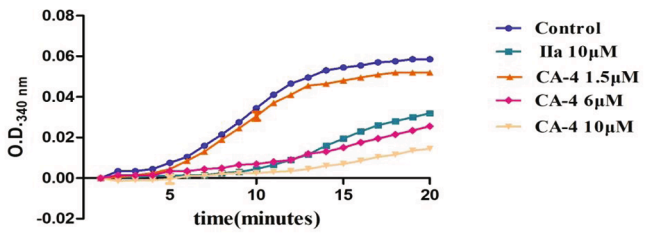


Fig. 3. Effects of **IIa** on tubulin polymerization *in vitro*. Tubulin in reaction buffer was incubated at 37 °C in the presence of the control (1% DMSO), compound **IIa** (10 μM) and CA-4 (1.5, 6, 10 μM). Absorbance at 340 nm was monitored at 37 °C every minute, and polymerizations were followed by an increase in fluorescence emission at 340 nm over a 20 min period. The experiments were performed three times.

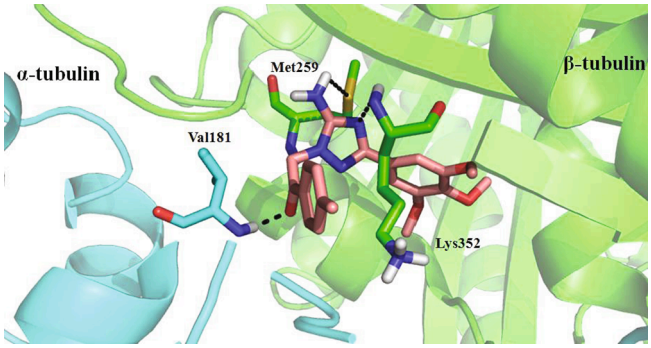


Fig. 4. Proposed binding model for **IIa** in colchicine-site of tubulin (PDB code: 5lyj). The backbone of tubulin is illustrated as ribbon representation (α-tubulin, cyan; β-tubulin, green). Hydrogen bonds are shown as dotted black lines.

In summary, based on our previous work, three new classes of 5-amino-1*H*-1,2,4-triazoles carrying 3,4,5-trimethoxyphenyl moiety were synthesized and evaluated for their antiproliferative activities. Compound **IIIm** displayed highest activity among all new derivatives against cancer cell growth and also demonstrated a significant inhibition of tubulin polymerization. Furthermore, **IIIm** concentration-dependently induced cell cycle arrest at the G_2/M phase in HeLa cells. Notably, **IIa** showed the most potent antitubulin activity with an IC_{50} value of 9.4 μM. Molecular docking studies revealed that **IIa** is likely to bind to the colchicine-binding site in a similar way with typical tubulin polymerization inhibitors. These preliminary results suggested that the novel 5-amino-1*H*-1,2,4-triazole scaffold has potential for further investigation to develop tubulin polymerization inhibitors with anticancer activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This present research was supported by the Natural Science Foundation of Guangdong Province, China (Grant No. 2018B030311067), and the Special Funds for the Cultivation of Guangdong College Students' Scientific and Technological Innovation. ("Climbing Program" Special Funds.) (No. pdjh2020b0117).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127880.

References

1 Coulup SK, Georg GI. Revisiting microtubule targeting agents: α-tubulin and the pironetin binding site as unexplored targets for cancer therapeutics. *Bioorg Med Chem Lett.* 2019;29:1865–1873.
2 Mirzaei H, Emami S. Recent advances of cytotoxic chalconoids targeting tubulin polymerization: synthesis and biological activity. *Eur J Med Chem.* 2016;121:610–639.
3 Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer.* 2004;4:253–265.
4 Bukhari SNA, Kumar GB, Revankar HM, Qin HL. Development of combretastatins as potent tubulin polymerization inhibitors. *Bioorg Chem.* 2017;72:130–147.
5 Govindaiah P, Dumala N, Grover P, Jaya PM. Synthesis and biological evaluation of novel 4,7-dihydroxycoumarin derivatives as anticancer agents. *Bioorg Med Chem Lett.* 2019;29:1819–1824.
6 Li Q, Jian XE, Chen ZR, et al. Synthesis and biological evaluation of benzofuran-based 3,4,5-trimethoxybenzamide derivatives as novel tubulin polymerization inhibitors. *Bioorg Chem.* 2020;102, 104076.
7 Chen P, Zhuang YX, Diao PC, et al. Synthesis, biological evaluation, and molecular docking investigation of 3-amidoindoles as potent tubulin polymerization inhibitors. *Eur J Med Chem.* 2019;162:525–533.
8 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.
9 Donthiboina K, Anchi P, Sri Ramya PV, et al. Synthesis of substituted biphenyl methylene indolinones as apoptosis inducers and tubulin polymerization inhibitors. *Bioorg Chem.* 2019;86:210–223.
10 Naaz F, Haider MR, Shafi S, Yar MS. Anti-tubulin agents of natural origin: targeting taxol, vinca, and colchicine binding domains. *Eur J Med Chem.* 2019;171:310–331.
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 Khodyuk RGD, Bai R, Hamel E, et al. Diaryl disulfides and thiosulfonates as combretastatin A-4 analogues: synthesis, cytotoxicity and antitubulin activity. *Bioorg Chem.* 2020;101, 104017.
13 Negi AS, Gautam Y, Alam S, et al. Natural antitubulin agents: importance of 3,4,5-trimethoxyphenyl fragment. *Bioorg Med Chem.* 2015;23:373–389.
14 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.
15 Mantipally M, Gangireddy MR, Gundla R, Badavath VN, Mandha SR, Maddipati VC. Rational design, molecular docking and synthesis of novel homopiperazine linked imidazo[1,2-*a*]pyrimidine derivatives as potent cytotoxic and antimicrobial agents. *Bioorg Med Chem Lett.* 2019;29:2248–2253.
16 Yang F, Jian XE, Diao PC, Huo XS, You WW, Zhao PL. Synthesis, and biological evaluation of 3,6-diaryl-[1,2,4]triazolo[4,3-*a*]pyridine analogues as new potent tubulin polymerization inhibitors. *Eur J Med Chem.* 2020;204, 112625.
17 Bommagani S, Penthala NR, Balasubramaniam M, et al. A novel tetrazole analogue of resveratrol is a potent anticancer agent. *Bioorg Med Chem Lett.* 2019;29:172–178.
18 Luo Y, Zhou Y, Song Y, et al. Optimization of substituted cinnamic acyl sulfonamide derivatives as tubulin polymerization inhibitors with anticancer activity. *Bioorg Med Chem Lett.* 2018;28:3634–3638.
19 Romagnoli R, Prencipe F, Oliva P, et al. 3-Aryl/Heteroaryl-5-amino-1-(3′,4′,5′-trimethoxybenzoyl)-1,2,4-triazoles as antimicrotubule agents. Design, synthesis, antiproliferative activity and inhibition of tubulin polymerization. *Bioorg Chem.* 2018;80:361–374.
20 El-Sherief HAM, Youssif BGM, Bukhari SNA, Abdel-Aziz M, Abdel-Rahman HM. Novel 1,2,4-triazole derivatives as potential anticancer agents: design, synthesis, molecular docking and mechanistic studies. *Bioorg Chem.* 2018;76:314–325.
21 Yang F, Yu LZT, Diao PC, et al. Novel [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives as potent antitubulin agents: design, multicomponent synthesis and antiproliferative activities. *Bioorg Chem.* 2019;92, 103260.
22 Jian XE, Yang F, Jiang CS, You WW, Zhao PL. Synthesis and biological evaluation of novel pyrazolo[3,4-*b*]pyridines as cis-restricted combretastatin A-4 analogues. *Bioorg Med Chem Lett.* 2020;30, 127025.
23 Ma W, Chen P, Huo X, et al. Development of triazolothiadiazine derivatives as highly potent tubulin polymerization inhibitors: structure-activity relationship, in vitro and in vivo study. *Eur J Med Chem.* 2020;208, 112847.
24 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.
25 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.
26 Yang F, He CP, Diao PC, Hong KH, Rao JJ, Zhao PL. Discovery and optimization of 3,4,5-trimethoxyphenyl substituted triazolylthioacetamides as potent tubulin polymerization inhibitors. *Bioorg Med Chem Lett.* 2019;29:22–27.
27 El-Sherief HAM, Youssif BGM, Bukhari SNA, et al. Synthesis, anticancer activity and molecular modeling studies of 1,2,4-triazole derivatives as EGFR inhibitors. *Eur J Med Chem.* 2018;156:774–789.